

**Souvenir and Abstracts**



# AMI-2019



## 60<sup>th</sup> Annual Conference of Association of Microbiologists of India

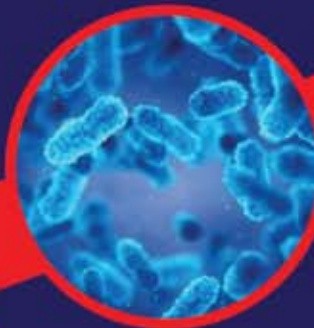
&

### International Symposium on Microbial Technologies in Sustainable Development of Energy, Environment, Agriculture and Health

15<sup>th</sup> - 18<sup>th</sup> November 2019

Organised by

Central University of Haryana  
Mahendergarh-123031, Haryana, India



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## MICROBIOLOGY

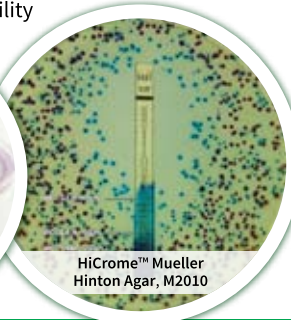
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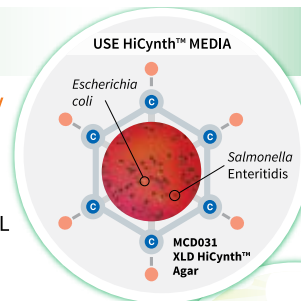
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**60<sup>th</sup> ANNUAL CONFERENCE**  
**of**  
**Association of Microbiologists of India**  
**&**  
**International Symposium**  
**on**  
**“Microbial Technologies in Sustainable  
Development of Energy, Environment,  
Agriculture and Health”**

**November 15-18, 2019**



**Organised by**

**Central University of Haryana**  
Mahendergarh, Haryana 123031



रमेश पोखरियाल 'निशंक'  
*Ramesh Pokhriyal 'Nishank'*



मंत्री  
मानव संसाधन विकास  
भारत सरकार  
MINISTER  
HUMAN RESOURCE DEVELOPMENT  
GOVERNMENT OF INDIA



### MESSAGE

It gives me immense pleasure to know that the School of Life Sciences, Central University of Haryana, Mahendergarh is organising 60<sup>th</sup> Annual Conference of AMI & International Symposium on "Microbial Technologies in Sustainable Development of Energy, Environment, Agriculture and Health" between 15 to 18 November, 2019.

The theme of the Conference is quite relevant and contextual for the students, faculty and scientists in the field of Microbiology, and would help the participate understand the varied nuances of the thrust areas of the event. I hope the Conference outcome will inspire the current and future generations of Microbiologists for advanced researches and studies in India and abroad. I am sure that the Souvenir being brought out on this occasion will highlight the outcome of the Conference for wider dissemination among the scientists and policy makers in the field of Microbiology.

On this occasion, I compliment the Vice Chancellor and his dedicated team for this special initiative. I also convey my best wishes for the launch of the Souvenir and wish the Conference a grand success.

(Ramesh Pokhriyal 'Nishank')



सबको शिक्षा, अच्छी शिक्षा ।

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हरियाणा राज भवन,  
चण्डीगढ़ - 160019

HARYANA RAJ BHAWAN,  
CHANDIGARH. - 160019

## संदेश

यह अत्यंत हर्ष का विषय है कि स्कूल ऑफ इंटरडिसिप्लिनरी एंड एप्लाइड लाइफ साइंसेज, केंद्रीय विश्वविद्यालय हरियाणा, महेंद्रगढ़ "कृषि और स्वास्थ्य" नामक विषय पर 15 से 18 नवंबर, 2019 तक "माइक्रोबायोलॉजिस्ट एसोसिएशन ऑफ इंडिया (ए.एम.आई) और इंटरनेशनल सिम्पोजियम ऑफ माइक्रोबियल टेक्नोलॉजीज इन सस्टेनेबल डेवलपमेंट ऑफ एनर्जी, एनवायरनमेंट" का 60वां वार्षिक सम्मेलन आयोजित कर रहा है।

इस अंतर्राष्ट्रीय सम्मेलन में सभी प्रतिभागियों को माइक्रोबायोलॉजी और लाइफ साइंसेज के क्षेत्र में अपने विचारों का आदान-प्रदान करने का अवसर मिलेगा। सम्मेलन में वैज्ञानिक और विशेषज्ञ अपने-अपने नए अनुसंधानों के अनुभव सांझा करेंगे, जिसमें कृषि और स्वास्थ्य से संबंधित नई जानकारी सामने आएगी। इस प्रकार से ये सभी जानकारी और सूचनाएं हितधारकों के लिए लाभदायक होगी। यह सम्मेलन कृषि के सतत विकास व आमजन के लिए मील का पत्थर साबित होगा।

मैं यह सम्मेलन आयोजित करने पर केंद्रीय विश्वविद्यालय हरियाणा की पूरी टीम को हार्दिक बधाई व शुभकामनाएं देता हूं और सम्मेलन की सफलता की कामना करता हूं।

(सत्यदेव नारायण आर्य)

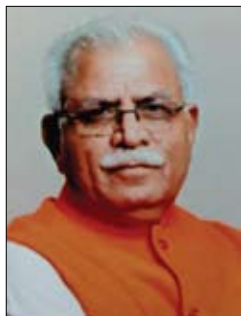




मनोहर लाल  
Manohar Lal



मुख्य मंत्री, हरियाणा,  
चण्डीगढ़।  
CHIEF MINISTER, HARYANA,  
CHANDIGARH.  
Dated.....11<sup>th</sup> November, 2019.....



### Message

It gives me immense pleasure to know that Haryana Central University, Mahendergarh is organising and International Symposium on the theme of "Microbial Technologies in Sustainable Development of Energy, Environment, Agriculture and Health" from 15 November to 18, 2019.

I appreciate the decision to coincide the international symposium with annual conference of Association of Microbiologists of India.

New findings in the field of microbiology have not only ushered in a revolution in many sectors including health sciences, medicine, energy and food industry, but have also posed some challenges to the scientific community. I am sure symposiums will update the knowledge of the participants, and also generate fresh perspective on various aspects concerning microbial technologies.

The deliberations of the annual conference will prove a step forward in acquainting the participants about scientific advancements made in this field and would also enhance their efficiency. Also, it would provide them an opportunity to find solutions to their common problems.

My best wishes.

  
(Manohar Lal)



दुष्यंत चौटाला  
DUSHYANT CHAUTALA



D.O. No. Dy. CM-2019/42897

उप मुख्य मन्त्री, हरियाणा,  
चण्डीगढ़

DEPUTY CHIEF MINISTER, HARYANA  
CHANDIGARH

Dated 07.11.2019

### MESSAGE

This is moment of immense pride for Central University of Haryana to host 60<sup>th</sup> Annual Conference of Association of Microbiologists of India-2019 and International Symposium on "Microbial Technologies in Sustainable Development of Energy, Environment, Agriculture and Health" on November 15-18, 2019. In the past three decades microbiology has grown widely in different specialized areas through research and development endeavors. Subsequently, numerous mysteries of microbes crucial in regulating human activities have been explored through researches at different levels and, therefor, it is quite pertinent to further discuss the scope and application of Microbiology for sustainable health, environment and development of human society.

The objectives of the Conference suggest that the 60<sup>th</sup> Annual Conference of Association of Microbiologist of India (AMI-2019) will cover a broad range of topics of Microbial-Industrial Microbiology, Bioinformatics, Agricultural Microbiology, Environmental Microbiology, Virology, Medical Microbiology, Food Microbiology etc. with an emphasis on 'microbes for sustainable development' to revolutionize future research in Microbiology to transform society.

On this pious occasion, I welcome all the participants, delegates, resource persons and guests, and wish the University a grand success for this international conference. I exhort all the participating microbiologists to stay connected with Central University of Haryana for future academic endeavours of similar nature.

I compliment Prof. R.C. Kuhad and his dynamic team for hosting this ambitious event with overwhelming participation from prestigious universities and research organisations. I am sure that the success of this event will surely motivate other universities of the state to conceptualise collaborative extension and academic activities.

Good Luck!

(Dushyant Chautala)





प्रो. धीरेन्द्र पाल सिंह  
अध्यक्ष

**Prof. D. P. Singh**  
Chairman



विश्वविद्यालय अनुदान आयोग  
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फैक्स Fax : 011-23239659, e-mail : cm.ugc@nic.in | web: www.ugc.ac.in



## MESSAGE

I am immensely delighted to know that Central University of Haryana is organizing and International Symposium on "*Microbial Technologies in Sustainable Development of Energy, Environment, Agriculture and Health*" and the Annual Conference of Association of Microbiologists of India (AMI), on November 15-18, 2019. It is an ambitious initiative of the University to bring together the Life Scientists from prestigious organisations of the world at one platform to discuss and deliberate the theme of the Symposium. It will give an opportunity to the young scholars and scientists to interact with the best of the scientists of the world, and shall connect the university fraternity with the outer world.

On this occasion, I compliment the Vice Chancellor of the University, Prof. R.C. Kuhad for having conceptualized this international Symposium at Central University of Haryana despite the constraints of a remotely located new university. It speaks volumes of the determination of the university leadership and the team to convert the challenges into opportunities.

I wish Prof. R.C. Kuhad and the organizing team all the very best for the organization of the Conference and the publication of the souvenir.

(Prof. D. P. Singh)

**11<sup>th</sup> November, 2019**





**हरियाणा केंद्रीय विश्वविद्यालय**  
(संसद के अधिनियम संख्या 25 (2009) के तहत स्थापित)  
महेंद्रगढ़ (हरियाणा)-123031

**Central University of Haryana**  
(Established vide Act No. 25 (2009) of Parliament)  
Mahendergarh (Haryana)-123031

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ईमेल/Email : vc@cuh.ac.in;

वेबसाइट/Website : www.cuh.ac.in

प्रो. आर. सी. कुहाड़,  
(एफएनएससी, एफएनएएएस, एफबीआरएसआई)

कुलपति

**Prof. R.C. Kuhad,**  
(FNASc, FNAAS, FBRSI)

**Vice-Chancellor**



क्रमांक /No \_\_\_\_\_

दिनांक /Date : 12 / 11 / 2019

## MESSAGE

It is a matter of immense delight for me to share that School of Life Sciences, Central University of Haryana is organizing 60<sup>th</sup> Annual Conference of AMI & International Symposium on “**Microbial Technologies in Sustainable Development of Energy, Environment, Agriculture and Health**” from November 15-18, 2019. Firstly, I welcome all the participants, delegates and resource persons from India and abroad to join us for this conference.

The field of Microbiology is vital and integral for sustainable development of human life. Its application in various fields has led to several discoveries crucial for resolving the complexities of life. I am happy that the theme and the scope of the conference has received wide appreciation among the community of life scientists in India and abroad. This international conference would provide a significant platform to discuss the vital issues of global significance. Some of the thrust areas of the conference include— effective use of microbes for the cost effective conversion of biomass into renewable energy as an alternate source of fossil energy; developing pest resistant agricultural crop varieties with high yield; microbial diversity in various ecosystems and their significance; exploration of gut microbiome in human health and physiology; and development of new drugs for combating microbial pathogens. The AMI-2019 conference would not only be helpful to generate mass awareness on the various Microbial Technologies in Sustainable Development of Energy, Environment, Agriculture and Health to encourage and promote entrepreneurship and startup enterprises, but would also provide a platform for interaction among life scientists, and young researchers.

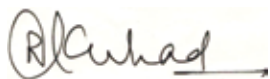
Entering into the eleventh year of its establishment, the Central University of Haryana has earned the reputation of an emerging institution in the National Capital Region. Gradually expanding the academic

horizons, the university now offers 60 academic programmes under various departments and schools of study, and three new programmes are queued to be introduced shortly after necessary permissions from the regulating authorities. The enrollment of 46% students from the states other than Haryana in the current academic year testifies the commitment of the University to promote diversity, inclusivity and quality in higher education. Similarly, the University has always been successful in maintaining encouraging enrolment of female students. The University has also expanded its physical and academic infrastructure to facilitate the learning activities on the campus. All the Science and engineering departments have developed adequate laboratory and experimentation facilities for UG/PG and research students. All the academic blocks have the facility of Digital classrooms to facilitate the creation and development of Massive Open Online Courses (MOOCs), and a couple of MOOCs offered by the University during the last semester were among the top-ranking courses on SWAYAM platform. To further motivate the faculty and students for optimum utilization of e-contents, the University has recently signed MoU with consortium of Educational Communication, Delhi. It will facilitate the University in access of e-contents available with CEC. Adopting innovative pedagogical advancements, the University conducts Global Initiative of Academic Networks (GIAN) programmes with the experts from prestigious foreign universities/organizations, as a regular feature. The faculty of the University has also been achieving significant milestones in publications, research projects, patents, consultancy projects and collaborative research initiatives. The University focuses on promotion of diversity, inclusivity and outreach which gets reflected through the best NIRF score of 56.18 in “Outreach and Inclusivity” parameter in NIRF Ranking-2019.

Promoting the culture of research and innovations, the University has institutionalized the Annual Best Researcher Award on the basis of outstanding research contribution by the individual faculty in a year. The University has also introduced the annual ratings of the departments assessed on the basis of faculty presentations, academic audit, and research outcome.

Dear participants, despite the remoteness of location, we have made the best possible arrangements to make your stay comfortable and fruitful, and I would personally urge upon you to make the best use of this rare opportunity to have the galaxy of great scientists, entrepreneurs and scholars at one place. I hope the participants will appreciate our humble offerings during the event.

I extend my best wishes to the organizing committee for the grand success of the conference and publication of Souvenir. Moreover, I expect some logical recommendations at the end of the conference, which eventually will benefit young researchers and research organisations in deciding their future priorities.



**(Prof. R.C. Kuhad)**



**J S. Viridi, Ph.D.**  
President AMI  
Professor, Department of Zoology  
University of Delhi  
Delhi 110007



### **Message**

It is my pleasure to welcome you all to the 60<sup>th</sup> Annual International Conference of Association of Microbiologists of India (AMI-2019) being hosted at the Central University of Haryana (CUH), Mahendergarh, Haryana.

By now, we all know that Microbes are present everywhere – almost all organs of the human beings, animals, plants, soil, water, in space, depths of the sea and in extreme environments. Besides being the causative agents of diseases, microbes are responsible for numerous beneficial activities such as industrial fermentation, biodegradation and geochemical recycling, antibiotic production, as hosts for cloning and production of recombinant proteins, and high-value applications such as vectors for tissue engineering and generation of transgenic.

The 60th Annual Conference of AMI will cover almost all the major areas of Microbiology and other important topics, which have direct bearing on human life and environment. The thrust areas of the conference include Agricultural Microbial Technology, Bioenergy, Environmental Microbial Technology, Food and Dairy Microbial Technology, Microbial Genomics, Proteomics and Metabolomics, Industrial Microbiology and Microbial Technology, Medical and Veterinary Microbiology, Microbial Diagnostics, Molecular Microbiology, and Industry-Student-Faculty interactions on “Microbiology Curriculum *versus* Industry Requirements” in consonance with the Learning Outcome-based Curriculum Framework (LOCF) initiated by the University Grants Commission (UGC). The organizing committee has finalized the schedule and technical sessions of the conference in consultation

with active members of the AMI and several external resource persons and experts, to ensure the utility of the conference for all participating delegates and students alike.

I strongly believe that the conference would act as a strong platform for the microbiologists to share and discuss their research that will, jointly, help address some of the challenging global issues like antimicrobial resistance (AMR), bioenergy and environmental pollution. The proceedings and the outcome of the conference shall be disseminated widely to apprise all stake holders about the current scenario of microbiology and how this knowledge may be used to mitigate some of the problems currently being faced by the human society.

I am delighted to acknowledge the efforts made by the local organizing committee in coordination and organization of this important event. All the members of the committee deserve applause and compliments for their un-tiring efforts. I am sure that the conference will be a grand success with the participation of delegates and experts from India and abroad.

Best wishes.



(J S Viridi)

**Yogendra Singh, Ph.D.**  
President Elect, AMI-2019  
Professor, Department of Zoology  
University of Delhi  
Delhi 110007



### **Message**

Dear AMI Members and Delegates

As the President elect of Association of Microbiologists of India, I would like to welcome all of you to the 60th Annual Conference of AMI and International Symposium on “Micobial Technologies in Sustainable Development of Energy, Agriculture and Health at the Haryana Central University, Mahendergarh, Haryana. AMI consists of a diverse group representing people from various backgrounds including microbiology, virology, environmental science, medicine, biotechnology, agriculture science, bioinformatics and science policy. The association serves the members and students by providing current scientific information through small meetings by AMI units and a annual meeting. We encourage interactions and networking among attendees. This is an opportunity where attendees can meet specialists of a particular area and discuss their problems and develop collaborations. I believe that such a diversified and multi-disciplinary forum will be a rich platform which will enrich all of us on the recent research being carried out in the area of micobiology throughout the world. I congratulate the organising committee for selecting the important topic such as “sustainable development of energy” as part of the theme of this meeting. I hope that all of you will enjoy and learn many new developments in several areas that will be covered in this meeting. I look forward to seeing you at this meeting and wish a grand success of the conference.

(Yogendra Singh)





**Prof. T. Satyanarayana**  
**Professor Emeritus**

**Division of Biological Sciences & Engineering**  
**Netaji Subhas University of Technology**  
**Azad Hind Fauj Marg**  
**Sector - 3, Dwarka**  
**New Delhi-110078, India**  
**Email: tsnarayana@gmail.com**

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#### **MESSAGE**

It gives me immense pleasure to appreciate that the Central University of Haryana will be organizing 60th Annual Conference of the Association of India and international conference on a very important topic 'Microbial Technologies in Sustainable Development of Energy, Environment, Agriculture and Health' during 15 - 18 Nov. 2019. The conference will give an opportunity for students as well as scholars in microbiology to present their research findings and listen to lectures by experts and interacting with them. I am very sure that Microbiology community will be immensely benefitted by participating in the conference.

It gives me great pleasure to wish CUH a success in organizing the well planned event and congratulate CUH for the same.

**Prof. T. Satyanarayana**  
**Ex-President, AMI**



**Prof Surjit Singh Dudeja**  
**Past President AMI**  
**Hisar 125001**



### **MESSAGE**

I am extremely happy to know that Central University of Haryana, Mahendergarh is organizing 60th AMI International Conference from Nov 15-18, 2019. During last one and a half decade the Association of Microbiologists of India (AMI) and during last five years Central University of Haryana has made tremendous progress. This annual AMI conference is considered to be one of biggest show of the association and it is proud moment for the young Central University of Haryana under the dynamic leadership of Prof R.C. Kuhad Vice Chancellor of the University are going to organize such a mega event.

Since this conference has included all the possible and relevant fields of Microbiology in the form of various symposia and eminent scientists from India and abroad including students are going to deliberate for four days. Therefore, I am sure important recommendations are bound to come in the different current fields of Microbiology like Industrial, Environmental, Molecular Biology, Bioenergy, Agricultural, Medical, Food, Microbial diagnostics, technology and diversity. Definitely these useful recommendations will benefit the policy planners and delegates in formulating renewed strategies for greater thrust in the respective disciplines.

I wish the venture a great success.

(S.S.Dudeja)





**Prof. Satish Kumar**  
*Chairman, AMI-2019*  
Department of Biotechnology  
Central University of Haryana  
Mahendergarh-123029, Haryana, India



### **MESSAGE**

It gives me immense pleasure to welcome you all at the 60th Annual Conference of Association of Microbiologists of India (AMI-2019) and International Symposium on “Microbial Technologies in Sustainable Development of Energy, Environment, Agriculture and Health” being organized by the Central University of Haryana from November 15-18, 2019.

Microbiology has been an area of intense research in the School of Life Sciences having more than 20 faculty working on variety of microbial model systems for researches on bioenergy, advanced biofuels, primary and secondary metabolite production, sustainable agriculture and environment, understanding host-pathogen interactions, antimicrobial resistance and elucidation of metabolic pathways.

During AMI-2019, the scientific discussions on microbial technologies and processes are envisaged to provide potential solutions to the global challenges related to energy, environment, agriculture, and health. This conference would make a bridge between academicians, health professionals and industrialists by providing a common platform for exchange of ideas. The participants will get a unique platform to present and discuss the most recent innovations, trends, concerns and practical challenges encountered and solutions adopted for sustainable development of energy, environment, agriculture, and health.

The conference will provide an opportunity to the students to interact with the world renowned scientists and researchers, as well as to network with their peers for exchange of ideas and to explore research and job opportunities. The special session on Industry-Student Interaction on “Microbiology Curriculum versus Industry Requirements” will help students to acquaint themselves with the industry needs, which will help in improving their technical skills and better explore the employment opportunities.

Team CUH has strived hard work to put forward quality science and hospitality to the delegates of the AMI-2019. I hope and wish that while you will enjoy your stay with us we shall have great opportunity to learn from all of you.



**(Satish Kumar)**

**Prof. Pratyoosh Shukla, FAMSc, FBRS**

*General Secretary,*

*The Association of Microbiologists of India (AMI)*

*Professor & Head, Department of Microbiology,*

*Maharshi Dayanand University, Rohtak-124001,*

*Haryana, India*

*Former ASM Indo-US Visiting Research Professor, University of*

*Cincinnati, College of Medicine, Cincinnati Ohio, USA*

*Bentham Science Ambassador (India)*

*Guest Professor, South China University of Technology, Guangzhou, China*



**Message**

It gives me immense pleasure to learn that the 60th Annual Conference of the Association of Microbiologists of India i.e. AMI-2019 and International symposium on microbial technologies in sustainable development of energy, environment, agriculture and health is organized by Department of Microbiology, Central University of Haryana, Mahendergarh, Haryana, India from November 15-18, 2019. The main objective of this conference is to encourage the exchange of scientific information on energy, environment, agriculture and health.

I congratulate the whole organizing committee and all the stakeholders of the University for their Efforts towards organizing a good conference and wish them all the best in their venture. I entirely urge all the participants and students to take full advantage of this opportunity, and thus, make this conference a spectacular success.

I wish to all AMI Members and participants of this conference a fruitful and pleasant stay at Central University of Haryana Mahendergarh, Haryana, India



**Prof. Pratyoosh Shukla**





**Dr. Surender Singh & Dr. Jitendra Kumar Saini**

*Organizing Secretaries, AMI-2019*

Department of Microbiology

Central University of Haryana

Mahendergarh-123029, Haryana, India



## MESSAGE

This is a moment of immense pride and pleasure for us to welcome all the participants, delegates, resource persons and guests at the Central University of Haryana for attending the 60th Annual Conference of Association of Microbiologists of India (AMI-2019) and International Symposium on “Microbial Technologies in Sustainable Development of Energy, Environment, Agriculture and Health” during November 15-18, 2019. In the past three decades, Microbiology has grown widely in different specialized areas through research and development endeavors. Subsequently, numerous mysteries of microbes crucial in regulating human activities have been explored through microbiologists at different levels, and it is quite pertinent to further discuss the scope and applications of Microbiology for sustainable development of environment and health in the welfare of human society.

The AMI-2019 International Conference will provide a common forum to discuss, deliberate and formulate recommendations about future thrust areas of research on various aspects related **microbial technologies in sustainable development of energy, environment, agriculture and health** for human welfare. The scientific discussions on microbial processes and technologies are envisaged to provide potential solutions to the global challenges related to environment and health. It will be a great opportunity to Microbiology and Life Sciences students to interact with some renowned scientists from different parts of the world.

AMI-2019 will cover all major sub-disciplines of Microbiology with an emphasis on “Energy, Environment, Agriculture and Health” to revolutionize future research to transform society. A total of 13 technical sessions ranging from agriculture and environment to bioenergy, from food and dairy to veterinary microbiology, from microbial diagnostics to genomics, proteomics and metabolomics will together constitute this mega event. Moreover, a special session on Industry-Student Interactions on “Microbiology Curriculum versus Industry Requirements” will enlighten budding

microbiologists by inculcating entrepreneurship ideas and scientific attitude for societal benefits. For promotion of young scientists and researchers, 4 young scientist sessions, one rapid fire Ph.D. student session and two poster presentation sessions will be organised during the conference. "Walk in Poster Session" has been introduced in the conference for the first time for those who have missed the registration deadline.

We truly acknowledge the whole-hearted support that we received in selection of Central University of Haryana to host the 60th Edition of the annual conference of AMI. We acknowledge the financial support received from government agencies like NFDB, IOCL, DST-SERB, CSIR, DRDO and private companies specially HiMedia laboratories without whose support it would have been difficult to organize such a mega event. Local organizing committee has worked tirelessly for past few months to take care of every minute thing to make this conference a success. We also take this opportunity to thank our colleagues, administrative staff, student volunteers and press who directly or indirectly helped for successful organization of the event. The financial assistance from Research and Development Fund of the National Bank for Agriculture and Rural Development (NABARD) towards the printing of abstract book of the conference is gratefully acknowledged.

We hope that you'll relish the feast of ideas during the conference, and stay connected with us.



**(Surender Singh)**



**(Jitendra Kumar Saini)**

## **ABOUT THE CENTRAL UNIVERSITY OF HARYANA**

The Central University of Haryana (established vide Central Universities Act 2009) is the only University of the state of Haryana to be funded and regulated by University Grants Commission and Ministry of Human Resource Development (MHRD), Government of India. Central University of Haryana is located at Jant-Pali villages of district Mahendergarh in South Haryana. Mahendergarh is now a part of the extended National Capital Region (NCR) and is around 125 kilometers away from Delhi. It is well connected to Delhi through railways.

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To develop enlightened citizenship of a knowledge society for peace and prosperity of individuals, nation and the world, through promotion of innovation, creative endeavours, and scholarly inquiry.

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To serve as a beacon of change, through multi-disciplinary learning, for creation of knowledge community, by building a strong character and nurturing a value-based transparent work ethics, promoting creative and critical thinking for holistic development and self-sustenance for the people of India. The University seeks to achieve this objective by cultivating an environment of excellence in teaching, research and innovation in pure and applied areas of learning, with a focus on social enquiry, democratic ethos and inclusive socio-economic development, community outreach initiatives, scientific endeavours and technological advancement.

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### **ABOUT THE ASSOCIATION OF MICROBIOLOGISTS OF INDIA (AMI)**

The Association of Microbiologists of India was established in 1938. AMI is one of the oldest and reputed scientific organizations in India. Since its inception, it has contributed significantly towards development and strengthening both basic and applied microbiology research and teaching in the country. At present, there are more than 5000 life and annual members and about 450 corporate members of the Association. AMI started publishing its own quarterly journal, ‘Indian Journal of Microbiology’ in the year 1960. The journal at present is published by Springer. Indian Journal of Microbiology publishes peer reviewed original research findings and research reviews from researchers in India and abroad and has acquired a respectable status among national and international scientific research periodicals in the world. The association holds one of the country’s largest conference annually at well-established centers of microbiology in the country.



# Note



# Note



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15	Stage, Bouquet and flower arrangement	Dr. Puja Yadav	Microbiology	Assistant Professor
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		Dr. Gurjeet	Political Science	Assistant Professor
		Dr. Ritu	Yoga	Assistant Professor
16	Poster session	Dr. Ashwani Kumar	Nutrition Biology	Assistant Professor
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		Dr. Hempal	Physics	Assistant Professor



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		Dr. Devendra Kumar	Statistics	Assistant Professor
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		Dr. Dinesh Chahal	Education	Assistant Professor
		Dr. Puja Yadav	Microbiology	Assistant Professor
		Dr. Savita	Nutrition Biology	Assistant Professor
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		Ms. Meenu	-do-	Nursing Officer
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		Dr. Renu Yadav	Education	Assistant Professor
		Dr. Gunjan Goel	Microbiology	Associate Professor



# INAUGURAL AND PLENARY TALKS



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### Abstract

The potential quantity of bio-ethanol that could be produced from cellulose is over an order of magnitude larger than that from corn. The cellulose to ethanol route has a clearly positive net energy balance. The rate-limiting step in the conversion of cellulose to fuels is the hydrolysis of the highly ordered structure of crystalline cellulose. *Clostridium thermocellum* is a thermophilic, anaerobic, cellulolytic, and ethanogenic bacterium capable of directly converting cellulosic materials into ethanol. The large amounts of attention and resources devoted over the past 30 years to the *Trichoderma-Saccharomyces* concept have perhaps interfered in the industrial development of the potential of the cellulolytic, thermophilic anaerobic bacteria such as *C. thermocellum* and their cellulases and hemicellulases. *C. thermocellum* is capable of converting biomass into ethanol, acetic acid, lactic acid, and hydrogen. The interest in this organism is due to several factors. First, *C. thermocellum* can directly convert lignocellulosic biomass to fuels and chemicals in a single step. Second, its anaerobic nature negates expensive oxygen transfer process for cellulase production. Third, its growth temperature at 60°C facilitates ethanol recovery and reduces the cooling requirement and potential of contamination in fermentation. The *C. thermocellum* cellulase system exists as a multi-protein complex called the cellulosome. More than 70 enzymatic subunits have been found to be associated with the cellulosome, which include endoglucanases, xylanases, other hemicellulases, pectinases, esterases, proteases, and exoglucanases. The core of the cellulosome is a 250-kDa non-catalytic polypeptide, CipA, which binds to cellulose and serves as a scaffold for the catalytic subunits. In addition, the bacterium produces free cellulases that are not associated with the cellulosome. The bacterium is thus capable of producing a large number of glycosyl hydrolases. The presentation will review the current knowledge of the *C. thermocellum* cellulase system.

**Keywords:** Cellulases; *Clostridium thermocellum*; Thermophilic; Lignocellulose

### About the Speaker

Professor Wu was born in Taiwan. He received a BS and MS degrees in Biochemical Science and Technology (formerly known as Agricultural Chemistry) from the National Taiwan University in 1976 and 1980, respectively. He earned his MS and PhD degrees in Biochemical Engineering from MIT in 1982 and 1987, respectively. He is a Professor of Chemical Engineering and of Biomedical Engineering at the University of Rochester in Rochester, NY. He is a leader in studying the biomass-degrading enzyme system of *Clostridium thermocellum*, a key bacterium in "Consolidated Processing" leading to bio-ethanol production. He directed a DOE-funded consortium to develop biomolecular strategies toward biofuel production employing this bacterium. His work has led to the discoveries of the modular structure of the now well-known cellulosomal scaffolding protein, the unique Family 48 of glycosyl hydrolases, the novel 3-D structure of the cellulosomal dockerin, and the first cellulase transcriptional regulator and operon in *C. thermocellum*. At the University of Rochester, he developed a novel 3-D bone marrow culture system conducive to multi-lineal blood cell differentiation. The 3-D culture system has been used as a model in three NIH- or BARDA-funded centers on developing countermeasures against bioterrorism, including vaccines and anti-radiation drugs. His research group continues to investigate the cellulosome mechanism, and transcription regulation concerning biomass degradation and bioethanol fermentation at the genome scale as well as molecular events governing blood cell formation.

Professor Wu is a Fellow of the American Academy of Microbiology (AAM), a Fellow of the Society for Industrial Microbiology (SIM), and a Fellow of the American Institute for Medical and Biological Engineering. He is a recipient of the SIM Waksman Outstanding Educator Award. He has twice won the awards for excellence in teaching from the Undergraduate Engineering Council of the University of Rochester. He

served as an editor for *Industrial Biotechnology* and was on the editorial boards of *the Journal of Bioscience and Bioengineering* and *Applied Microbiology and Biotechnology*. He is also an editor for *The ASM Manual of Industrial Microbiology and Biotechnology* (Second Edition). He served as the ASM Div. O Chair in 1997/1998 and was the Divisional Lecturer in 2002. He has served as a reviewer for various federal programs on bioenergy or tissue engineering, including those of DOE, DOE-GTL, NSF, NIH, and NREL. He also served as a Scientific Advisor to NYSTAR (the New York State Foundation for Science, Technology & Innovation), and a Program Co-Chair for the SIM Annual Meeting. Professor Wu has also been involved in interactional collaborations. He served as a Visiting Professor to the International Biotechnology Center of Osaka University in Japan and an Adjunct Professor of National Cheng-Kung University in Taiwan. He is currently an Adjunct Professor of Jiangsu University in China. In addition, he has given lectures in many different countries.

### **Genome editing of methanotrophs for improved phenotypic properties: Greenhouse gas to value-added products**



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#### **Abstract**

Methane remains the second largest contributor to radiative forcing of climate change. Its global warming potential is 34-fold larger than that of carbon dioxide over a 100-year period. Globally, 60% of methane emissions are related to anthropogenic sources, most which are attributed to microbial methanogenesis. A large gap in scientific knowledge is associated with methane emission and oxidation from earth's deep biosphere. To fill this knowledge gap, this talk will discuss i) roles of unexplored methanotrophic and non-methanotrophic microorganisms present in the deep biosphere (300 to 5,000 ft. deep levels) of Sanford Underground Research Facility (SURF) at Homestake Gold Mine (SD, USA); ii) *in-silico* characterization of active sites of regulatory proteins responsible for methane oxidation to determine underlying molecular mechanisms; and iii) genome editing of methanotrophs for improved phenotypic properties and conversion of methane to value-added products e.g., methanol, electricity, and polyhydroxyalkanoates. Recent activities of BuG ReMeDEE consortium (Building Genome-to-Phenome Infrastructure for Regulating Methane in Deep and Extreme Environments) will also be presented.

**Keywords:** Methanotrophs; BuG ReMeDEE consortium; Regulatory proteins; Value added products

#### **About the Speaker**

Dr. Sani is a Professor in the Departments of Chemical and Biological Engineering and Applied Biological Sciences at South Dakota School of Mines and Technology, South Dakota, USA. His research expertise includes Extremophilic Bioprocessing, Biocatalysis, Biomaterials, Gas to Liquid Fuels, Genome Editing of Extremophiles, Metabolic Engineering, and Space Biology. Over the past 13 years, he has been the PI or co-PI on over \$18.39 million in funded research. He has one patent, seven invention disclosures, and published over 73 peer-reviewed articles in high impact factor journals and have contributed in over 18 book chapters. He has edited five books and one proceedings for Springer International Publishing AG.

## Plant-microbe interactions- from basic microbial ecology to function



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### Abstract

Interactions with its microbiome are crucial for plant development and health. Understanding of the genetic pathways involved in host-microbe interactions will allow us to interfere and promote desired processes. Recent advances in sequencing and data analysis have propelled the field of microbiology, providing valuable information not only on the community composition, but also on the dominant factors shaping it, and the functions of this community. Examples presented will include the use of these tools for determining community composition associated with different plant roots, describing community potential function and interfering with community structure, in order to solve ecological problems. Additionally, the use of microbiome structure and function as a biosensor for host stress will be demonstrated.

**Keywords:** Plant-microbe; Microbial communities; Plant stress; Functional microbiome

### About the Speaker

Dr. Dror has completed his Masters in M.Sc. in 1991 in Microbiology, *Summa Cum Laude* and PhD in 1995 in Microbiology, Department of Molecular Microbiology and Biotechnology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel. He had done Post Doctoral studies with David A. Stahl (Northwestern University, Evanston, IL), Gerard Muyzer (Max-Planck Institute for Marine Microbiology, Bremen, Germany) and Yehuda Cohen (Hebrew University, Jerusalem). His research interests are Microbial Ecology, Environmental Microbiology, Rhizosphere microbiology, Microbial communities structure and function in soil and water systems, Biofilm microbiology, Biocontrol of plant pathogens.

### Phylogenomic Characterization of Plant-associated *Cronobacter*

**Jang Hyein, Gangiredla Jayanthi, Patel R. Isha, Negrete Flavia, Finkelstein B. Samantha, Weinstein M. Leah, Wang Z. Caroline, Mammel K. Mark, Jean Junia Beaubrun Gilles, Addy Nicole, Gopinath R. Gopal, and Tall D. Ben\***



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Office of Applied Research and Safety Assessment, Laurel, MD 20708*

### Abstract

The genus *Cronobacter* consists of a diverse group of foodborne pathogens that cause both intestinal and systemic human disease in individuals of all age groups. Taxonomically, the genus consists of seven species including: *C. sakazakii*, *C. malonaticus*, *C. turicensis*, *C. muytjensii*, *C. universalis*, *C. dublinensis* (with three subspecies: *dublinensis*, *lausannensis*, and *andlactaridi*) and *C. condimenti*. Although *C. sakazakii*, *C. malonaticus*, and *C. turicensis* cause most infections, all species, except *C. condimenti*, have been found associated with clinical infections. Historically, infantile illnesses have been epidemiologically linked with consumption of temperature abused, intrinsically or extrinsically contaminated reconstituted powdered infant formula (PIF). However, source attribution of adult illnesses has been more elusive. Furthermore, reports from numerous surveillance studies show that *Cronobacter* species are found in a variety of other foods including dried foods (spices, herbs, flour, and cereals) and fresh ready-to-eat vegetables. This

increasing body of evidence suggests that the preferred niche, besides that of man-made environments, (e.g., PIF manufacturing facilities) for *Cronobacter* species may be eukaryotic plants; this is supported by the increased reports of isolating *Cronobacter* species from plant-origin foods. However, relatively little genomic information is available for these strains. The goal of this report is to describe the more salient genomic features possessed by plant-associated *Cronobacter* species such as plasmids, phage, and several predicted genomic islands that have come about through robust microevolutionary processes. For example, over 96% (771/801) of *Cronobacter* strains possess the virulence plasmids pESA3/pCSCS09a and pCTU1/pCMA1, which share a common backbone consisting of an IncF1B origin of replication gene (*repA*), and two iron acquisition gene clusters, *eitCBAD* and *iucABCD/iutA*; yet each species also possess plasmidborne specific traits. For example, *C. sakazakii* strains possess both *cpa* and a 17-kb type 6 secretion system (T6SS), while *C. turicensis* and *C. malonaticus* strains instead possess a 27-kb region encoding for a *Bordetella pertussis*-like filamentous hemagglutinin. Parallel whole genome sequencing and microarray analyses were useful in characterizing the total genome content of 538 *Cronobacter* strains showing that all species including *C. sakazakii* [found only in sequence type (ST) 64 strains] possess both a malonate and xylose utilization operon; these comprise genes to utilize two important plant-associated classes of metabolites. The nine-gene, ~ 7.7 kbp malonate utilization operon was located between two conserved flanking genes, *gyrB* and *katG*. In malonate-negative *C. sakazakii* strains the malonate operon is instead replaced by a 323–325 bp nucleotide region. In contrast, a metabolic island comprised of a variably-sized (~17,000 kbp) xylose utilization operon was found within a spice-associated group of *C. sakazakii* strains. Several sequence repeats (inverted repeats, palindromes or direct repeats) were found throughout the xylose operon suggesting that these are binding sites for regulatory proteins or that they may be evidence of past transpositions. FASTA data sets from nineteen *Cronobacter* strains, which were isolated from flies and *C. sakazakii* strain BAA-894 were uploaded to the PHASTER web server and pipeline to identify phage sequences. One hundred and fourteen incomplete, questionable, and intact phage-encoding regions were found; 50 of these gene clusters were identified as being intact and contained genes encoding for noted phage components of phages from *Salmonella*, *Aeromonas*, *Edwardsiella*, *Haemophilus*, and *Mannheimia*. The parallel genomic analyses reported here also showed that *Cronobacter* possess other species-specific attributes such as genes for fimbriae and ST-specific significant phylogenetic orthologues for efflux pumps and toxin-antitoxins. These attributes demonstrate the genomic diversity and phylogenetic relatedness among these strains. In summary, this report proffers examples of important traits associated with *Cronobacter* that describe their inter- and intra-species phylogenetic relatedness that may augment their physiological survival strategies and provides further insights into the phylogenomic complexity of this important foodborne pathogen.

**Keywords:** Phylogenomic; Virulence; Sequence Type; Diversity; *Cronobacter*

### About the Speaker

Dr. Ben D. Tall currently serves as Acting Branch Chief of the Virulence Mechanisms Branch, of the Division of Virulence Assessment in the Office of Applied Research and Safety Assessment. He oversees a group of nine researchers and support scientists engaged in a research program focused on the development of microbiological and molecular genetic approaches for detecting, identifying, and differentiating bacterial foodborne pathogens such as *Salmonella*, *Cronobacter*, *Bacillus cereus*, *Vibrio* and *Listeria* spp. He is a Senior Research Microbiologist and since 2003 have been with the Virulence Mechanisms Branch, Division of Virulence Assessment, Office of Analytical Research and Safety Assessment, Center for Food Safety and Applied Nutrition (CFSAN), U. S. Food and Drug Administration (FDA). He has completed his Ph. D. training at the Univ. of Maryland at Baltimore (UMAB) in 1988. His postdoctoral training took place at the Center for Vaccine Development (CVD), Univ. of Maryland Sch. of Med., UMAB during 1988-1989; and as a senior staff fellow during 1990-91 with the Div. of Microbiol. (DMS), Microbial Ecol. Br. (MEB), CFSAN, FDA. He has held several academic and research positions. Most recently as a Special Member of the Graduate Faculty of the Department of Plant Science and Landscape Architecture, University of Maryland College Park, College Park Maryland. (5.24.2017). He had served as President-elect (1992-1993), President (1993-1994) and



Past-President (1994-1995) of American Society for Microbiology (ASM), District of Columbia Branch, and (1995-1999) served as Membership Coordinator for that ASM Branch. Recent honors and awards include: a total of 32 awards including several FDA Group Recognition Awards, U.S. Health and Human Services Secretary's Award for Distinguished Service; CFSAN Individual Problem Solving/Creativity Award, CFSAN Excellence in Laboratory Science Scientific Achievement Award, CFSAN Exceptional Achievement Award, and a FDA Commissioner's Special Citation award. Lastly, he was most recently inducted into the ASM's American Academy of Microbiology. He has advised over 95 undergraduate, graduate and post-doctoral fellows. His significant contributions to food safety and public health have been through 140 manuscripts/book chapters, and 310 invited seminars and posters. He has studied various foodborne pathogens, most recently focusing on the emergence of *Cronobacter* spp. in outbreak investigations and in understanding its persistence in low moisture foods. He sits on the editorial boards of Applied and Environmental Microbiology, GSL Journal of Public Health and Epidemiology, and Microorganisms.

### The Enterococcus: A tale of survival and success of a second-rate pathogen



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#### Abstract

Enterococci are common inhabitants of the gastrointestinal tract of many species. They have also been given as probiotics to animals and man for decades. However, enterococci have also been a well known cause of infective endocarditis for over 100 years and, in the past 40 or so years, enterococci have become a common cause of healthcare-associated infections. This presentation will address the many roles of enterococci and will then focus on pathogenesis and antimicrobial resistance.

Virulence factors described in enterococci include the *Enterococcus faecalis* (*Efs*) and *E. faecium* (*Efm*) collagen (CN) adhesins, Ace (*Efs*) and Acm (*Efm*), each shown to be important in infective endocarditis and/or urinary tract models. We have shown that Ace immunization protects against *Efs* endocarditis and UTI and have delineated the molecular mechanism of Ace's binding to CN and to C1q. My group's other work on LPxTG "MCRAMMS" that will be discussed include the Ebp (**E**ndocarditis, **b**iofilm and **p**ilus) genes, *ebpABC* (which are *Efs* core genes, as are *ace/acm*), and Emp (*Efm***p**ili), also important for biofilm, UTI and endocarditis. Monoclonal Ab to EbpC (shaft pilin) inhibits biofilm and is protective against *Efs* endocarditis; we also have shown that Ebp regulation involves RnjB, Fsr, and a rare ATT start codon and that these pili cause *Efs* to co-aggregate with fusobacteria and they promote conjugation and antibiotic transfer between *Efs* strains.

In general, enterococci are intrinsically more resistant to beta-lactam antibiotics than streptococci (enterococci were considered part of the genus *Streptococcus* until the 1980s) but *Efs* is generally susceptible to penicillin and ampicillin. A rare cause of penicillin resistance in *Efs*, described in the early 1980s, was due to the acquisition of the staphylococcal penicillinase, although little enzyme is produced by these strains and, after some years, they diminished in frequency. *Efm* now accounts for ~35% of healthcare-associated enterococcal isolates in hospitals in the USA, and is one of those **multi-drug resistant (MDR)** bacteria from which we have "no ESKAPE". An interesting feature of *Efm*, unlike *Efs*, is that it contains two distinct clades that separated thousands of years ago: the healthcare-associated clade A (with most clinical *Efm* isolates) and the community-associated, clade B (comprised mostly of human commensals). A very important phenotypic difference is that community-derived human commensals (clade B) are readily inhibited by Amp (MICs 0.125-2 ug/ml), while healthcare

associated isolates are Amp-R (MICs often >64 ug/ml). PBP5 of *Efm* is the low-affinity penicillin-binding protein (PBP) that is essential for Amp R. In hospitals in the USA, >80% of clinical *Efm* isolates are vancomycin resistant (VRE) and almost all of these are highly ampicillin resistant (Amp-R), the traditional drugs of choice. Resistance to newer agents (linezolid, daptomycin) is well documented and increasingly reported and some *Efm* infections are untreatable medically. An interesting therapeutic or preventative strategy, shown to be effective in a murine model of peritonitis, is the use of bacteriophage, as has recently been applied to other serious infections caused by MDR bacteria.

**Keywords:** Enterococci; Multi-drug resistance; Virulence

### About the Speaker

Barbara E. Murray, M.D. is the J. Ralph Meadows Professor of Medicine and director of the ID division. She is a 1969 cum laude graduate in mathematics from Rice University in Houston, Texas and graduated from the University of Texas Southwestern Medical School in Dallas in 1973. She joined the faculty at McGovern Medical School at The University of Texas Health Science Center at Houston (UTHealth) as an assistant professor in 1980 and became Professor in 1990, Director of the Division of Infectious Diseases in 1995, and the J. Ralph Meadows Professor in 2003. Dr. Murray's laboratory's broad interests involve the genetic and biochemical mechanisms of resistance to antibiotics and bacterial pathogenicity, particularly relating to enterococci, and molecular epidemiologic typing. Recent acquisition of antibiotic resistance traits have led enterococci to be called antibiotic resistant "super bugs" because of the lack of commercially available effective antibiotics. Work in her laboratory has included the first description of enterococci producing beta-lactamase and of enterococci with high-level resistance to all aminoglycosides. Her research has resulted in approximately 300 papers in peer-reviewed journals, as well as many reviews and editorials. She also writes for UpToDate, Harrison's Textbook of Internal Medicine, and Mandell's Principles and Practice of Infectious Diseases.



### Fungal degradation of antimicrobial phytochemicals: the case of ellagitannins

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### Abstract

The system of protection of plant tissue against microbial attacks is formed not only by structures that function as a physical barrier, but of a complex system of phytochemistry compounds, among which tannins and alkaloids stand out. This work describes a research effort related to understanding the process of fungal degradation of the ellagitannin compounds, a complex family of polyphenolic phytomolecules. To achieve this, the extraction conditions of a model molecule were optimized. Subsequently, the ellagitannins were used as the only source of carbon and energy in a fungal crop. The compounds and enzymes involved in the biochemical pathway of degradation of the ellagitannins are described.

**Keywords:** Tannins; Fungi; Biodegradation; Enzymes

### About the Speaker

**Prof. Cristóbal Noé Aguilar** is vice-chancellor of Research and Postgraduate Programs at Autonomous University of Coahuila, México. He is Chemist (1992) by the same institution; his MSc Program in Food Science and Biotechnology (1995) was held in the Autonomous University of Chihuahua, México. His PhD Program in Fermentation Biotechnology (2000) was given by the Metropolitan Autonomous

University, Mexico. He was working in a Postdoc stay (2001) at IRD-Marseille, France. Dr. Aguilar has published more than 260 papers published in indexed journals, more than 50 articles in Mexican journals; 16 book chapters, 8 mexican books, 7 editions of international books, 34 proceedings and more than 350 contributions in scientific meetings (His *h index* = 35 by Scopus/Mendeley & 45 by GoogleScholar). Prof. Aguilar is member level III of the National System of Researchers of Mexico (S.N.I.) from 1998. He has awarded with several prizes including: Outstanding Researcher Award 2019 (International Bioprocessing Association, IBA-IFIBiop 2019); Prize of Science, Technology and Innovation Coahuila 2019; National Prize of Research 2010 of the Mexican Academy of Sciences, the Prize-2008 of the Mexican Society of Biotechnology and Bioengineering, National Prize AgroBio-2005 and the Mexican Price in Food Science and Technology. CONACYT-Coca Cola México 2003. From 2014 is member of the Mexican Academy of Science. Prof. Aguilar has developed more than 25 research projects, including 6 international exchange projects. He has been advisor of 25 PhD thesis, 35 MSc thesis and 50 BSc thesis. Prof. Aguilar is member of the International Bioprocessing Association, Mexican Academy of Sciences, Mexican Society for Biotech & Bioeng, Mexican Association for Food Science & Biotechnology. His research areas are the design of bioprocesses for bioproducts production (of importance in: Hort/Food/Pharma/Environ sectors) & fermentation & enzymatic technologies. He is associate editor of **Heliyon (Elsevier)**, **Frontiers in Sustainable Food System (Frontiers)** and member of several editorial boards of International Scientific Journals.



# INVITED TALKS



## An Insight to the Impact of Climate Change on Bioremediation



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### **Abstract**

Bioremediation is microorganism dependent process which helps in breaking down the pollutants and is crucial for reducing the soil pollution. Since the pollutant degrading abilities of microorganisms depend on various environmental factors, the climate change is bound to have an impact on bioremediation as it can alter the soil temperature, moisture, atmospheric carbon dioxide concentrations etc. For example, warming can increase the microbial degradation of the pollutants but only under optimum soil moisture content. Warming can also increase the toxicity of some pollutants and mobilization of volatile organic pollutants influencing the bioremediation and further increasing the environmental pollution. Prolonged warming can lead to reduction of labile carbon sources, microbial biomass and activity. Similarly, soil moisture can affect the contaminant binding and distribution. Higher precipitation induced increased moisture content can decrease the rate of pollutant degradation due to lower oxygen supply in soil as free pore space may be blocked by the water. To the contrary, low level of soil moisture may affect the solubility and bioavailability of the pollutant and reduce the microbial activity and pollutant degradation. Moreover, changes in soil pH can affect pollutant solubility as acidic conditions generally increase pollutant solubility and reduce their sorption on soil particles. Further, heterotrophic bacteria prefer to have neutral pH conditions and are affected by change in pH, however, fungi are less adversely affected by small pH change. Changes in pH can also affect the enzymatic reduction of metal ions in soil. Apart from this, the climate change induced alteration in plant growth and physiology will also have effect on plant-microbe mediated degradation of pollutants in the rhizosphere. However, understanding the impact of climate change on bioremediation is extremely complex and attention must be devoted to advancing the research in this domain for improving the efficiency of bioremediation under changing climate conditions.

**Keywords:** Bioremediation; Climate change; Bioavailability; Temperature; Soil moisture; Pollutant mobility

### **A Microbiologist role in the development of sustainable cities**



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### **Abstract**

Approximately 50% of the world's population now lives in the urban habitat and projections from the United Nations Department of Economic and Social Affairs indicate that urban population will increase to 68% by 2050. In India, urban population is estimated to grow to 40% by 2030. Making cities sustainable is therefore a priority. A sustainable city can be defined as that which works towards consideration of social, economic, and environmental balance. In short, we need to re-orient the biosphere towards

sustainable development goals. From preventing biodiversity loss, to climate change mitigation; from generation of green fuels and bio-products to urban green spaces; from solid waste management to ecosystem rejuvenation; from ecosystem services of soil, lakes and rivers to basic health, microbes are the major driving forces. Hence, a sustainable city needs to draw up a systematic green plan to harness the potential of microbes in meeting sustainable goals, where the microbiologist needs to be part of the policy making team. Microbes have been a part of industrial productivity and are an integral part of ecosystem rejuvenation and restoration. However, as stress levels on the ecosystem increase, the rate of rejuvenation needs to match the rate of deterioration. To be on par, there is an urgent need to understand the bottlenecks and develop improved systems and tracking tools.

**Keywords:** Sustainable city; Ecosystem; Microbiologist; Waste management

### A case study on restoration of oil spills site in Kuwait oil field



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#### Abstract

The petroleum industry effluents and oily sludge and oil spills cause a serious threat to the environment as their constituent compounds have toxic, mutagenic and carcinogenic properties. With the stringent regulatory norms and environmental obligations, the petroleum corporates have taken initiatives for proper management and treatment of these hazardous wastes. Different approaches have been tried since early times to overcome this problem. Some of them are known as, Land filling, Incineration, Air Sparging, Natural Remediation (like evaporation of VOCs, auto oxidation, and photo oxidation, etc.), Land farming, Surfactants, and Other conventional methods like chemical dissociation, dumping in injection wells, surface impoundment, waste piles, underground injection wells, etc. However the common drawbacks in all those conventional methods are that they are not the permanent solution for the environmental pollution and sometimes they are not cost effective. Therefore, environment friendly technologies are increasingly in demand today for oily sludge management. After extensive research an indigenous bacterial consortium was developed by assembles of selected bacteria species, isolated from oil contaminated sites of Kuwait Oil Company (KOC), Kuwait, which could degrade different fraction of total petroleum hydrocarbon. This bacterial consortium is designated as KT-Oilzapper. Biodegradation is the most ecofriendly and economically viable among all the available methods of sludge management. As a case study, a pilot demonstration project on bioremediation was carried out at an area of 70 m x 70 m of oil contaminated site, both at surface and subsurface level (upto 1.5 m depth), near Gathering Centre 2 (GC-2), Burgan, South Kuwait Oil Field of KOC, Kuwait, using KT-Oilzapper. The surface level of the site was divided in three blocks as Block A, B & C and the subsurface level of 1m x 1m area was considered as Block D. One control plot of 10 m x 10 m area was also selected for the demonstration project where KT-Oilzapper was not applied. At the experimental site the total petroleum hydrocarbon (TPH) in the oil contaminated soil was biodegraded by 98.04%, 98.42%, 98.00% and 99.29 % in Block A, B, C and D respectively in 60 days time. After bioremediation, the TPH content in all the experimental blocks were less than 1000 ppm, whereas, at the control plot the degradation of TPH was only 0.68% in the same time period. The soil after bioremediation was tested in laboratory in Kuwait and found to be equivalent to agricultural soil. Various vegetable and flower plants were grown on the oil contaminated soil after bioremediation.

**Keywords:** Bioremediation; Biodegradation; Oily sludge; KT-Oilzapper; Total Petroleum Hydrocarbons



## Genome and metagenome studies of the subsurface microbial world of the gas fields of the Great Artesian Basin (GAB) aquifer of Australia using cloud infrastructure



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### Abstract

The Great Artesian Basin (GAB) of Australia is one of the largest subsurface aquifers in the world occupying 1.7 million km<sup>2</sup> (22% of the Australian land mass). Distinctly different microbial communities have evolved and adapted to a variety of physiochemically heterogeneous ecosystems of the GAB which include temperatures (ambient to boiling), pH (neutral to alkaline), chemicals (iron, chlorides, carbonates, sulfates) and gasses (methane, carbon dioxide, carbon monoxide, hydrogen sulfide, hydrogen and oxygen). Over the past 30 years our lab has cultured a range of taxonomically new and standard microbes including extremophiles but their diversity, evolution and adaptation mechanisms and physiology has remained poorly understood. The introduction of high throughput Next Generation Sequencing (NGS) in 2010 has not only revolutionised the development of bioinformatics and computational infrastructure but results of analysed data has challenged many of our views on biological systems and life on earth. This paper will discuss the microbiology, and the microbial communities of GAB using genome and metagenome bioinformatics tools installed on cloud computing infrastructure. The first part of the paper will discuss the advantages of using cloud bioinformatics for microbiologists and the second part of the paper will discuss the use of this infrastructure for studies on the GAB microbial communities, new taxonomic cultured microbes, and the depth of taxonomic diversity of uncultured *Bacteria* identified in the metagenomic assemblages of different physiochemically distinct bore wells and their run off channels by NGS rRNA gene sequencing and sequence analysis Furthermore, the metagenomic communities present in metal-petroleum-methane-dominated gas fields and a gas industry waste-water settling pond in relation to the presence of overall gene families / metabolic pathways, and genomes of the most dominant microbes, their metabolic pathways and potential functions in this specific environments using NGS of fragmented environmental DNA (shotgun NGS sequencing) will be discussed.

**Keywords:** Metagenomics, Ecosystem, Microbial communities, Diversity

### Metagenomics in microbial ecology: Microbial community structure and its functional implications



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### Abstract

Microbial bioremediation has been well-demonstrated as an ecofriendly and cost-competitive strategy for elimination of xenobiotic compounds and or anthropogenic compounds from the environments. Implementation of efficacious bioremediation strategies relies heavily on innate microbial community dynamics, structure and function. Any one particular microorganism is incapable of processing all the metabolic reactions to degrade environmental pollutants, however a group of diverse organisms

form a community and collectively process all the metabolic reactions for bioremediation. Therefore, metagenomics based analyses of entire microbial community genomes becomes imperative to delineate the metabolic pathways responsible for biodegradation. The essential genes for bioremediation may be present, however to ascertain how many of them are involved in bioremediation we need high throughput metatranscriptomics and metaproteomics, transcriptome and proteome analyses of entire community respectively. Metametabolomics, analyses of the entire repertoire of microbial community metabolites and fluxomics, real time flux analysis of molecules/metabolites over a time period provide the missing links about regulation of metabolism of anthropogenic/xenobiotic compounds. Here, we discuss the potential of recent innovative breakthroughs in molecular and '-omics' technologies such as molecular profiling, ultrafast pyro-sequencing, microarrays, mass spectrometry and other novel techniques and applications along with bioinformatics tools to gain insights of indigenous microbial communities and their mechanism in bioremediation of environmental pollutants.

**Keywords:** Metagenomics; Bioremediation; Xenobiotics; Microbial communities

### **Translational repression of Plasmodium apicortin by human micro RNA impairs malaria parasite growth and invasion: a new host factor inspired strategy for designing of an anti-malarial molecular medicine**



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#### **Abstract:**

Mature human erythrocytes contain a rich pool of microRNAs as a result of differentiation process of the erythrocytes in course of hematopoiesis. Recent studies have elaborated the effect of the erythrocytic microRNAs on the invasion and growth of the human malaria parasite *Plasmodium falciparum* during the asexual blood stage of its life cycle. In this work, we have identified two erythrocytic microRNA, miR-150-3p and miR-197-5p, showing favorable *in silico* hybridization with Plasmodium Apicortin possessing putative microtubule stabilizing properties. Co-expression of PfApicortin and the two microRNAs mentioned earlier in cell line model resulted in down-regulation of Apicortin in both RNA and protein level. In order to create a disease model of erythrocytes containing microRNAs, chemically synthesized mimics of miR20 150-3p and miR-197-5p were loaded in erythrocytes and subsequently used for invasion by the parasite. Growth of the parasite was hindered followed by impaired invasion in miRNA loaded erythrocytes along with reduction in microneme secretion, particularly in case of miR-197-5p. PfApicortin expression was found to be reduced in miRNA loaded erythrocytes. Interpretation of the effect of down-regulation of Pfapicortin was made by checking the expression of another cytoskeletal parasite protein MTIP (MyoA tail interaction protein) which was found to co localize with apicortin. Reduction in expression of MTIP in miRNA mimic exposed parasite confirmed the role of apicortin in cytoskeletal assembly stabilization and invasion. Overall, this study identifies a novel target apicortin in parasite along with its miRNA inhibitor miR-197-5p signifying it as unconventional nucleotide-based therapeutics and adds a new host factor inspired strategy for designing of an anti-malarial molecular medicine.

**Keywords:** Malari; *Plasmodium falciparum*; Micro-RNA; Apicortin



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### Abstract

Bacteria contain a peptidoglycan (PG) cell wall which plays pivotal role in maintaining cell shape and combating osmotic stress. Maintaining the integrity of the cell wall is essential for cell viability and its importance is evident by the number of antibiotics that target the PG metabolic pathway, for example, the  $\beta$ -lactam antibiotics (e.g. penicillins and cephalosporins) inhibit the so-called penicillin binding proteins (PBPs) involved in PG synthesis and remodeling. All bacteria gram-positive and gram-negative harbor enzymes which cleave bonds in the cell wall, known as PG hydrolases. The activities of these enzymes are essential for both growth and division of bacterial cells. The hydrolases have to be tightly regulated in the cell as overproduction of these enzymes leads to cell lysis. In recent years, *Caulobacter crescentus* has emerged as a powerful model to address the questions pertinent to spatio-temporal regulation of enzymes. *C. crescentus* is a crescent shaped bacterium that undergoes asymmetric cell division at each reproductive cycle, giving rise to two genetically identical but morphologically different daughter cells: a sessile stalk cell and a motile swarmer cell. Highly dimorphic feature provides many markers which can differentiate between the two cell poles. Recent work has shown three different set of divisome components: LytM like endopeptidases, soluble lytic transglycosylases (SdpA) and amidase, are recruited at midcell at distinct stages in *C. crescentus*. Amidases cleave the peptide moiety from the *N*-acetylmuramic acid moiety in peptidoglycan and lytic transglycosylases cleave sugar backbone of peptidoglycan. Our research explores the cross talk between these PG hydrolases during cytokinesis in *C. crescentus*. Many questions regarding the spatial and temporal regulation PG hydrolases in the cell remain unanswered and insights into the regulation of these important enzymes will close a fundamental gap in our understanding of bacterial cell morphogenesis.

**Keywords:** Peptidoglycan; Amidase; Cell division; *Caulobacter crescentus*

### Acidic phytase of the yeast *Pichia anomala*: Production and Applications



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### Abstract

Among several yeasts isolated from dried flowers of *Woodfordia fruticosa*, *Pichia anomala* produced a high titre of cell-bound phytase. The optimization of fermentation variables led to formulation of media and selection of cultural variables that supported enhanced phytase production. The enzyme productivity was very high in fed batch fermentation in air-lift fermentor as compared to that in stirred tank fermentor. Amelioration in the cell-bound phytase activity was observed when yeast cells were permeabilized with Triton-X-100. The enzyme is thermostable and acid stable with broad substrate specificity, the characteristics that are desirable for enzymes to be used in the animal feed industry. The phytase encoding gene was cloned and expressed in *Pichia pastoris*. The 3D structure of the enzyme was proposed by comparative modelling using phytase of *Debaryomyces*

*occidentalis* (50% sequence identity) as template. When broiler chicks, and fresh water and marine fishes were fed with the feed supplemented with yeast biomass containing phytase, improvement in growth and phosphorus retention, and decrease in the excretion of phosphorus in the faeces were recorded. The cell-bound phytase of *P. anomala* could effectively dephytinize wheat flour and soymilk, thus useful in reducing phytate content of bread and Indian flat breads like tandoori and nan.

**Keywords:** Yeasts; Phytase; Bioprocess; Phosphorus

### Genotypic diversity of arid rhizobia retrieved from South West Haryana and Rajasthan state



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#### Abstract

A total of 639 rhizobial isolates belonging to mungbean, mothbean, clusterbean, pigeonpea, pea, chickpea and Cowpea were obtained from semi-arid, arid and hyper arid zones of South-West Haryana and Rajasthan state. Most of these isolates were able to grow up to 40°C, however few isolates showed growth at 45°C. These isolates were also able to grow at 30 or 40% PEG concentration, thus indicating that these isolates are temperature and drought tolerant. Most of the rhizobial isolates were found to have different plant growth promoting traits like IAA production, P-solubilization, bacteriocin & siderophore production and ACC deaminase activity. The authenticity of these isolates was checked by amplifying the *nodC* gene *nifH* gene. ARDRA analysis of these rhizobial isolates using *MspI* and *HaeIII* showed wide diversity among themselves. Two mungbean (MuJs52b and MuJs72a), three of clusterbean (CIBk 43b, CIJs74b and CIJs87a) and two mothbean (MoCh17b and MoBr99b) rhizobial isolates retrieved from hyper-arid zones of Rajasthan performed better at high temperature (45°C soil temperature) and high drought (25% FC) conditions in terms of nodulation efficiency and plant growth parameters as compared to their respective reference strains and un-inoculated control both in Leonard jars and pots. Three rhizobial isolates viz. MR63, MB17a and MR54 were selected as promising multi-trait mungbean rhizobia, which showed 7.1, 6.7 and 4.8% increase in seed yield as compared to RDF under rain-fed field conditions. These isolates also resulted in better nodule no. and nodule fresh wt. as compared to reference strain. Six rhizobial isolates (GB14c, GB32a, GB32c, GH1a, GH2b and GM16b) showed significant increase in seed yield, which varied from 13.53-14.06 q ha<sup>-1</sup> as compared to RDF (12.00 q ha<sup>-1</sup>) & commercial strain, GSS (12.92 q ha<sup>-1</sup>), with clusterbean variety HG 220 at recommended dose of fertilizer (RDF) under field conditions. Most of these isolates also showed significant increase in nodulation efficiency as compared to control and commercial strain. Four pigeonpea rhizobial isolates (PPM37D, PPM33B, PPB25A and PPH10B) were found to be most efficient isolates, which resulted in 7.1, 5.9, 5.4 and 4.7% increase in seed yield, respectively as compared to RDF under rain-fed condition at CCS HAU, farm. Four chickpea rhizobial isolates (CK15, CH25, CS12 and CK 16) isolated from South-West Haryana performed better than control (RDF) and the commercial chickpea strain, 1233 in terms of nodule number per plant, nodule weight per plant and seed yield under field conditions. These isolates resulted in 7.99 to 11.73 % increase in chickpea seed yield, while the commercial strain, 1233 showed 6.78 % increase in seed yield as compared to RDF.

**Keywords:** Rhizobia; Chickpea; Diversity

## *Chlamydomonas reinhardtii*: a unicellular biflagellated chlorophyte as a model microorganism for studying stress and motility



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### Abstract

The unicellular green alga, *Chlamydomonas reinhardtii* is a soil-dwelling microorganism that enjoys a strategic position in the phylogenetic tree of life. For its ease in growing as a mixotroph, and well-developed genetic toolkit, it is easily tractable experimentally and has been the most sought model system for studying the processes of photosynthesis, cell cycle, flagellar assembly-disassembly, motility, phototaxis, mating, cell wall biogenesis and more recently for the production of biofuel and biopharmaceuticals. The past two decades have seen an interest in developing this microorganism towards addressing the question of stress-induced death and survival. Using osmotic and oxidative stress conditions on logarithmically grown vegetative cells of *C. reinhardtii*, we have shown that both a mitochondrion-mediated, caspase-dependent and -independent apoptotic mechanism exists in this species (Sirisha *et al*, 2014; 2015; 2016). In two interestingly exceptional situations, this alga exhibits a spectra of responses. It undergoes necrosis at concentrations of >200 mM NaCl; while, at those of 100 and 150 mM, it form palmelloids, a strategy for survival (Khona *et al*, 2016). When exposed to KCl, a mixture of two physiological states have been observed, almost 60% of the cells undergo a mitochondrion-mediated, caspase-independent apoptotic death, and (2) rest of cells form clusters that are akin to 'multicellular' forms. Taken together, it appears that (1) a simpler form of the apoptotic machinery has evolved with this unicellular eukaryote that provides an easier molecular platform to dissect the mechanism, (2) the triggers that might have led to the formation of multicellular organisms during the course of evolution can be explored using this alga, and (3) the molecular underpinnings in the tug-of-war between survival and death *vis-a-vis* stress can also be studied. Earlier investigations indicated that flagella are the first organelles to experience the wrath of stress; these are immediately paralyzed and lost within minutes of exposure to stress (Gudipati *et al*, 2005; Dharmadhikari *et al*, 2006). In parallel, we showed that a flagellar associated protein 174 (FAP174) was upregulated 90-fold (maximum in this study) over the control in the spent medium from cells de-stressed of NaCl. In order to explore the role of flagella in the battle of survival and death, and delve into the molecular details, we took this investigation further. FAP174 gene was cloned, and protein overexpressed protein in *E. coli*, purified the 6XHis-tagged version, and used specific antibodies to immunoprecipitate a ~2 MDa multiprotein complex that is localized to the central pair apparatus of the flagella. The FAP174 protein is helical in nature (Yogesha *et al*, 2018) and binds strongly to an A-Kinase Anchoring Protein (AKAP240), also present in the flagella (Rao *et al*, 2017). The *fap174* mutant cells are immotile and exhibit short flagella. Coincidentally, cells treated with NaCl also exhibit short flagella (Khona *et al*, 2016). FAP174 appears to play a role in motility, stress and deflagellation.

**Keywords:** *Chlamydomonas reinhardtii*; Stress; Motility; Model organisms

## Anti-HCV roles of mTORC1



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### Abstract

Mammalian target of Rapamycin (mTOR) is a well known regulator of several cellular activities including protein translation, autophagy and lipid metabolism. Here, we demonstrate the antiviral effects of mTOR due to its ability to restrict HCV replication. Pharmacological inhibition of mTOR caused activation of HCV replication. mTOR assembles into two entirely distinct complexes known as mTORC1 and mTORC2. Specific knockdown of unique components of these complexes revealed that mTOR restricts HCV replication through mTORC1 and through one of its substrates, ULK1. We further demonstrate that ULK1 restricts levels of a key host factor for HCV, miR122, as ULK1 knockdown caused elevated levels of the molecule. Interestingly, high titers of HCV during chronic HCV infection coincided with severe suppression of mTORC1, indicating the anti-HCV roles of mTORC1. mTORC1 inhibition also resulted in higher IFN expression and STAT phosphorylation, demonstrating a novel mechanism of regulation of antiviral activities by mTORC1.

**Keywords:** HCV; mTOR; Anti-viral

## Molecular Tactics of HIV to hit the Blood Brain Barrier



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### Abstract

HIV-1 infection leads to the development of HIV Associated Neurological Disorders among HIV infected patients. HIV-1 Tat protein has been reported to exert an adverse effect on Blood-brain barrier integrity and permeability. Blood Brain Barrier is a barrier separating peripheral blood circulation and the central nervous system. Brain microvascular endothelial cells are an integral part of the Blood-brain barrier, which regulate the flow of substances into and out of the brain. Blood-brain barrier injury is a concerted effect of direct insult and or /bystander effects to the human brain microvascular endothelial cells in the cases of neurotropic viruses utilizing the Blood-brain barrier route to enter into brain. Cell culture, Recombinant Protein Expression, Western Blotting, Overexpression and knockdown strategies, Co-Immunoprecipitation and Luciferase Assay have been used to study the bystander effects of HIV-Tat protein on human brain microvascular endothelial cells. HIV 1-Tat C affects the barrier permeability by microRNA via post-transcriptional regulation of VE-cadherin as well as via the Redox-sensitive kinase mediated tyrosine phosphorylation of VE-cadherin and  $\beta$ -catenin. Impairment of human brain microvascular endothelial cell's functions contributes to changes in vascular permeability. We demonstrated the molecular mechanism of HIV-1 Tat C protein; affecting the junctional integrity human brain microvascular endothelial cells. The study suggests that the microRNA mediated posttranscriptional regulation of adherens junction and tight junction proteins and redox sensitive kinase mediated post-translational modifications of adherens junction and associated catenins lead to the disruption of barrier integrity in human brain microvascular endothelial cells.

**Keywords:** HIV; NeuroAIDS; HIV Neuropathogenesis; Molecular Virology; Neuroinflammation

## Enhancement of uptake and translocation of micronutrients in Wheat by using endophytes



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### Abstract

Plants exhibit differential potential for uptake of nutrients which depends on the genetic makeup of the plant and on the availability of nutrients in the soil. A study was carried out with an aim to isolate and identify efficient endophytes for wheat to further enhance the genetic potential of low accumulating wheat genotypes for uptake and translocation of iron and zinc in plant parts and grains. With the view to identify low and high iron and zinc accumulating genotypes of wheat, 13 genotypes were screened for uptake of these micronutrients in soils sufficient and deficient for iron and zinc. Three genotypes (4HPYT-404, GW-07-112, HD-3086) accumulating  $<20 \text{ mg kg}^{-1}$  zinc and 12 genotypes except GW-07-112, accumulating  $<45 \text{ mg kg}^{-1}$  of iron in grains were designated as low accumulator of zinc and iron respectively. A total of 213 endophytes obtained from different genotypes were screened for zinc solubilization and siderophore production. For the plant microbe interaction studies carried out in deficient soils and using genotypes identified as low accumulator for zinc or iron, 8 endophytes (6 bacteria and 2 fungi) each efficient for zinc solubilisation or siderophore production were used as inoculants. Due to inoculation of endophytes, the percent increase in zinc uptake ranged from 15.2-130.4% and iron uptake ranged from 15.16-156.85% respectively. Among the endophytes, isolate DS178 and DS-179 were more potent for zinc acquisition whereas endophytes DS-68 and DS-163 were efficient for iron acquisition in grains. On the basis of 16S rRNA gene sequencing these four endophytes were identified as *Bacillus subtilis* DS-178 and *Arthrobacter* sp. DS-179; *Arthrobacter sulfonivorans* DS-68 and *Enterococcus hirae* DS-163. These four strains were used to inoculate low and high Fe and Zn accumulating genotypes respectively in soils sufficient and deficient for Fe-Zn. The data on different root morphological parameters, yield and accumulation of Fe and Zn indicate distinct variations among genotypes; soil type and also among endophyte inoculated, un-inoculated and chemical fertilized treatments. In general, the amount of Fe and Zn in grains due to inoculation of endophytes was 2 folds higher as compared to un-inoculated control. The low and high Fe or Zn accumulating genotypes responded in almost identical manner to endophyte inoculation irrespective of the soil type. The mechanism/s of enhanced uptake of Fe or Zn in wheat genotype due to inoculation of siderophore producing and zinc solubilizing endophytes was also studied. Three different parameters, that is root anatomical features, qualitative and quantitative production of organic acids and sugars in root exudates and expression of *TaZIP* genes were analysed. TEM studies revealed that the endodermis, cortical region, root hair extension, xylem and xylem vessels, pericycle and vascular bundles were more pronounced and thicker in inoculated treatments as compared to control. The organic acid profile showed five types of organic acids in root exudates with citric acid being the predominant acid produced. The amount of total organic acids was 5 fold and 8 fold higher due to inoculation of *Arthrobacter sulfonivorans* and *Arthrobacter* sp respectively as compared to control. The concentration of citric acid, succinic acid and acetic acid increased many fold in root exudates of inoculated treatments. Four *TaZIP* genes were targeted for expression studies using gene specific primers and expression was achieved only for *TaZIP3* and *TaZIP7* genes in wheat genotype 4HPYT-414. The results clearly indicated endophyte mediated over expression of these two genes in roots and shoots. These endophytes were also evaluated under field conditions for biofortification of wheat grains with Fe and Zn. The grains were also analysed for phytic acid content, protein, carbohydrate and phosphorus concentration. Endophytes inoculation significantly increased the fresh weight and dry weight of root and shoot, yield and yield component (number of grains/m<sup>2</sup>, number of grains/

spike and seed weight of 1000 grains. Both low and high Zn and Fe accumulating wheat genotypes had the significant results for Fe or Zn accumulation in wheat grains under field conditions with respect to endophytes inoculations.

**Keywords:** Wheat; Endophytes; Translocation

### Phase variation in a bacterial pathogen of rice

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#### Abstract

Phase variation in bacteria is characterized by reversible switching between alternative phenotypic and genotypic states. This phenomenon has been well characterized in animal pathogenic bacteria where it is reported to help in evasion of the host immune system. We have recently discovered that this phenomenon occurs in the rice pathogen, *Xanthomonas oryzae* pv. *oryzae* (Xoo) which causes bacterial blight, a serious disease of rice. It appears that, during its life cycle, Xoo undergoes periods of boom and bust in terms of the availability of nutrients. We had previously reported that Extracellular polysaccharide (EPS) deficient mutants of Xoo, called Spv mutants, accumulate in stationary phase cultures of the bacterium and that some of them carry mutations in the EPS biosynthetic cluster. We have recently found that a number of Spv mutants carry mutations in lipopolysaccharide (LPS) biosynthetic genes. These mutations also cause EPS and virulence deficiencies. The mutations are caused by either insertion of endogenous Insertion Sequence elements or through base insertion at a particular hexamer tract of G residues. These mutations revert spontaneously to variants that are wild type in terms of phenotype and genotype. The possible significance of this phase variation in the life cycle of Xoo in terms of adaptation to nutrient deprivation will be discussed.

**Keywords:** Phase variation; Extracellular polysaccharides; Virulence; Mutations

### Prevalence of bacteria with multitasking capabilities in diverse soil samples

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#### Abstract

Soil is a rich source of bacteria which can be used for applications in agriculture, soil remediation and biofuel production. Diverse soil samples from different locations in Punjab and Mizoram were screened to identify the proportion of phosphate solubilizing, indole acetic acid producing, heavy metal resistant and antibiotic resistant bacteria out of total culturable bacteria. Commonalities and differences in their prevalence with respect to the above attributes were observed. Ribosomal DNA sequencing analysis of bacterial isolates which harbor all the attributes of plant growth promotion,



heavy metal remediation and biological control revealed the identity of these bacteria. The biological significance and the potential applications of these bacteria which are present in relatively large numbers are discussed.

**Keywords:** Soil microbiology; PGPR; DNA sequencing; Soil remediation

### Endophytic bacteria as potential agents against Phytoviruses

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#### Abstract

Viral diseases and their damage cause the significant loss to economically important crops have increased several folds during last decade especially in tropical and subtropical countries. More importantly these viral diseases are very critical in cash crops which directly affect the farmers of the affected regions. Use of wrong chemical pesticides further aggravates the issue as farmers do not have proper idea about the viral infections. Till now the known methods against viral protection are limited to limited to breeding resistant varieties, obtaining virus-free micro-plants, plant genetic modification with viral genes, using the RNA interference phenomenon or editing the plant genome with CRISPR/CasN cassettes. As an alternative and eco-friendly method, we consider the possibility of using endophytic microorganisms that produce a wide range of protective enzymes, including extracellular RNases, antiviral effects of which is known.

Based on this, we investigated the RNase activity of endophytic bacteria isolated from potato, as well as deposited in the collection of the laboratory of plant immunity biochemistry of the Institute of Biochemistry and Genetics of Ufa Federal Scientific Centre RAS (<http://ibg.anrb.ru/naychnaya-deyatelnost/bioesyrs/kollekciya-simbioticheskix-mikroorganizmov>). The enzyme activities were detected visually in the agar plates on Petri dishes by the formation of the halo zone around the bacterial colony, introducing in the Luria Bertani nutrient agar with yeast RNA (Sigma, USA). In other experiments, the activity of RNases in the cultural media was determined spectrophotometrically. Among the 130 isolates and strains of bacteria, 27% are identified as *Bacillus* genus, 6% as *Pseudomonas*, and 48% as *Enterobacter* spp.. The remaining 19% of isolates have not yet been identified. When *Bacillus* cultivated in a liquid media they secreted RNases, ranged with activity from 2 to 6 units/mg protein. The greatest activity in the cultural media was produced by *B. thuringiensis* strain (6 units/mg protein) and *B. subtilis* 26D (4 units/mg protein). It has been established that the used bacteria strains effectively colonize the intercellular tissues of potato plants and forming compatible relationships with plant cells, can influence the spread of viruses in the potato crops.

The conference materials will present the results of studies on the effect of plant treatment with endophytes on the reproduction of viruses in plant tissues. It has been suggested that *B. subtilis* strains with high RNase activity could be used as the base of preparation for potato protection against viral infection.

The work was carried out by a joint international grant from the Russian Science Foundation and the Department of Science and Technology (DST) of the Government of India No. 19-46-02004.

**Keywords:** Veridical; Endophytic bacteria; Plant viruses; RNase

## Cyanobacteria as eco-strategists and *green saviours* in the climate change scenario



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### Abstract

Cyanobacteria or blue green algae (BGA) represent one of the earliest life forms on the earth which have converted the earth's reducing atmosphere into an oxidizing one, thereby, facilitating the explosion of biodiversity. They are model systems for evolutionary biologists not only as the first colonisers, but also because they have undergone a series of genomic, functional and structural modifications with unique and highly-adaptable traits. They also possess tremendous potential for producing a wide range of secondary metabolites, including cyclic peptides, hepatotoxins and alkaloid neurotoxins exhibiting algicidal, antifungal, cytotoxic and enzyme inhibitory activities. Cyanobacteria probably evolved as a group of organisms about 2,000 million years and their contributions to the biogeochemical cycles of carbon, nitrogen and oxygen and primary productivity, mostly in aquatic environments are well known. With an impressive ability to inhabit infertile substrates such as volcanic ash, desert sand and rocks, besides colonising diverse aquatic, terrestrial and aerial habitats, cyanobacteria are ubiquitous and omnipresent. The agronomic benefits of cyanobacterial biofertilizers for rice as nitrogen supplements are well established; however, their role as environmentally friendly options, especially as plant growth promoting / biocontrol agents is less explored in other crops. Recent research has illustrated their cross talk with other PGPR, soil microbial communities and plant species. Advances in developing novel cyanobacteria-based formulations, including biofilm preparations, with agriculturally beneficial bacteria and fungi, which simulate natural microbial communities, represent a more viable inoculation option. Macro and micronutrient sequestration, UV-tolerance, ability to under high/low light or oxygen environments and adaptations to stress - akinetes, photo-pigments and DNA repair, enable cyanobacteria to proliferate in a widespread manner. Such eco-physiological traits may not only be useful for harnessing their potential in nutrient and pest management strategies, but also as resilient inoculant options in the changing climate scenario.

**Keywords:** Cyanobacteria; Climate change; PGPR; Formulations

## Bioethanol production from sugarcane bagasse using developed thermotolerant yeast for enhanced xylose utilization



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### Abstract

Second generation bioethanol is a clean and renewable energy source, which is produced by fermenting the sugars of the lignocelluloses using a range of microbial diversity. Both pentose and hexose sugars must be utilized for economical production of lignocellulosic ethanol. *Kluyveromyces* sp. has a great potential for ethanol fermentation by utilizing broad range of sugars. But ethanol fermentation ability is still low

on xylose as compared to *Pichia stipitis* and *Candida shehatae*. Satisfactory uptake of pentose sugar (xylose) in cells is the priority for enhanced ethanol production to avoid fuel insecurity in future. Therefore, the present study was carried out to study the potential for bioethanol production from sugarcane bagasse (SCB) using developed thermotolerant yeast *K. marxianus* NIRE-K3.2 for enhanced xylose utilization in comparison of native strain *K. marxianus* NIRE-K3. SCB was pretreated with 15 % liquid ammonia using 10% solid loading at 85 °C for 24 h. The neutralized bagasse was hydrolyzed using commercially available enzyme Cellic Ctec 2 with concentration of 15 FPU/g-db pretreated bagasse with 10 % solid loading at 50°C and pH 5.5. The maximum glucose, and xylose concentrations in hydrolysate were found to be 35.45 g/l, and 14.92 g/l, respectively, which was further fermented at 45 °C, and pH 5.5 using native *K. marxianus* NIRE-K3, and adapted *K. marxianus* NIRE-K3.2 individually. After fermentation, native strain *K. marxianus* NIRE-K3 showed 43.23 % xylose utilization with 12.98 g/l, 0.31 g/g, 0.81 g/l/h and 2.34 g/l of ethanol concentration, yield, productivity, and xylitol concentration, respectively, whereas adapted strain *K. marxianus* NIRE-K3.2 showed 75.06 % xylose utilization with 21.92 g/l, 0.47 g/g, and 1.37 g/l/h of ethanol concentration, yield, and productivity, respectively. The yield obtained by adapted strain *K. marxianus* NIRE-K3.2 was equivalent to 92.15 % of the maximum theoretical yield, whereas it was 60.78 % in case of native strain *K. marxianus* NIRE-K3. The adapted strain *K. marxianus* NIRE-K3.2 showed 34.04 % improvement in ethanol yield by utilizing xylose in addition to glucose as compared to that of native strain. It is concluded from the study that the novel adapted strain *K. marxianus* NIRE-K3.2 has potential for bioethanol production utilizing pentoses in addition to hexoses of lignocelluloses such as sugarcane bagasse as feedstock.

**Keywords:** Lignocellulosic biomass; Sugarcane bagasse; Bioethanol; Hexoses; Pentoses

### Biorefinery approach for management of Agri and Horti residues

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#### Abstract

Biorefinery is a holistic approach for conversion of biomass to energy, chemicals and other value added products. Agricultural residues are rich in cellulose, hemicellulose, lignin and even ash in certain feedstocks, while the horticultural residues by and large are composed of cellulose, hemicellulose, pectin and lignin. Bioethanol and Biobutanol are the most sought after products from the lignocellulosic biomass (LCB) that are produced through biochemical and thermochemical routes. Biochemical conversion employs enzymes and microbial cells, in addition to heat and chemicals, to convert biomass into an intermediate sugar mix stream for its subsequent conversion to ethanol or butanol. Thermochemical conversion technologies depend on heat and/or physical catalysts for the conversion of biomass into a gaseous intermediate comprising of H<sub>2</sub> and CO and a subsequent chemical or biological conversion of such an intermediate to biofuels. During the bioconversion process, only the cellulosic and hemicellulosic fractions are converted to intermediate compounds and finally to biofuels, while lignin having industrial applications is not being put to any significant commercial use. It is therefore important to devise strategies to separate the lignocellulosic biomass into the cellulosic, hemicellulosic and lignin fractions and develop value-added products, such as glucose or nanocellulose from the cellulosic fraction, xylitol and arabitol from the hemicellulosic fraction and nanolignin from the lignin fraction. There also exists a possibility to produce nanosilicates from rice straw. Pretreatment of the LCB is a highly energy and cost intensive process while hydrolysis, fermentation and downstream processes for production of biofuels through the biochemical route are highly cost intensive. Thermo-mechanical

extrusion process could be thought about as an energy saving pretreatment process in combination with mild chemicals and enzymes. Use of ionic liquids and/or organic solvents for pretreatment, high substrate loading, employing thermophilic cellulase consortium and thermotolerant fermenting microorganisms could help in reducing the energy and cost involved in the upstream process during the biochemical conversion of LCB. Horticultural residues, such as mango peels, orange peels and apple pomace, can be utilized for extraction of pectin and the residues could be used for production of variety of value-added products, such as sugars, dietary fibre and organic acids, thereby ensuring their complete utilization. Similarly, grape prunings, jackfruit rind, pineapple rind and banana peels could be effectively utilized for production of bioethanol. Banana pseudostem and okra stem are rich in fibre and hence can serve as a good source for biobased materials with an opportunity of using residues after fibre extraction for production of biofuels. Huge possibility also exists in the production of biohydrogen from both agri and horti residues. Coconut coir because of its good water holding capacity is being extensively used for production of fermented cocopeat. Biorefinery approach is the need of an hour and better suited to ensure sustainability of the biobased economy.

**Key words:** Biorefinery; Lignocellulose; Agro-residues

### Evaluating the biotechnological potential of an Indian marine cyanobacterium with high polyglucan content through genome-scale metabolic modeling



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#### Abstract

Cyanobacteria are promising microbes to capture and convert atmospheric CO<sub>2</sub> and light into biomass and valuable industrial bio-products like platform chemicals, biofuels, biodegradable plastics etc. Marine cyanobacterial species are attractive because they do not require freshwater which is scarce in India. In this work, we show that an Indian marine cyanobacterium has biomass accumulation comparable to a well-studied *Synechococcus* strain and accumulates much higher levels of polyglucans. These characteristics make it an attractive organism for feedstock applications and as a platform for production of various bio products through metabolic engineering. We present a genome scale metabolic model (GSMM) of this organism, which we have named as iSyn706. The model includes 706 genes, 908 reactions and 901 metabolites. The model was utilized to understand the internal metabolism of the organism under different trophic conditions as well as to identify the essential reactions. Finally, we analyzed the GSMM to determine the potential of the strain for the production of various industrially useful products. This cyanobacterium and its model will be helpful to researchers interested in developing this organism as a feedstock as well as to design metabolic engineering strategies for the production of industrially relevant compounds.

**Keywords:** Marine cyanobacterium; Biomass; Polyglucans; Systems biology; Metabolic engineering; Genome scale model



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### Abstract

In search of alternate energy sources, lignocellulosic biomass including forestry and agricultural residues available in abundant at low price/no price seems to be a promising raw material for its transformation into the desirable bio-products in the so-called Biorefineries. It is estimated that by 2031-32, the power generation capacity would mark up an increase from 183 GW to 800 GW. On the contrary to the demand, the native energy reserves of India are inadequate, therefore dependent on imports of crude oil (>80 %). Owing to the oil geo-politics and environmental concern, it has become necessary to innovate alternate 2G renewable energy options. In this regard, being an agrarian tropical nation, crops generate considerable amounts of leftover residues that exhibit not only resource loss but also a missed opportunity to improve farmer's income. Currently, about 3.7 billion tonnes of LCB is being supplied globally by grasslands, and 1.3 billion tonnes from agricultural residues (Pitrowski *et al.*, 2015). India produces 686 MT of gross crop residues both on the farm and off-farm annually, of which 234 MT (34% of gross) is estimated as surplus that can be tapped for bio-energy generation and other platform chemicals (Devi *et al.*, 2017).

Though LCB is a potent energy reserve, the lignocellulosic feedstock bio-refinery is still in infancy stage due to: i) the difficulty in raw material availability ii) feasibility in product supply chain iii) scalability of the model hampering its development at commercial scale. LCB is a renewable organic material and is the major structural component of all plant cell wall, which consists of three major components: cellulose (40-50%), hemicellulose (20-40 %) and lignin (20-30 %). The cellulose fibrils are entangled with hemicellulose and lignin, forming a complex matrix rendering plant biomass largely inaccessible to cellulolytic enzymes in the native state. Such complex material needs to be broken down by various pre-treatment processes including bio-delignification and depolymerization strategies to make the cellulosic and hemicellulosic fraction of LCB, available for enzymatic hydrolysis. Hyper active and highly stable laccases at diverse conditions (Uthandi *et al.* 2010, Kandhasamy *et al.*, 2016) are of importance in bioprocessing and selective removal of lignin and their derived aromatics which leads to yield different platform chemicals.

Enzymatic saccharification and pre-treatment require robust enzymes that are more stable at high temperatures and a wide range of pH with enhanced catalytic efficiency. Bioprospecting microbes from a thermophilic niche, hot springs of Himachal Pradesh that harbors a rich diversity of GHs producers with the multi-substrate utilizing ability and survive in temperatures above 50°C would a step forward in biocatalyst development. Thermophiles having unique macromolecular properties can possess high metabolism growth at high temperatures and, physically and chemically stable enzymes and higher-end product yields than mesophilic species. Thus, the rate of cellulolysis is presumably more rapid at elevated temperatures. Additionally, a robust microbial system that can secrete enzymes to hydrolyze both the C5 and C6 sugars is of great challenge, and such microbes are yet to be explored.

Considering the energy platform for LCB residues is already well established in co-generation of heat and power (CHP), mining various platform chemicals benefits modern LCB based biorefineries. Fractionation of LCB (chemical and biological process) such as hydrogenation and oxidation of glucose into sorbitol and gluconic acid respectively, acid dehydration of xylose to furfural and other emerging products such as levulinic acid, furfurals, adipic acid, and other lignin-based products are more avenues to revitalize biorefinery commercialization. The cellulose biopolymer is hydrolyzed by an enzyme complex such as cellulases, beta-glucosidase, xylanases in a cascade process into simple monomers. The hydrolytic performance of cellulases is enhanced by augmenting with xylanases, since deconstruction of hemicellulose layers between cellulose microfibrils further improve the accessibility of cellulose to hydrolytic enzymes.

Our initial efforts in *in-situ* enrichment and development of thermophilic GHs producer resulted in potential exoglucanase and endoglucanase producing *Chaetomium thermophilum* EDWF1 (Saranya, 2017), beta-glucosidase producing *Bacillus tequilensis* (Thangappan et al., 2018) xylanase producing *Bacillus licheniformis* (Thangappan et al., 2017). With this enzyme cocktail, a modified saccharification process developed for corncob, and *Erianthus* stalks enhanced the saccharification efficiency of 51 and 87 %, respectively (Patent filing under progress). Developing a suitable separation strategy using NF membranes would be helpful in achieving the full potential of biorefinery for multiple products.

Lignin ranks second only to cellulose as the most abundant natural product on earth. When such lignin containing biomass are used for bioethanol production, large amounts of lignin is generated as waste which is up till now the only renewable resource, potentially available in enough quantities, for the industrial production of aromatics. In this context, one of the important technologies required for the successful biological conversion of lignocellulosic biomass to biofuel and chemicals is delignification to liberate cellulose and hemicellulose from their complex with lignin (Thangavelu et al., 2018). Whatever may be the method used for delignification, lignin is generated as a major by-product and is comprised of cross-linked, branched aromatic monomers. As this lignin-based waste has generally been utilized as an industrial by-product that is burned to supply steam and electricity, any attempt to convert the lignin into value-added products is expected to improve process economics of an ethanol biorefinery. More importantly, success of LC biomass as renewable carbon sources is dependent on the development of economically feasible technologies by employing advanced techniques for different steps and utilization of by-products like lignin generated during pre-treatment. Recovery, characterization and application of lignin can lead to sustainable production of ethanol from lignocellulosic biomass.

Compared to chemical processes (acid/alkali), which liberate lots of inhibitors/reduces sugar recovery, enzymatic delignification processes would be more specific, catalytic, use less chemicals, would be less likely to damage the fibre than chemical methods, and allow higher hydrolysis yields under less severe conditions. By enzymatic catalysis, development of novel routes to high-value aromatic chemicals (vanillin, phenol, styrene, etc.) from lignocellulose will be of considerable interest to industries of plastic manufacture, food industry, personal care, etc. The common ligninolytic enzymes (laccases, peroxidases) from bacterial sources are more powerful and operate by generation of free radicals that initiate cleavage of linkages in lignin and results in generation low molecular weight phenol and derived aromatic chemicals.

**Keywords:** Lignocellulosic biomass; Saccharification; Thermophilic enzymes

## Multi-omics-based drug discovery and characterization of future drugs from marine microbes



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### Abstract

Gigantic technological advancements in next-generation DNA sequencing methods opened up wide avenues in discovering and characterization of various aspects of biological science in a cost-effective manner. These methods have revolutionary impacts on genetic and genomics applications in the areas of biology and medicine. Herein, I summarize my experiences in developing bioinformatics and genomic applications in unravelling future drugs from marine fungi. I developed the multi-omics-based methods for discovering drug-like compounds using marine fungal genomics and transcriptomics. Marine fungi are potent producers of secondary metabolites. Genomics provides comprehensive overview of the secondary metabolite producing biosynthetic gene clusters and hint

for their regulatory systems which can be modified and can be used for higher production of drugs in the heterologous systems like yeast or *Aspergillus*. At the moment, I have characterized largest datasets with more than 10 genomes of marine fungi and about 300 drug-like compounds produced by marine fungi with the notably anti-cancerous compound, scopularide from marine *Scopulariopsis brevicaulis*. Towards end, I will be presenting my future projections of 5 years for setting of OMICS approaches for drug-discovery from marine microbes.

**Keywords:** *Scopulariopsis brevicaulis*; Marine fungi; Omics; Secondary metabolites

### Topoisomerase I and Nucleolin modulate the stability of G4 DNA-forming genomic loci in yeast.



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#### Abstract

Genomic loci containing repetitive sequences are prone to secondary structure formation. The four-stranded G-quadruplex (G4) DNA consisting of multiple guanine-runs is one such structure. Although a significant correlation between the manifest enrichment of G4 sequence motifs and the leukemia/lymphoma-associated translocation breakpoints has been shown repeatedly, how G4 DNA-motifs instigate chromosome abnormalities leading to malignant transformation is yet to be resolved. We developed quantitative assays to measure and characterize multiple types of G4 DNA-induced genome instability events in the context of the yeast genome. This system allowed us to demonstrate that in addition to the level and direction of transcription, the topological change ensuing in the absence of Topoisomerase I (Top1) is a critical determinant of the extent of genome instability at actively transcribed G4 motifs. The G4-associated instability in *top1Δ* yeasts was suppressed partially by the bacterial topA (*ecTopA*), which removes negative supercoils and not at all by the gyrases (*ecGyrase*), which removes positive supercoils. The expression of a Top1 catalytic mutant (Y727F or Y740STP) aggravated the G4-associated instability further than the absence of functional Top1 (*top1Δ*). We showed that these Top1 mutants can form a stable complex with a G4 DNA forming-oligo which indicates that mutant forms of Top1 can possibly bind and stabilize G4 DNA further hindering DNA replication and/or transcription. We also showed that the highly conserved nucleolar protein Nucleolin (Nsr1 in yeast) is important in rendering the G4 DNA-loci unstable either in the absence of functional Top1 or in the presence of mutant Top1. Deletion of Nsr1, a known G4 DNA-binding protein, surprisingly led to a significant decrease of G4-induced recombination. We present a model where the accumulation of transcription-associated negative helical tension in the absence of functional Top1 shifts the equilibrium between B DNA and G4 DNA toward stable G4 DNA and thereby allows the formation of the replication-blocking G4 DNA-Nsr1 complexes. Our model also speculates that Top1 catalytic mutants that can nonetheless bind DNA such as Y727F and Y740STP can also generate the replication-obstructing G4 DNA-protein complex.

**Keywords:** G-quadruplex; DNA binding proteins; Top1; Genome stability

## Transcriptional modulation of transport and metabolism gene clusters leads to preferential utilization of aromatics over glucose in *Pseudomonas putida* CSV86



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### Abstract

*Pseudomonas* strains play an important role in carbon cycling in the environment and display a hierarchy in carbon utilization: organic acids first, followed by glucose, and aromatic substrates last. This limits their exploitation for bioremediation. Thus for effective removal of aromatic pollutants from the environment can be achieved by efficient degradation of these compounds by microorganisms even in the presence of simple carbon sources like sugars or organic acids. *Pseudomonas putida* CSV86, a soil isolate, displays a unique ability to degrade aromatic compounds prior to glucose. Metabolic and proteomic studies suggest that enzymes and transport proteins responsible for the glucose metabolism are suppressed in the presence of aromatic compound like naphthalene or benzoate. The draft genome and transcription analyses revealed that glucose uptake and benzoate transport and metabolism genes are clustered at the *glc* and *ben* loci, respectively, as two distinct operons. When grown on glucose+benzoate, CSV86 displayed significantly higher expression of genes at *ben* locus in the first-log phase and genes of *glc* locus in the second-log phase. Kinetics of substrate uptake and metabolism substantiate the observed transcription profiles. The inability of succinate to suppress benzoate transport and metabolism resulted in co-utilization of succinate and benzoate. Benzoate and succinate failed to interact with or inhibit the activities of glucose transport components or metabolic enzymes. The data suggest that succinate and benzoate suppress glucose transport and metabolism at the transcription level, enabling *P. putida* CSV86 to preferentially metabolize benzoate. Strain CSV86 can serve as an ideal host to engineer and facilitate efficient removal of recalcitrant pollutants even in the presence of simpler carbon sources.

**Keywords:** Aromatic compounds; *Pseudomonas putida*; Transcription; Metabolic engineering

## The PIP box of DNA replication protein Cdc45 mediates direct interaction between Cdc45 and PCNA and is essential for cell survival in the protozoan parasite *Leishmania donovani*



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### Abstract

*Leishmania donovani* is the causative agent of the dreaded kala-azar or visceral Leishmaniasis that afflicts the world's poor. Every year approximately half a million new cases are reported, 90% of which occur in Sudan, Brazil, and the Indian subcontinent. Though there are drugs to treat the disease, due to emerging drug resistance the search for new sites for therapeutic intervention continues. The parasite is transmitted to humans by the bite of the sandfly, and it reproduces by binary fission in both hosts, with DNA replication of the genome being central to the process. Eukaryotic DNA replication is a conserved process involving a host of proteins. Cdc45 is a component of the eleven subunit replicative helicase, whose other components are the heterohexameric Mcm2-7 and the heterotetrameric GINS. Together, this CMG complex is responsible for unwinding the DNA helix ahead of the advancing replication fork. Our analysis of *Leishmania* Cdc45 revealed the presence of a PIP box, a motif that is usually involved in interactions with PCNA. The homotrimeric PCNA interacts with several replication proteins, and all of the interactions are vital for the replication



process. Thus far there are no reports of interaction between Cdc45 and PCNA. This study was taken on with the aim of determining if *Leishmania* Cdc45 interacts with PCNA. Our data indicates that Cdc45 and PCNA in fact interact directly, via the Cdc45 PIP motif, and this interaction is essential for cell survival. The role of the interaction in the *in vivo* context is examined, and our data suggest that this interaction, though unlikely in humans, is not restricted to *Leishmania*. Further studies will be directed towards determining if this interaction is seen in other model eukaryotes.

**Keywords:** *Leishmania donovani*; Drug resistance; PCNA; DNA replication

### Functional metagenomics reveals repertoire of oxidative stress tolerance genes from microbial communities



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#### Abstract

Majority of the bacteria present in any environment cannot be cultivated in laboratory. Traditionally, microbiologists relied on culture to study the microorganism or for their exploitation for biotechnological application. This allowed access to only a small proportion of the microbial diversity. Molecular diversity analysis revealed that these uncultivable bacteria represent previously unrecognized classes and divisions in bacterial and archaeal domain and are expected to possess novel physiologies and genes. Culture-independent methods give access to the vast gene pool of the uncultivable bacteria.

Microorganisms inhabit various niches and face different abiotic stress like oxidative stress, salinity stress, toxic heavy metals etc. Uncultivable bacteria present in different environments are expected to possess unique stress tolerance mechanisms. We have identified number of previously uncharacterized genes for oxidative stress tolerance from microbial communities of different niches and their detailed characterization helped in elucidation of the mechanisms of stress tolerance.

**Keywords:** Metagenomics; Abiotic stress tolerance; Microbial communities

### Microbial enzyme based processes for functional molecules production



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#### Abstract

Microbes and microbial enzymes are being explored for functional molecules productions. In this regard an efficient process based on xylanase, produced by *Acinetobacter pittii* MASK25, hydrolysis of only physically treated rice straw and corn cob has been developed for the production of xylooligosaccharides. Process was further improved by developing magnetic-xylanase CLEA. Crude xylanase and magnetic-xylanase CLEA could convert respectively more than 45% and 60% xylan of the powdered rice straw and corn cob into xylooligosaccharides. Interestingly, hydrolysis by both types of enzymatic forms was found to produce predominantly xylopentose and xylohexose. Hence, the process has predominantly produced

xylopentose and xylohexose that could find unique prebiotic applications. A novel hybrid nanoflower of manganese and l-arabinose isomerase was developed and explored for its use in the synthesis of d-tagatose, a rare sugar of high commercial value. The hybrid nanoflowers exhibited excellent reusability and reproducibility during reaction cycle analysis. Hence, the developed manganese hybrid nanoflowers of l-arabinose isomerase shows promise for commercial production of the rare sugar d-tagatose. Similarly, several residues from agro-forestry industries such as rice straw acid hydrolysate, corn cob acid hydrolysate, tomato juice, cane molasses and orange pulp were evaluated as the economical source for the production of bacterial cellulose by a newly identified bacterium *Acetobacter pasteurianus* RSV-4. The produced bacterial cellulose was characterized through FTIR, SEM, TGA and DSC and found to be of very good quality. The bacterial cellulose produced by identified strain on these various agro-waste residues could be a cost effective technology for commercial its production.

**Keywords:** Nanoflowers; Enzymes; Bioprocess

### Evaluation of biocatalysts for their efficient use and re-use in bioprocesses



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#### Abstract

The biocatalysts play a very important role in a system for the continuation and completion of the process in most desirable way. The performance of biocatalyst determines the rate and efficiency of the bioprocess and therefore, the selection of a suitable biocatalyst controls the overall yield in the process. However, the biocatalyst can be designed for the convenience of their use and then re-use for an economical process to run in a more practical way. The re-use of biocatalyst is important in several systems such as fermentations and bio-transformations. In this presentation an evaluation of such strategically designed biocatalysts will be discussed, which were modified by several immobilisation techniques like entrapment of microbial cells in four ways, and encapsulation of cells in two different ways. The performance of these purposely designed biocatalysts has been evaluated in batch and continuous fermentation systems and will be discussed in reference to their stability, and fermentation-efficiency of the process.

**Keywords:** Biocatalysis; Bioprocess; Fermentation; Biotransformations

### Cost-effective synthesis of L-Theanine using $\gamma$ -Glutamyltranspeptidase from *Bacillus licheniformis*: A step towards greener technology



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#### Abstract:

L-theanine ( $\gamma$ -glutamylethylamide) is an emerging nutraceutical with multi- facet health benefits. Besides strengthening immune system and cognition power, it enhances anti-tumor activity. Presently it is largely extracted from green tea, however, it has several limitations such as seasonal variations, cumbersome extraction with low yields leading to high cost. Chemical synthesis too has many limitations

including synthesis of racemic mixer of L- and D- forms that are difficult to separate. Enzymatic synthesis of L-theanine overcomes most of these limitations and  $\gamma$ -glutamyltranspeptidase (GGT) can be utilized in its green synthesis. China is the mainland manufacturer of theanine followed by United states and Singapore and enzymatically synthesised L-theanine is sold under the brand name 'Suntheanine' only by one US based industry present world-wide. However, there is not a single manufacturer of L-theanine in India and pays upto 150% import duty on L-theanine import from other countries leading to a very high cost. Owing to its huge demand world-wide there is a need for initiating indigenous manufacture for its sustainable production.

In this respect L-theanine was synthesized enzymatically using GGT from *Bacillus licheniformis* in the laboratory and now efforts are being made to address different upstream and downstream steps to bring down overall cost of the finished product. The presentation will include various strategies employed to enhance its production by recombinant DNA technology and media optimization followed by protein engineering for increased catalytic efficiency. Further sustained production was obtained by standardizing enzyme immobilizing with recyclability and improved shelf-life. This was followed by optimizing L-theanine synthesis and its extraction. So overall the presentation will reflect step-wise development of a sustained process for L-theanine synthesis.

**Keywords:** L-theanine; Green technology; rDNA technology; Bioprocess

### **A novel cellulase from indigenous fungal strain NSF-2 using agro industrial waste and its use in waste paper recycling**



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#### **Abstract:**

The study, present the production of cellulase using agro-industrial waste, its purification and characterization. A fungal strain NSF-2 isolated from the plant litter produce high amount of cellulase. 15.59 fold purified enzyme achieved specific activity 71.421 U/mg. Under the optimum condition,  $V_{max}$  and  $K_m$  for the enzyme were 1.5 mg/ml/min and 3.2 mg/ml respectively. Purified cellulase stable in non-ionic surfactant like TWEEN -20, Triton X-100 on the other hand salt like urea, EDTA and SDS inhibited the enzyme activity up to 65%, 50% and 55% respectively. The study also reveals that  $Hg^{2+}$  is a non-competitive inhibitor of cellulase. The  $V_{max}$  was decreasing with increasing concentration of metal inhibitor. The purified and crude cellulase produce by NSF-2 strain is well suitable in industrial application such as pulp and paper for waste paper recycling which also reduces the use of chemical thus help in waste water management also.

**Keywords:** Cellulase; Agrowastes; Paper recycling

### **Analysis of post translational modifications during bacterial exposures in the eukaryotic model system, *Caenorhabditis elegans***

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## Abstract

Studying the Host pathogen interaction in a definite model organism is being employed by the researchers for the past few decades. By using the OMICS technologies, the underlying mechanisms of the pathogenesis can be studied very well during host pathogen interactions. Most importantly, functional proteins of a living system play a crucial role in all types of cellular mechanisms (such as defense mechanism, cell-signalling, homeostasis, etc.). Undeniably, the translated proteins will be activated or inactivated soon after undergone for a biological phenomenon called post translational modification (PTM). Hence, studying the importance of PTMs will give a clear view on pathogen mediated immune response in the host system. Previously, host pathogen interaction studies have been reported that *C. elegans* can be used as a better model system to study the bacterial pathogenesis. Our research group is currently focusing on the impact of various PTMs such as phosphorylation, glycosylation, acetylation, etc., against various bacterial exposures. By studying the impact on *C. elegans* phosphoproteome using TiO<sub>2</sub> column chromatography followed by MALDI-ToF-ToF analyses, we identified the involvement of molecular chaperone, HSP-90 and ubiquitin mediated proteolysis pathway during *S. Typhi* exposure. Meanwhile, the analysis of O-GlcNAcylation revealed that it confers protection against *S. aureus* through ubiquitination. Subsequently, the analysis of acetylated proteins and Sumoylated proteins are also currently on progress to understand their involvement during bacterial pathogenesis. The identification and characterization of the molecular mechanism and the responsible PTMs during host pathogen interactions is a challenging task which will pave the way for future therapeutic applications.

**Keywords:** 2DGE; Glycoproteins; Phosphoproteins; MALDI-ToF-MS; STRING analysis; Bioinformatics



## Single-species and Multi-species Biofilms and its Impact in Disease

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## Abstract

A biofilm is defined as a community of bacteria attached to a surface and surrounded by a complex extracellular matrix. Biofilms are an important topic in medical research mainly due to their high resistance to antimicrobial therapy and ability to evade the immune system. Importantly, some harmless commensal organisms can become virulent when growing within a biofilm. With the alarming global increase in antimicrobial resistance, it is now urgent to better understand biofilm physiology, in order to develop novel antimicrobial agents.

Biofilm-related infections often occur associated with the utilization of indwelling medical devices, such as the ones caused by *Staphylococcus epidermidis* in central venous catheters. However, other biofilm-associated infections occur naturally in the human body, and often those infections involve multiple bacterial species, such as the case of bacterial vaginosis.

In this lecture, I will start by addressing what strategies *S. epidermidis* uses to enhance its tolerance to antibiotics and survival in the host. Starting from a global transcriptomic analysis, my research group highlighted key pathways that allow this commensal microorganism to thrive in the host, causing one of the most prevalent nosocomial infections. Several mutant strains have been constructed and their phenotype characterized, regarding both the ability to growth as biofilms as well as their interaction with the host immune system.

I will also address the topic of bacterial vaginosis, in particular the aspects associated with the microbial interactions that can occur in the human vagina, from the protective role of lactobacillus interference with bacterial vaginosis species, to the enhancement of biofilm formation by particular species that, in

cooperation, can enhance virulence and contribute to the high recurrence rates associated to bacterial vaginosis.

**Keywords:** Nosocomial infections; *Staphylococcus epidermidis*; Bacterial vaginosis; *Gardnerella vaginalis*; Gene expression

### Small molecules to tackle antibiotic resistance in Gram negative bacterial pathogens



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#### Abstract

Antibiotic resistance is one of the biggest threat to public health. Without an urgent action, the mankind is headed towards a post-antibiotic era, in which common infections and minor injuries can be deleterious. A growing list of infections – such as pneumonia, tuberculosis, blood poisoning, gonorrhoea, and foodborne diseases – are becoming harder, and sometimes impossible, to treat as antibiotics become less effective. The pace of antibiotic discovery has inadvertently failed to keep up with the mounting crisis of antibiotic resistance. Most pharmaceutical companies are not interested in developing new antibacterial agents due to reduced economic incentives and challenging regulatory requirements. No new antibiotics have reached the market in the last two decades for Gram negative pathogens. My research group employs chemical genetic strategies to address various aspects of this phantom problem. Recently we have discovered a lead series of molecules (such as IITR06144 series) that exhibits all the features of an ideal antibacterial. We have also discovered small molecule adjuvants to revive the activity of “ineffective antibiotics”. A few antibiotic - antibiotic combinations have also been characterized for their synergistic interactions against ESKAPE pathogens. My talk will focus on these recent findings and their molecular and biochemical mode of action against bacterial pathogens.

**Keywords:** Antibiotic resistance; ITTRs; ESKAPE pathogens

### The Enigmatic PE\_PPE Protein Family of *Mycobacterium tuberculosis*



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#### Abstract:

The pathogenesis of *Mycobacterium tuberculosis* (*M.tb*), the causative agent of human tuberculosis, involves the co-ordinate action of multiple bacillary components that modulate host immune responses to ensure its survival. One such group of factors is the multigenic PE\_PPE protein family, which occupy almost 10% of the coding potential of *M.tb*. Although some members of this family have been implicated in host immune evasion, the precise roles of these proteins remain largely undefined. To gain insights into their functions, we are investigating the physiological significance of several proteins of this family. Our work has shown that these proteins possess specialized functions and potentially play pivotal immunomodulatory roles in regulating the pathophysiology of *M.tb*. Our findings have greatly expanded the repertoire of proteins that *M.tb* utilises to alter host immunity and establish infection.

**Keywords:** *Mycobacterium tuberculosis*; PE\_PPE protein; Immunomodulation; Infection

## Rotavirus vaccine development : The India story



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### Abstract

Rotavirus is the leading cause of diarrhoea-associated hospitalisations and deaths in developing countries, estimated to account for approximately 610,000 deaths annually. It is a particularly important problem in India where nearly one-fourth of these deaths occur and it is estimated that about 1 child in 250 in India will die of this disease. Accordingly, development of vaccines for rotavirus infections has been accorded high priority. As part of the Indo-US Vaccine Action Program, a Department of Biotechnology (DBT), Government of India sponsored activity to promote new vaccine development, two rotavirus strains were identified that demonstrated interesting properties as potential candidate vaccines. These strains were obtained from outbreaks of asymptotically infected newborns in Delhi (116E) and Bangalore (I321). 116E is a human strain with a single gene segment encoding VP4 derived from a bovine rotavirus. By contrast, strain I321 was a bovine strain with two non-structural gene segments derived from a human strain. Safety and immunogenicity studies of two orally administered human rotavirus AGMK cell-adapted vaccine candidates 116E and I321 were carried out in ninety healthy infants aged 8 weeks who received a single dose of 116E ( $10^5$  ffu), I321 ( $10^5$  ffu) or placebo. There were no significant differences in the number of adverse events. These studies showed that 116E strain was attenuated, clinically safe and highly immunogenic.

The neonatal rotavirus candidate vaccine 116E was subsequently tested in a double-blind, placebo-controlled dose escalation trial in India. Two doses of the Vero cell-adapted vaccine were evaluated. There were no vaccine-related serious adverse events. A 4-fold increase in rotavirus IgA titer was observed in 66.7% and 64.5% of infants after the first administration and in 62.1% and 89.7% of infants after 3 administrations of doses of  $10^4$  ffu and  $10^5$  ffu, respectively. Subsequently, a randomised double-blind, placebo-controlled, multicentre trial of  $10^5$  ffu 116E vaccine was conducted at three sites in Delhi (urban), Pune (rural), and Vellore (urban and rural) that enrolled 6799 infants aged 6-7 weeks. Of these, 4532 infants were assigned to receive the 116E vaccine and 2267 to receive placebo. The incidence of severe rotavirus gastroenteritis per 100 person-years was 1.5 in the vaccine group and 3.2 in the placebo group. Prevalence of immediate, solicited, and serious adverse events was similar in both groups. These studies demonstrated that the monovalent human-bovine (116E) rotavirus vaccine was effective and well tolerated in Indian infants. Having established the safety and efficacy, the 116E rotavirus vaccine 'Rotavac' produced by BBIL, Hyderabad was launched by the Prime Minister of India for use in India.

**Keywords:** Rotavirus; Vaccine; 116E; Clinical trial

### Bacteriophage Banks - A unique therapeutic platform providing personalized medicine against emerging infectious agents



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## Abstract

Severity of multi-drug-resistant (MDR) bacterial infections has necessitated novel antibacterial strategies amongst which bacteriophage therapy has been understood and exploited the most. Phage therapy has been revitalized after phage cocktails and encoded products have been demonstrated to be safe and efficacious as evidenced in recent past where bacteriophage therapy has proved useful in treating human cases of MDR infections. Bacteriophages are widely distributed in nature and exist in abundance in locations where bacteria reside *i.e.* soil, sewage, water bodies, sludge, etc. Bacteriophages are specific in their action and have fine tuned their biological evolution to match the rapidity in evolution of their host. They offer an affordable alternative strategy for combating bacterial infections. The entire world has realized the importance of bacteriophages and many international culture collections worldwide such as ATCC, USA; NBRC, Japan; DSMZ, Germany; NCCB, Netherlands; NCIMB, Scotland; Korea National Research Resource Center, China Center for Virus Culture Collection, Felix d'Herelle Reference Center for Bacterial Viruses, Canada etc. are maintaining bacteriophage collections. Apart from direct delivery for disease treatment, the bacteriophages have been exploited for their immense potential in various other applications *viz.* anticancer therapy, vehicles for the recombinant and subunit vaccines, biocontrol agents in agriculture and petroleum industry, display system for many proteins and antibodies etc. "Phage Therapy" is now turning out to be a ray of hope in personalized medicine for chronic infections also. As such, the phage repositories may play an important role by timely presenting a panel of biologically active phages which may be screened against case-specific infection causing pathogens to select the most virulent or active phage against the provocative infectious bacteria. Phages have a critical edge in therapeutics owing to their inherent properties such as comparative easy isolation, rapid growth kinetics, survivability till availability of host and increase in number during therapy without disturbing the natural microflora besides being plentiful in nature. Due to coevolving arms race amongst bacteria and phages, the chances of infective phages against the '**then emerging bacteria**' will probably always remain in comparison to searching an altogether new antibiotic.

**Keywords:** Multi-drug resistance; Phage therapy; Biocontrol; Virulence

### **Fulfilling the mission of drought and salinity tolerance in crop plants by harnessing the diversity of extreme halophilic microorganisms**



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## Abstract

The existing horizon of drought and salinity affected areas globally will rapidly expand due to predicted change in climate, affecting food security of estimated 9.6 billion world population by 2050. This has necessitated the urgent attention for developing crop varieties tolerant to drought and salinity stresses. To cope with these situations, genetic enhancement is considered to be a major option, yet spectacular progress in developing truly drought- and salinity- tolerant cultivars have not taken place. The bottleneck of such efforts, probably, is our inability to provide a solution to the reduced photosynthesis during drought- and salinity stress, and compensate them suitably, when plants resort to minimize stomatal opening or even shut them down during daytime to reduce transpirational losses resulting in drastic reduction in photosynthesis and carbon gain during drought. In salinity, unless transpiration is minimized by regulating the opening and closing of stomata, there is chance of inflow and accumulation

of Na inside plant tissues during salinity stress, affecting cellular integrity. If the situation persists for a long period, unless alternative methods are not made available for carbon gain, the plants will eventually die due to lack of sufficient reserves to overcome the situation.

Therefore, the question remains as to how the concerns of salinity and drought can be addressed so that more area can be brought under cultivation to sustain agricultural growth. This needs stable signatures/traits that might have expressed and transferred evolutionarily across domains of life which can impart salinity and drought stress tolerance. Such 'treasure trove' might be present in a system which would have evolved and witnessed all events of evolution and has transferred the stable signatures/traits into new and newer forms of life in each step of evolution. As salinity tolerance is almost synonymous to drought tolerance, clues can be obtained from strains of extreme halophilic bacilli and their derived genera, archaea, and fungi that can survive the extremities of salinity as prevalent in the otherwise undisturbed ecosystem of the Rann of Kutch of Gujarat with the history of stable evolutionary lineages. While studying the diversity of the extreme halophiles, it was found that these groups of organisms can survive and perpetuate their races at upto saturated levels of NaCl concentration, the most inhospitable level of possible salinity.

The sequencing of the genomes and metagenomes of the representative organisms and samples of the Rann of Kutch and their diversity provided the much needed information about the strategies required to be adopted to circumvent such level of stresses and the ways and means of gaining valuable carbon required for growth and multiplication, when there is very limited availability of nutrients and carbon sources in the growth environment. Genes reported to have capability of imparting osmotolerance could not be detected in metagenomes and many of the genomes of the extreme halophiles obtained from the Little and Great Rann of Kutch. Then how did all these organisms survive in such inhospitable salinity? Critical evaluation of the sequence data of the metagenomes revealed that there are more than 20000 species of organisms that can survive and multiply in more than 35% of salt concentration. When the salt concentration reaches around 50% level, the species richness reduced to around 15000 species with predominance of extreme halophilic archaea! Contrary to the reported involvement of methyl aspartyl pathway in helping *Haloarcula marismortui* to thrive in the extreme salty settings prevalent in the Dead Sea, we found possible involvement of serine-glyoxylate, acetyl CoA, methyl malonyl- and ethyl malonyl-CoA pathways in a novel haloarchaeon 3A1-DGR and metagenomes in imparting salinity tolerance by helping the organisms to gain carbon and channelizing the intermediates to other anabolic reactions resulting in net positive carbon balance. This begins with the uptake of dissolved CO<sub>2</sub> as HCO<sub>3</sub><sup>-</sup> which is then converted into phosphoenol pyruvate- oxaloacetate- malate- pyruvate/ phosphoenolpyruvate-glyoxylate-serine-, etc. by overexpression of key enzymes like phosphoenolpyruvate carboxylase, malate dehydrogenase, isocitrate lyase, malate synthase, decarboxylases, serine hydroxymethyl transferase, etc. in a series of reactions to capture carbon from the environment and also to utilize respiratory CO<sub>2</sub> and eventually diverted for synthesis of key intermediates required for sustaining life processes. What is important is to have the intact network of the pathways and the expression of all the necessary enzymes in coordination. In addition to carbon gain, the organisms expressed Na/K/H pumps to remove most of the Na trying to enter into cytoplasm with solute. All the machineries required for such expression are present in bacilli and fungi (archaea-eubacteria-eukaryote pipeline) obtained from the Rann of Kutch. The big question is whether such pathways have been acquired by plants while transition from aquatic to terrestrial ecosystems during the process of acclimatization and evolution and divergence? If yes, whether it would be feasible to identify all these footprints or orthologs in plants so that crop plants truly tolerant to salinity can be identified which can address the issue of reduced photosynthesis during salinity stress by ensuring steady supply of carbon through alternative route/mechanisms to anabolic reactions to occur leading to net positive carbon gain. Many of the orthologs of the genes involved in the alternate route of carbon gain has been found in *Arabidopsis*. Recent studies in groundnut indicated the collinearity in expression that has been found in the diverse group of extreme halophiles of the Rann of Kutch. The alternate route of carbon gain and expression of many of the above enzymes in C3 background has the chance of ensuring photosynthesis behind closed stomata, during daytime, in drought- and salinity- stress, without much affect on biomass.



Thus, evolutionary clues and mechanisms of alternate route of carbon gain obtained from the diversity analysis of extreme halophiles would suffice the future strategies for ensuring drought and salinity tolerance in plants.

**Keywords:** Halophiles; stress; Carbon cycling; genome analysis



### OMICS As a tool for Microbial Biodiversity Study

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#### Abstract

Microbes are the key providers of various ecosystem services and play an important role in biogeochemical cycling of materials, pollutant remediation, solid waste management, greenhouse gas formation and generation of breathable oxygen. They are crucial components for plant growth and work as bio-fertilizers and biological control agents for suppression of pathogens. Culture independent metagenomic studies have shown that immense microbial diversity of agricultural, industrial, environmental and clinical importance is not yet cultured. Despite all the innovation in cultivation of the microorganisms and development of novel cultivation tools; only 1-10% microbial diversity has been cultured and rest 90-99% is not yet cultured. Culture independent metagenomics approach can only tell who they are but unable to say what they are doing. In order to understand their functionality cultivation is must. I present discussion I would like to discuss how to use different tools of OMICS for cultivation, of not yet cultured microbes to bring them in laboratory pure culture for their industrial, agricultural, environmental and clinical applications.

**Keywords:** Metagenomics; Microbial diversity; Omics

### Nitrogen, Nitrogen, everywhere, nor any efficient use: Adaptation and shifts in rhizosphere microbiomes of rice



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#### Abstract

Nitrogen is everywhere, making up 78 percent of the earth's atmosphere. The microbial power to fix dinitrogen enables higher plants to use the reactive nitrogen ( $N_r$ ), primarily for producing proteins and other building blocks. The Haber-Bosch's discovery and the ensuing industrial N production has given humans the enormous power to feed their increasing populations. In intensive agriculture, the application of nitrogenous fertilizers has become necessary to achieve higher productivities of crops, including rice. But the Haber-Bosch nitrogen (N) can influence several processes depending on the inherent physiologies and abundance of different microbial players, the major workhorses of the soil N cycling. The soils from the experimental rice fields of Indian Agricultural Research Institute [New Delhi (IARI) and Aduthurai (ADU)], and farmers' fields of Kuttanad (KUT), Rajasathan (RAJ), Umiam (UMI) and Karnal (KAR) along with subsurface (SUB) soil of non-agricultural soil from Delhi were analysed for the microbial communities. The gene copies of eubacteria, archaea, and anammox and those of *nifH*

ranged from  $7.26 \times 10^7$  to  $1.47 \times 10^{10}$ ,  $4.53 \times 10^3$  to  $9.53 \times 10^5$ ,  $4.22 \times 10^7$  to  $7.41 \times 10^9$ , and  $1.54 \times 10^3$  to  $2.94 \times 10^4$  g<sup>-1</sup> soil, respectively, suggesting the characteristic differences in the soil microbial communities. Varying levels of N, when added to these soils, brought out different shifts in the microbial communities, with increases in anammox bacteria but decreases in archaeal populations. Higher amounts of N had adverse effects on the nitrogen-fixing bacteria. Both the fertilizer N management practices including the application of biofertilizers, and the stages of plant growth, strongly influenced the rhizosphere microbiomes of field grown rice. The abundance of *nifH* gene copies in the rhizosphere was only about  $10^3$  to  $10^6$  g<sup>-1</sup> soil, more at the flowering stage than at the vegetative stage. The gene copies of *narG*, which encodes the membrane-bound nitrate reductase, ranged from  $5.11 \times 10^7$  to  $1.52 \times 10^9$  g<sup>-1</sup> soil, suggesting higher rates of denitrification. The perils of excess nitrogen include the cascading effect not only on the rhizosphere microbiome but also on the planetary boundaries on the biogeochemical flows. Hence, the precise, rapid quantification of functional genes and linking with the composition of N-cycling microbial communities are critical to identifying, designing and powering sustainable agricultural practices within the planetary boundaries.

**Keywords:** Nitrogen; Rhizosphere; Biofertilizer; Soil microbiology

### Structural diversity and molecular basis for prebiotic potential of short chain $\beta$ -mannooligosaccharides in probiotic *Lactobacillus* sp.



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#### Abstract:

A recombinant endo- $\beta$ -1,4-mannanase (ManB-1601) from *Bacillus* sp. CFR1601 hydrolyzed locust bean gum, a galactomannan, to  $\beta$ -mannooligosaccharides ( $\beta$ -MOS) mixtures. Size exclusion chromatography of oligosaccharides mixtures, led to separation of oligosaccharides with various degrees of polymerization (DP 2, 3, and 5). ESI-MS, FTIR, XRD, TGA, and NMR (<sup>1</sup>H and <sup>13</sup>C) showed that DP2 oligosaccharide was composed of two species, (A) mannopyranose  $\beta$ -1,4 mannopyranose and (B)  $\alpha$ -1,6-galactosyl-mannopyranose, while DP3 oligosaccharide showed the presence of only one species, i.e.  $\alpha$ -D-galactosyl- $\beta$ -Dmannobiose. Immobilized ManB-1601 in cross-linked aggregated form and novel chitosan magnetic nanocomposites of MB-C were able to repeatedly hydrolyze locust bean gum till 12 cycles and generated predominantly di-, tri-and tetra- species of  $\beta$ -MOS. DP2 and DP3  $\beta$ -MOS improved the growth of probiotic *Lactobacillus* sp. while, DP5 resulted in poor growth of all *Lactobacillus* spp. tested under in vitro conditions. Attempts were made to identify the genetic loci involved in the transport and catabolism of locust bean gum derived  $\beta$ -MOS in *Lactobacillus plantarum* WCFS1, a human probiotic strain. Studies on substrate depletion showed that *L. plantarum* WCFS1 can metabolize only short chain  $\beta$ -MOS (degree of polymerization; DP $\leq$ 3). Global transcriptome microarray profiling of *L. plantarum* WCFS1 revealed differential expression when  $\beta$ -MOS or control sugars (glucose, galactose, and mannose) were used as a sole carbohydrate source. The cellobiose (~3.2 kb) and oligo-sucrose (~7.3 kb) utilization in *L. plantarum* WCFS1 were found to be highly up-regulated up to 8.3 and up to 6.7-fold, respectively by  $\beta$ -MOS utilization. High correlation was observed between qRT-PCR studies of the selected gene clusters and microarray data. Our overall transcriptome and qRT-PCR studies of *L. plantarum* WCFS1 suggest that proteins encoded by the cellobiose operon might metabolize un-substituted mannobiose (DP2) while, oligo-sucrose utilization loci encoded proteins were responsible for the transport and catabolism of substituted DP2 (galactomannose) and DP3 (galactomannobiose).  $\beta$ -MOS also showed industrial potential by enhancing the survival of *Lactobacillus* sp. in low-fat ice creams under simulated gastrointestinal stress.

**Keywords:** Prebiotic; Probiotics; Oligosaccharides

## Microbiological Food Safety: Indian Regulatory Perspective

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### Abstract

With the advent of the globalization of the food supply chains, there is an impact on the food safety and quality aspects of food across global markets. Together with the removal of tariff barriers and quantitative restrictions, quality and safety of food has become extremely important in international trade. Not only the consumers all over the world have become quality & safety conscious but the governments have also realized their role in protecting the health and safety of their populations by imposing stringent regulations for ensuring food safety. Among various hazards, microbiological hazards have potential to pose serious threat to food safety.

Although it is difficult to estimate global burden of food-borne diseases, World Health Organization (WHO) in 2015 provided first estimates of global foodborne disease incidence, mortality, and disease burden in terms of Disability Adjusted Life Years (DALYs). As per report, 31 global hazards caused 600 foodborne illnesses and 420,000 deaths in 2010. The most frequent causes of foodborne illness were diarrhoeal disease agents, particularly norovirus and *Campylobacter* spp. Other major causes of foodborne deaths were *Salmonella typhi*, *Taenia solium*, Hepatitis A virus, and aflatoxin. The 40% of the foodborne disease burden was among children under 5 years of age. Microbiological hazards represent more than 90% of the incidents of food-borne illness and are the major problems not only in developing countries but also in developed countries.

The Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) began in 2000 in response to requests from the Codex Alimentarius Commission and FAO and WHO Member Countries and the increasing need for risk based scientific advice on microbiological food safety issues. The JEMRA aims to develop and optimise the utility of Microbiological Risk Assessment (MRA) as a tool to inform actions and decisions aimed at improving food safety. Codex has developed some very useful documents to address the Microbiological Risk for the benefits of member countries.

Food Safety and Standards Authority of India (FSSAI) has been established in terms of Food Safety and Standards Act, 2006, as a single reference point for laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, storage, distribution, sale and import, to ensure availability of safe and wholesome food for human consumption. In Pre-FSSAI era, various regulations specified microbiological requirements/limits in food products to ensure food safety, however, the FSSAI has tried to reorient the food regulation to emphasize and ensure food safety, food hygiene and food quality as a holistic approach. Microbiological requirements have been specified in Appendix B of food safety and standards (food products standards and food additives) regulations, 2011. Subsequently several amendments have been done in the requirements. The FSSAI Notified revised microbiological standards for fruits and vegetables and their products in March 2018 and microbiological standards for milk & milk products have also been revised in December 2018. Appropriate Surveillance Mechanism to understand the food borne disease status in India and emergency preparedness for microbiological risk are some of the concerns which require attention and FSSAI is committed to work on these aspects.

**Keywords:** Food safety; Regulations; FSSAI; Food additives

## Metagenomics and predictive functionality of ethnic fermented foods of North East India



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### Abstract

Ethnic fermented foods and beverages of North East India are unique and unappreciated. People of North East India have been practicing crude knowledge of "ethno-microbiology" to preserve the functional microbiota for fermented food and alcoholic beverage production for more than 4000 years ago. Traditional knowledge of ethnic people on food fermentation has significantly contributed to enrich the dietary culture, unique culinary, nutritional security and preservation of vast microbial resources comprising both culturable and unculturable microbiome and mycobiome. Functionalities including probiotics, antioxidants, synthesis of some bioactive compounds, and other health-promoting benefits in some ethnic fermented foods of NEI have been studied. Metagenomics approach has profiled the entire microbial community in some fermented foods and beverages with several health-promoting benefits through predictive functionality to consumers.

**Keywords:** Ethnic food; Metagenomics; Microbial communities; Probiotics

## Cobiotics prevent diet induced obesity through modulating gut bacteria and short chain fatty acid production in mice



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### Abstract

Energy dense foods contribute to weight gain and if not controlled leads to obesity besides other contributing factors like genetics and decreased physical activity. Excess calories stored in the body, especially in the adipose tissue, leads to weight gain, obesity and associated comorbidities due to low-grade inflammation, oxidative stress, altered adipose tissue secretome and dysbiosis of beneficial gut microflora. Drugs treating obesity control the host physiological process involving brain and endocrine hormone system and while doing so might cause untoward effects on chronic usage. This necessitates the need for alternate and safe strategies for the prevention or treatment of obesity and that may be achieved by various food ingredients such as dietary fibers, prebiotics, polyphenols, omega fatty acids and several others. High fat diet (HFD)-obesity in rodents are indispensable models for evaluating the role of various dietary interventions that could control obesity. Such diets induce dysbiosis in the gut microbiota and results in 'leaky gut' that promotes low-grade systemic inflammation, metabolic endotoxemia and ectopic fat deposition. We have developed a rationale combination of a prebiotic and an anti-inflammatory agent, designated as COBIOTIC and evaluated its preventive effect in diet induced obesity (DIO). Systemic adiposity, gut bacterial V3-V4 region based 16S rRNA metagenomic sequencing, ectopic fat deposition, liver metabolome analysis, systemic and tissue inflammation, and energy homeostasis markers along

with gene expression analysis in multiple tissues were done in mice supplemented with each agent or their combination. The “cobiotic combination” effectively prevented HFD-induced adiposity and lipid accumulation in liver and muscle while normalizing fasting blood glucose and related hormones. This combination also prevented leaky gut phenotype and HFD-induced pro-inflammatory stress. Most importantly, this combination improved beneficial gut microbiota abundances, improved Firmicutes to Bacteroidetes ratio and Prevotella/Bacteroides proportions. In particular, this cobiotic combination has shown improved beneficial effects on multiple parameters studied. Details will be presented in the talk during conference.

**Keywords:** Obesity; Prebiotics; Beneficial bacteria; Cobiotics

### **Mycotoxigenic fungi in Food chain and their novel biocontrol agents**



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#### **Abstract**

Mycotoxins are toxic metabolites of fungi, which are chemically stable and survive in all food processing. *Aspergillus*, *Penicillium* and *Fusarium* species produce toxins leading to contaminations emerging in the food chain. More than fifty genera of fungi are known to produce toxins and unfortunately, identification of closely related toxigenic species is overwhelming task even for taxonomic experts. Further, polyphasic approach is necessary in any taxonomic study and important facets of fungal biology in addition to morphology, nucleotide sequences that creates molecular markers for the straightforward identification of fungal species to solve taxonomic difficulties within species. Furthermore, molecular detection of mycotoxins, can serve as good alternatives to conventional methods targeting their biosynthetic pathway gene regarded as an efficient method for spotting of toxigenic *fungal species in fields, food and feed*, to ensure the food safety and quality. Aflatoxins (AFs), ochratoxins (OTs), Trichothecenes, Zearalenone (ZEA), Fumonisin (FUM), and citrinin (CIT) are significant toxins in food safety concern in the grain supply chain. At present, the use of synthetic chemicals/antifungal agents for control of fungi is in practice and is an abundant hazard to the humans and animals due to increasing in numerous drug resistant microbes, and its residual toxicity to animals and humans. Therefore, there is a tendency to use bio-agents to control the fungal growth and the elimination of mycotoxins in food commodities by several agricultural and food industries. Toxigenic fungi and toxin detoxifying agents produced by diverse source of fungi used for the management of mycotoxins are important for the food safety. Furthermore, detailed studies need to be undertaken in mycotoxin-contaminated food in developing countries including India.

**Keywords:** Mycotoxins; Detection; Biocontrol; Food safety

### **Diet and the intestinal microbiome**



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#### **Abstract**

Diet influences the intestinal microbiome in terms of composition and functionality. The nutrients that reach the largest intestine in greater proportion are non-digestible carbohydrates accessible to

the microbiota. The presence of these carbohydrates in the diet has proven to be relevant for a greater diversity of species in the intestinal microbiota, which in ecological terms brings stability and resilience to the ecosystem, thus contributing to intestinal homeostasis. Moreover, diet and derived microbial metabolites have strong implications with the development of food associated diseases such as obesity and metabolic syndrome, among others. In turn, healthy diets and specific nutritional interventions, including consumption of probiotics and prebiotics, could be valuable for restoration of beneficial bacteria and microbiota diversity. A key mechanism by which prebiotics exert benefits for human health is the production of short chain fatty acids, such as acetate, propionate and butyrate, as a consequence of the microbiota fermentation activities. These compounds have antimicrobial activity, reduce intestinal pH and exclude potentially pathogenic bacteria, exerting a modulating role in numerous metabolic and immunological activities of the organism. Among the applications of prebiotics in human health, it is worth highlighting the improvement of intestinal function, regulation of appetite and decrease in the postprandial glycaemic response. Laboratory dynamic simulators of the colon microbiome are relevant for microbial ecological studies since they allow long-term experiments needed to evaluate the spatial and temporal adaptation of the colonic microbiota to dietary ingredients without ethical constraints. *In vitro* models are particularly well suited for screening prebiotics or probiotics for special functions in the gut. The capability of *in vitro* fermentation models to closely mimic the gut microbial environment can offer remarkable insights into gut microbiota functions or microbial metabolites associated with a disease state and could be predictive for *in vivo* situations.

**Keywords:** Microbiome; Diet; Gut model; Probiotic; Prebiotic

### Novel approaches for detection of virus and virus like pathogens for indexing and certification



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#### Abstract

Plant viruses and virus like organisms are one of major challenges in Indian agriculture due to their non-cultural nature and difficulty in their management. Their management in vegetative propagated crops is often achieved by indexing and certification. Enzyme linked immunosorbent assay (ELISA) and PCR/RT PCR are two most common methods used for indexing. ELISA is a simplified, cost effective, sensitive method that can be subjected for large scale screening. However, availability of diagnostic reagents such as polyclonal antibodies and conjugates are major constraints for development of ELISA. Expressed recombinant coat protein of several viruses has been utilized as an alternative to purified virus preparation for production of diagnostic reagents. Lateral flow assay has been developed for quick detection of plant viruses but has not been very popular. PCR and variants of PCR based assays including IC-PCR, multiplex PCR and isothermal PCR based assays have been developed for indexing of viruses and virus like organisms. An isothermal sequence independent rolling circle amplification has been used to detect and characterize circular DNA viruses. A novel and rapid isothermal recombinase polymerase amplification (RPA) assay has been developed which can be easily performed in less than 30 min using a very small amount of crude sap extract at a constant low temperature (37°C-42°C) dispensing the use of expensive thermal cycler. RPA is superior to other isothermal amplification techniques such as loop mediated isothermal amplification (LAMP). RPA has been successfully used for detection of few plant DNA/RNA viruses and can be an ideal technique for plant virus indexing on a large scale in resource

constrained laboratory. For parallel detection of multiple infections of viruses and viroids in crops like Grape, DNA microarray chip has been developed. Using DNA chip several new reports of viruses and viroids have been made in India. Recently, next generation sequencing has been used for identification of several viruses in grapevine. These diagnostic approaches have helped Indian agriculture to prepare for production of clean planting material.

**Keywords:** Plant viruses; PCR; LAMP; DNA chips

### **Development of a panel of transcriptionally responsive whole cell bacterial biosensors for applications in metabolic engineering, synthetic biology and antimicrobial screening**

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#### **Abstract**

Whole cell bacterial bio-reporter systems provide easy, cost-effective and field applicable solution to identify and screen array of metabolites and complex natural compounds. These reporter systems are based on innate cellular response circuit composed of transcriptional fusion of gene promoter and non-invasive output signal called reporters. The promoters are activated either by a single target molecule or compounds of similar structural families present in complex environmental samples. The output signals are generated through bioluminescence/fluorescent proteins or enzymes, which generate optical readouts. Bacteria possess various stress signaling systems to cope-up with antimicrobial compounds. To understand the various stress signaling pathway involved, we developed a battery of whole cell biosensors from a combinatorial stress responsive library inspired by  $\sigma^E$ ,  $\sigma^{54}$ ,  $\sigma^{32}$ ,  $\sigma^{24}$  and  $\sigma^S$  promoters. We tested this battery against essential oils, metal nanoparticles, antibacterial peptide capped metal nanoparticles and metabolites central to human metabolic disorder diseases like phenylketonuria and homocysteinemia. Results of our endeavor in this biosensing field will be discussed.

**Keywords:** Biosensors; Whole-cell biosensor; Biological applications; Bacterial signal system

### **Rapid pathogen identification and phenotypic antimicrobial susceptibility testing**



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#### **Abstract**

Unavailability of rapid diagnostics for pathogen identification and antimicrobial-susceptibility-testing has been the significant factor contributing towards unnecessary/inappropriate use of antibiotics resulting into emergence/spread of antimicrobial-resistance (AMR) among pathogens. We are developing a syringe compatible, single-use, affordable and user-friendly combination of device that can capture bacteria from whole-blood/urine samples, enrich, culture, identify and establish their antimicrobial susceptibility profile(s) for multiple drugs simultaneously within 90-minutes. The proposed solution

would help in guided clinical-decision making and minimize empirical use of antibiotics and emergence/spread of AMR. This would ultimately reduce duration and cost of treatment as value proposition to the customers.

**Keywords:** Rapid pathogen capture; Micro culture; Rapid antimicrobial susceptibility testing

### **Novel strategies to combat emerging metal-induced antibiotic resistance and to develop rapid diagnostic tools with special reference to *Salmonella enterica* serovar Typhi**



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#### **Abstract**

The global emergence of multidrug resistant pathogens is a growing threat to antibiotic therapy. Active efflux of the antibiotics by the pathogens is emerging as a challenge for the clinicians. Active efflux of both antibiotics as well as heavy metals by multidrug efflux transporter of pathogenic bacteria has been documented. Heavy metals are widespread in sewage as a consequence of unregulated industrial and anthropological activities. Thus, in natural habitats, bacteria are continuously exposed to different metals, thereby giving rise to survival of the metal tolerant cells due to mutations. *Salmonella* is known to persist for longer period in sludge of sewage treatment plant (STP) where it encounters the heavy metal selective pressure. The pathogen penetrates into the food chain and water through agricultural practices where effluents and minimally treated sewage sludge are used in order to re-circulate nutrients from sludge to arable land, thereafter entering the human host through contaminated food and water.

In view of the reports on co-selection of metal and antibiotic resistance, recently we have reported that increased cadmium accumulation in *Salmonella* Typhi Ty2 leads to increased antibiotic resistance. Such an association has been substantiated using the clinical isolates. On cadmium accumulation, antibiotic(s) sensitive isolates were rendered resistant and the resistant isolates were rendered more resistant as per their minimum inhibitory concentration(s). The results indicated that cadmium, if acquired from the environment, being non-degradable can exert a long-lasting selective pressure on *Salmonella* in the host which may display antibiotic resistance later on, as a result of co-selection. Therefore, appropriate strategies need to be developed to inhibit such an enduring pressure of heavy metals, as these represent one of the factors for the emerging antibiotic resistance in pathogens. In this context, underlying molecular mechanisms associated with both the metal and antibiotic co-selection have been explored and selected chemical and biological inhibitors have been evaluated in order to disarm the cadmium induced antibiotic resistance in *Salmonella enterica* serovar Typhi.

In the diagnostic field, a need to develop fast, reliable and easy to perform sero-diagnostic tests (with higher sensitivity and specificity) is being felt in order to advocate the antibiotic therapy at an early stage so as to reduce the need for frequent hospital visits. In this context, detection of infections using the pathogens themselves or their components as specific biomarkers of disease through application of nanoparticles has become a thrust area in microbial biotechnology which provides the platform for development of various biosensors. Encouraging observations made in these two areas will be discussed.

**Keywords:** Antibiotic resistance; Heavy metal accumulation; Food chain; *Salmonella enterica*



## Arboviruses of livestock importance: precise diagnosis and control

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### Abstract

Arboviruses (arthropod-borne viruses) have been responsible for some of the most explosive epidemics of emerging infectious diseases over the past decade and are a major threat to human and animal health, international trade and food security. Several arboviruses have emerged in new regions of the world lately, like West Nile virus (WNV) in the Americas, Usutu virus (USUV) in Central Europe, or Rift Valley fever virus (RVFV) in the Arabian Peninsula. There have been several reports on Kyasanur Forest diseases (KFD), Dengue (DENV), Japanese Encephalitis (JE), Chandipura virus, West Nile virus (WNV), Chikungunya virus (CHIKV), and various alpha viruses in India. Arboviruses that are zoonotic or primarily livestock pathogens need serious attention. Bluetongue (BT) is one of the important arbovirus of livestock importance, transmitted by *Culicoides* biting midge with more than 26 BTV serotypes recognised till date. The segmented nature of BTV genome can lead to the generation of mixture of viruses through independent reassortment of different genome segments leading to changed phenotypic characteristics of the virus. Recent analysis of European and Indian strains revealed non-random associations between genome segments for reassortant phenomenon.

The early detection either through surveillance or diagnosis of virus will be a critical feature in responding and resolving the emergence of such epidemics in the future. The recent advances in sequencing technologies and molecular assays make feasible to identify any virus in a rapid and high throughput fashion. In addition, reverse genetics and gene synthesis technologies, together with *in silico* analyses allow us to link viral genetic signatures with potentially altered phenotypes such as virulence, antigenicity and host range. Significant scientific input is required to achieve the goal of characterization, early diagnosis, and define optimal control strategies for emerging arboviral diseases.

**Keywords:** Arboviruses; Bluetongue; Livestock; *Culicoides*; Diagnosis; Vaccines; Control

## Nutritional interventions targeting rumen microbiome to improve feed efficiency of livestock



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### Abstract

In ruminants, rumen, can be designated as the powerhouse, as it is the rumen microbiome which bio-converts the fibrous feed taken by the animal into usable nutrients like volatile fatty acids, a major source of energy and microbial protein, a major source of protein for the host animal. The rumen microbiome is highly rich and diversified microbial eco-system including bacteria, protozoa, archaea, fungi and bacteriophages, all working in cohesion with each of them performing specific role in feed fermentation. The rumen microbiome dictates the health status, production and reproduction performance of the animal, therefore to improve the animal performance rumen microbiome is one of the main targets.

Previously, antibiotics were used to modify the rumen microbiome community structure but due to its adverse effects, alternatives were searched. Probiotics (microbial feed additives) are the first to come in picture as a successful alternative and its beneficial effects on health, reproduction and production are well documented. Use of prebiotic, which act as a substrate for the proliferation of the beneficial bacteria in the gut has also been worked out and is very effective during pre-ruminant stage. Plant secondary metabolites, attracted animal nutritionists most, due to their antimicrobial characteristics. PSMs being naturally occurring substances are therefore more acceptable among the livestock owners. PSMs are boom for livestock industry as they have been effective rumen modifiers when used as feed additive. Now days, feed processing for its better utilization is a common practice. The studies have demonstrated that due to processing, the physical and chemical characteristics of the feed changes which in turn modify the rumen microbiome community structure leading to improved feed utilization. But modification of the rumen microbiome is a herculean job and is a great challenge for the nutritionists and rumen microbiologists to bring changes in the rumen microbial community structure through nutritional interventions, as the rumen microbial community is surprisingly resilient when changes are introduced in the feed. As said above, rumen microbiota is to be targeted to make rumen fermentation more efficient and for that in-depth knowledge of rumen microbes is a prerequisite. Advanced nucleic acids based techniques has made possible to have deep understanding of the functioning of rumen microbiome and the interactions among the various populations. The information generated on rumen microbiome will enable us to develop more effective technologies to improve livestock performance.

**Keywords:** Rumen microbes; Prebiotics; Plant secondary metabolites; Animal productivity

### **EchAMP: A broad spectrum antimicrobial protein from monotreme milk**



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#### **Abstract**

Monotremes, oviparous mammals, exhibit prototherian lactation process, indicating them as representatives of early mammals. Milk is the single source of nutrition and protection for the hatchlings which are immunologically naive. Sequencing the cDNA of cells from echidna milk led to identification of novel echidna anti-microbial protein (EchAMP). *In silico* analysis showed putative antimicrobial effects that was further confirmed using *in vitro* assays. Further, we expressed EchAMP in *E. coli* and the purified protein showed broad-spectrum anti-bacterial activity by attacking the bacterial cell membrane. Also, structural studies using NMR spectroscopy revealed purified protein has intrinsically disordered structure and can undergo conformational change resulting in bacterial cell lysis. Furthermore, to investigate the impact of EchAMP against mastitis, EchAMP transgenic mice were generated. Mastitis model created by infecting the mammary gland with four bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*) displayed significant reduction in bacterial load in transgenic mice injected with *S.aureus* and *B. subtilis*. On further confirmation, histomorphological analysis showed absence of necrosis and cell infiltration in the mammary glands of transgenic mice. To understand the role of EchAMP against inflammation, we employed LPS injected mastitis mouse model. In LPS treated EchAMP transgenic mice, phosphorylation levels of inflammation mediators namely - NF- $\kappa$ B, p38 and ERK1/2 levels were significantly downregulated. Taken together, these data suggest that EchAMP has an anti-inflammatory response and it is effective against *S.aureus* and *B. subtilis*. We suggest

that EchAMP may be a potential prophylactic protein against mastitis in dairy animals by expressing this gene in their mammary gland.

**Keywords:** Antimicrobial peptides; Transgenic mice; Mastitis; Dairy animals

### Multidrug resistant bacteria in seafood: A new crisis in seafood safety



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#### Abstract

The presence of antibiotic-resistant bacteria in the coastal environment and seafood is not only a threat to human health, but also can result in the transfer of resistant determinants to other clinically important bacteria leading to wider dissemination of resistance genotypes. The coastal environment in Mumbai, India and the seafood harvested from it harbour enteric pathogens such as *Escherichia coli* and *Salmonella enterica*. The threat due to the presence of enteric pathogens in seafood is more confounding when such bacteria are multi-drug resistant (MDR). The antibiotic resistance patterns of clinical isolates of enteric bacteria is alarmingly high in India, and with the discovery of the New Delhi Metallo- $\beta$ -lactamase (NDM)-producing enteric bacteria, the concerns on the rapid spread of such bacteria *via* food chain and water is increasing. Our study has found widespread occurrence of multiple drug-resistant *Enterobacteriaceae* in wild-caught seafood in Mumbai, the resistance levels of which are comparable with the clinical isolates. Nearly, 78.60% of the seafood isolates of enterobacteria produced multiple extended spectrum- $\beta$ -lactamase (ESBL) enzymes. These were predominantly *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp. and *Erwinia* spp. The  $bla_{NDM}$ -harboring enterobacteria isolated from fish were resistant to all  $\beta$ -lactam antibiotics, quinolones, trimethoprim-sulfamethoxazole, chloramphenicol, and tetracycline, and were susceptible to colistin, polymixin B, fosfomycin, and tigecycline only. These isolates harbored  $bla_{NDM-1}$ ,  $bla_{NDM-2}$  and  $bla_{NDM-5}$  genes. The  $bla_{NDM}$ - harbouring plasmid could be easily transferred to a recipient strain by conjugation. MDR bacteria such as the  $bla_{NDM}$ - harbouring *E.coli* survive in seawater, frozen and chilled fish for prolonged periods of time and transfer the  $bla_{NDM}$  plasmid by conjugation, both in seawater and chilled fish. MDR bacteria are an emerging threat to seafood safety and can potentially hamper the seafood trade. Proper treatment of domestic sewage before releasing into the sea, hygienic handling of seafood on board and in the landing center and control of non-clinical application of antibiotics are necessary steps to prevent contamination of fish and shellfish with MDR bacteria.

**Keywords:** Antibiotic resistance; Seafoods; Enteric bacteria

### Role of microbes in arsenic pollution in Bengal delta & its possible bioremediation in human and animals

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#### Abstract

Arsenic contamination of ground water is the worst geogenic pollution in human history. More than 100 million people are at the risk from groundwater having >50  $\mu\text{g/L}$  arsenic in Bangladesh and India.

Beside West Bengal, arsenic contamination of ground water has been detected in the states of Uttar Pradesh, Bihar, Jharkhand, Assam in India. Soil of Bengal delta is rich in arsenic, which remains bound with sulfur and iron of soil particles, however, dissimilatory arsenic respiring microbes (DARM) and/or iron respiring microbes in deep subsurface soil have been shown to cause reductive release of the soil bound arsenic which freely percolates down the aquifer and contaminates groundwater. Contrastingly, profuse arsenic release has been detected in near-surface sediments, rather than deeper soils, and dissimilatory arsenic respiring bacteria have not been reported from arsenic contaminated Bengal delta raising doubts on the role of DARM in arsenic mobilization in Bengal delta. To identify the bacteria responsible for arsenic mobilization the present study has isolated and characterized arsenic reducing bacteria from water and soil of ponds and canals, deeper pond sediments and soil up to 120 feet depth. The study showed that all depths of sediment and aquifer soil tested had bacteria causing significant arsenic reduction. Arsenic reducing bacterial diversity were different in every studied environment with dominance of *Chryseobacterium*, *Comamonas*, *Acinetobacter* and *Pseudomonas* in superficial pond sediments; *Paraclostridium*, *Clostridium* and *Exiguobacterium* in deeper pond sediments; *Paraclostridium* towards the surface & *Exiguobacterium* at greater depths of soil. Nearly all the strains caused profuse arsenic reduction in culture medium as well as in sediment microcosm. Many of the bacteria carried arsenic resistance gene *ArsC* and only one strain carried arsenic respiring gene *Arr* indicating that arsenic resistance rather than arsenic respiration was the principal mechanism of arsenic release in soil and aquatic sediment.

It is further interesting to note that animals were equally or even more severely exposed to arsenic in the Bengal delta than human, yet clinical manifestations and mortality due to chronic arsenic toxicity are negligible raising suspicion that animals are somewhat resistant to arsenic. To validate this, our animal experiments of arsenic administration *per os* showed much higher amount of arsenic excreted through faeces in ruminants than in monogastric animals. We hypothesized that gut microflora might play some role in this low bioavailability of arsenic in ruminants. Isolation of gut bacteria from ruminants and arsenic exposed yet healthy human subjects in the contaminated area showed that bacterial diversity were grossly different between these two groups, although they all were arsenic resistant. Bacteria from ruminants caused oxidation of arsenic and the oxidized form of arsenic has reduced bioavailability which might explain higher resistance of ruminants to arsenic exposure in this region. Administration of few strains in to monogastric animals enhanced arsenic excretion suggesting possible use of these bacteria in reducing arsenic toxicity in human.

**Keywords:** Arsenic bioremediation; Monogastric; Toxicology; Dissimilation of arsenic

### Emerging and evolving enteric viruses, challenges for animal health



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#### Abstract

A spike increase in the emergence or re-emergence of deadly viral infections has been noted in the recent past that are affecting human and animal health significantly. Several factors are complicating this situation including the growing population density, urbanisation, poor hygiene and sanitation measures, altered climatic conditions with increase in arthropod-borne infections. As of now, with the technological advancements in biomedical knowledge, still we are only able to decipher a little fraction of knowledge on the mammalian viruses accounting for infections in humans and animals. We predict that there are several evolving viruses which are untapped and could infect and adapt to new host

species. There exist restricted information on the emerging novel viruses, their possible reservoirs, and modes of common and uncommon transmission. The major obstacles in assessing the prevention and control modalities worldwide include capricious nature of the emerging infections, limited epidemics, lesser numbers of confirmed cases, concurrent and inapparent occurrence, and disease occurrence in resource-poor locales. The connection of wildlife and livestock is very important, and information on the maintenance of diseases between them is imperious for understanding the disease ecology. Thus, this is one specific area which needs special attention with decoding of information on epidemiology of novel infections in places where humans, livestock, and wildlife interactions are more. The information would help us in understanding new epidemics and capacity building for adopting adequate control measures. The research in recent years has established that animals (farm, wild) act as the most common reservoirs of several human viruses. This complicated "host-pathogen-environment" association is the crucial point in understanding the novel pathogenic viruses. An estimate shows that "Of the known viruses that infect humans, about 80% perpetuate naturally in non-human "reservoirs," largely farm mammals and poultry and, to a lesser extent, in wild animals and arthropods". The zoonotic infections constitute nearly 60% of the known human pathogens and 75% of the emerging pathogens. Of the note, still our knowledge on such zoonotic infections is limited including their evolution and genetic diversity. In this presentation my focus is to discuss the impact of enteric viral infections in animals, various evolving viruses and their divergence possessing challenges in combating the growing number of infectious diarrhoea cases.

Infectious diarrhoea is a widespread problem and associated with colossal losses worldwide. Its impact is much higher among < 5 years children and is reported to cause ~800,000 (~10.5%) of global deaths in this age group. As per the estimates, approximately two-thirds of deaths documented from South Asia and sub-Saharan Africa. Amongst various causes of infectious diarrhoea, the viral aetiology is of much more significance. Since the first report on rotavirus being the cause of acute gastroenteritis during 1972 by Ruth Bishop, list is being expanded with the discovery of several other viruses (picobirnavirus, astrovirus, calicivirus, kobuvirus, Bocavirus, sapelovirus, teschovirus, coronavirus, etc.) which can easily traverse through human and animal population with inferior immune status by virtue of malnutrition and pose serious challenge to mankind. High mutation rates in some viruses like picobirnavirus and rotavirus enable enteric viruses to overpower the out-dated preventive measures and emerge as a new type with entirely new genetic makeup. Researchers are also highlighting extra-intestinal detection of enteric viruses for example; rotaviruses causing encephalitis, astrovirus associated with meningitis and disseminated infections etc. Moreover, to stop airborne spread of enteric virus such as Noro- and Rotavirus is another new type of challenge that is being experienced in developed countries and is closely related to urban lifestyle. To better understand and tackle such existing and emerging enteric viruses there is an urgent need to acquire more scientific knowledge of these pathogens including their reservoir hosts, immunopathobiology, genetic diversity and the development of state-of-art diagnostic tools. As animal hosts are known to play important role in the maintenance and possible transmissibility of enteric viruses to humans, there is always a need felt to assess enteric infections in animals.

Worldwide, rotavirus (RV) is considered as the main cause of diarrhoea affecting young animals of different host species. Classification of rotaviruses into ten distinct serologic groups designated A to J is based on the capsid VP6 protein. Group A rotaviruses (RVA) are most often responsible for diarrhoea in animals and humans. Due to the difficulty in diagnosis of diarrhoea caused by other rotavirus groups, non-group A rotaviruses are rarely reported. Since the discovery of first group A rotaviruses in the diarrheic faeces in the 1970s, several serologically distinct non-group A rotaviruses, such as RVB, RVC, RVE and RVH, have been encountered around the world. Still, little attention has been paid on the circulation of rotaviruses of different groups in India. In contrast, prevalence studies in many countries around India reported a relatively frequent occurrence of RVs infections. At this moment it remains unclear to which extent RVA, RVB, RVC, RVE and RVH are circulating in the Indian animal population. Recently, Picobirnavirus (PBV) has emerged as a potent cause of neonatal diarrhoea. It is the single genus identified under newly identified family *Picobirnaviridae* and carries a dsRNA genome. Based on RNA polymerase gene, PBVs have been further classified into genogroups. The rearing of pigs in the organised farms or as backyard farming in the close vicinity to the human population also possesses a possible

chance of viral zoonosis. According to earlier reports pigs are considered to be the potent carriers of PBV infection. We plotted a comprehensive picture of the PBV strains circulating in the porcine population of different North Eastern States of India. Furthermore, an in-depth assessment of the impact of these viral infections on animal health and production becomes indispensable and thus necessitates the development of precise diagnostics for appraising the diagnostic algorithms of enteric pathological conditions. During the presentation, some important differentials and the impact of enteric infections on animal health will be presented.

**Keywords:** Enteric viruses; Picobirnavirus; Prevalence; Animals

# **AGRICULTURAL AND MICROBIAL TECHNOLOGY**





# ORAL PRESENTATION



**ACC deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in French bean (*Phaseolus vulgaris*) plants**

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**Abstract**

Plant growth promoting rhizobacteria (PGPR) with 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity has potential to promote plant growth and development under adverse environmental conditions. In the present study, rhizobacterial strains were isolated from Garlic (*Allium sativum*) rhizosphere and were screened *in vitro* ACC deaminase activity in DF salt minimal media supplemented with 3 mM ACC. Out of six isolates, two could degrade ACC into  $\alpha$ -keto butyrate, exhibiting ACC deaminase activity more than  $\sim 1500$  nmol  $\alpha$ -ketobutyrate mg protein<sup>-1</sup> h<sup>-1</sup> and assessed for other plant growth promoting (PGP) functions including Indole acetic acid production (greater than  $\sim 30$   $\mu$ g/ml), siderophore, Ammonia, Hydrogen cyanide production and inorganic Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> ( $\sim 85$ mg/L) and ZnSO<sub>4</sub> solubilization. The priming of seed with ACC02 and ACC06 revealed that treatment of plants with bacterial isolates in the form of consortia significantly declined ( $\sim 60\%$ ) stress stimulated ethylene levels and its associated growth inhibition by virtue of their ACC deaminase activity. The consortia treatment alleviated the negative effects of salinity stress and increased root length (110%), root fresh weight ( $\sim 45\%$ ), shoot length (60%), shoot fresh weight (255%), root biomass (220%) shoot biomass (425%) and total chlorophyll content ( $\sim 57\%$ ) of French bean seedlings subjected to salinity stress.

**Keywords:** ACC deaminase; PGPR; Salinity stress; Siderophore; Indole acetic acid

**Multifarious role of bacterial mediated novel volatile organic compounds**

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**Abstract**

Rhizosphere microorganisms have been reported for their beneficial effect on plant growth and yield by production of plant growth hormones, antibiotics and many other secondary metabolites. These help to control pathogens and infectious organisms for plants and animals. Such microbes also produce many volatile compounds and they play an important role for long distance signaling in microbe- microbe and microbe-plant interaction. There is a lack of knowledge about the functional role of many of these volatile organic compounds. In this study, we report the role of biogenic volatile organic compounds to kill plant pathogenic fungi and bacteria, plant growth promotion and root associated microbial communities. The novel volatile organic compounds from rhizosphere microbes through gas mass spectrometry. These compounds were characterized for their role in killing plant pathogenic fungi, enhancement of *Arabidopsis*

*thaliana* and *Brachypodium distachyon* growth and their influence on rhizosphere and soil microbial communities. They are nontoxic to plant, human and animals and can be used as fungicides instead of chemical pesticides. The identified compounds are volatile in nature and disperse uniformly in the air so they can be used for soil fumigation and in large food storage units to control post-harvest fungal growth and spoilage. These microbes/compounds also enhance plant growth and induce systemic resistance making them attractive alternatives for agrochemicals.

**Keywords:** Volatile organic compounds; Rhizosphere communities; Phytopathogens

OP/AMT-37

**Success Story of Rice-Specific Endophytic Diazotrophic *Azotobacter Chroococcum* Avi2  
Formulation: A Substantial Substitute of Nitrogenous Fertilizer in Rice**

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**Abstract**

Nitrogen (N) is deprived in most of the world's soil which would directly hamper the sustainability of crop production. More than 50% of exogenous applied N-fertilizer is lost in rice field, causes negative impacts on soil environment thereby affecting its yield and nitrogen use efficiency. Nitrogen fixing microbes are one of the possible alternatives of chemical N; however potentiality of these microbes is really a question at field level mainly due to environmental constraints. Endophytes are less prone to environmental factors, therefore, *Azotobacter chroococcum* Avi2, a native endophytic diazotrophic has been explored as a potential diazotrophs for rice crop. Avi2 was isolated from Swarna root and its endophytic nature has been proved through FRET-based technique. Further, *in vitro* and *in vivo* analyses of Avi2 were done in rice to prove its diazotrophic efficacy. Finally, a liquid formulation of Avi2, having shelf life of more than one year was developed. Continuous evaluation of Avi2 in 30 different locations of rice field in Odisha, revealed that it can save - 25% of chemical-N without compromising its yield. Chlorophyll imaging study revealed the efficacy of Avi2 in terms of quantum yield of PSII. Molecular data also supported the result as higher abundance of *nifH* gene was found in Avi2-treatment compared to the recommended N (RDN). The data from *Flagship Experiment* on "Nitrogen Use Efficiency in rice" conducted at NRRI, Cuttack for last two seasons also showed *at par* yield in Swarna and CRDhan310 in Avi2-treatment compared to RDN. Overall, the present study showed the potentiality of Avi2 to save around 25% of chemical-N fertilizers without compromising the rice yield. Besides subsidy given by Indian Government, input cost of nitrogenous fertilizers for rice production is too high which can bring down drastically through intervention of Avi2 and will help to enhance the farmers' income.

**Keywords:** Endophytic *Azotobacter chroococcum* Avi2; Liquid formulation; Chlorophyll imaging; q-PCR;

OP/AMT-73

***Azospirillum* in rainfed agriculture: benefits beyond  
nitrogen fixation**

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### Abstract

Diazotrophic bacteria under genus *Azospirillum* are known to form association with cereals and grasses. Besides nitrogen fixation, *Azospirillum* can exhibit other plant growth promoting traits like phytohormone production, nutrient acquisition, exopolysaccharide production, biofilm formation etc. These properties along with its cyst forming nature make *Azospirillum* a suitable candidate for application under rainfed production systems that witness intermittent dry spells during the cropping season. Indole acetic acid production by this bacteria helps in extensive root hairs and lateral root formation that can help the plant in acquiring nutrients and water from the lateral root zone. Besides, exopolysaccharide production and biofilm formation by these bacteria on the root surface and in the rhizosphere soil can prolong plant survival under abiotic stress conditions. The present study demonstrates the potential of these bacteria in promoting the growth of pearl millet, one of the major cereal crops of semiarid tropics in India that faces nutritional as well as abiotic stresses mainly drought under rainfed conditions. Fifty strains of *Azospirillum* isolated from rhizosphere soil of rainfed pearl millet collected from eight locations across five states of India could exhibit nitrogenase activity (upto 200nmoles hour<sup>-1</sup>), IAA production (upto 28µg/ml), siderophore production and mannitol tolerance (800mM) *in vitro*. Seed germination in the presence of 10%, 20% PEG6000 revealed positive effect of bacterial inoculation on lateral roots and root hair formation in pearl millet. Microscopic observations revealed bacterial colonization on the root surface as well as in the root hairs. The selected strains identified as *A. formosense* evaluated under rainfed field conditions could improve root biomass, plant physiological status and grain yield (upto 27%). The selected strains are being characterized for biofilm formation and exopolysaccharide production. Multilocation trials with the selected strains may lead to a product for boosting productivity in rainfed crops.

**Keywords:** Drought; Exopolysaccharide; Biofilm; Pearlmillet

OP/AMT-90

### Accelerating composting process by modification in physical, chemical and biological environment during composting of paddy straw

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### Abstract

The north-western states of India constitutes very productive rice-wheat region in the Indo-gangetic plains. However, the management of huge amount of paddy straw left behind after harvesting is of major concern. Being a cost effective method farmer prefers to burn paddy straw in the field but several environmental implications are associated with this practice. To address these challenges, an experiment was conducted by modification in physical, chemical and biological environment during *ex-situ* composting at three different time intervals (mid january, first week of february and mid-february). The modification in physical environment was done using plastic sheet to cover windrows, chemical by supplementing the windrows with jaggery (1%), FYM and urea (1&2%) and biological environment by using microbial consortium. The experiment was conducted for two months and samplings were done at 0, 7, 15, 30, 45 and 60 days for analysis of moisture content, temperature, microbial diversity, pH, EC, N, P, K, micronutrients (Mn, Zn, Cu and Fe) content and phytotoxicity. The application of plastic sheet over the windrows did not affected composting process however, urea, jaggery and microbial consortium accelerated the composting process. Among treatments the best treatment was FYM+straw+urea(2%)+microbial consortium+Jaggery was found to be most suitable *ex-situ* management practice for rapid composting and improving microbial activity and nutrient content of compost.

**Keywords:** Composting; Microbial consortium; Microbial diversity; Nutrient content; Paddy straw

## Microbial production of biosurfactants: potential applications and challenges

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### Abstract

The term 'surfactant' represents a class of molecules which are decisive towards the operation of both cleaning along with formulated products and also represent a monetary value of \$32-36 billion yearly market. A major proportion of surfactants currently dominating the market finds its origin from either oil or from natural products via chemical reactions. Biosurfactants are their biological analogues which are produced by different microorganisms and possess several advantages as compared to chemical surfactants. The application of biosurfactants in enhanced oil recovery, bioremediation of agrochemicals and petroleum hydrocarbons, agriculture, food production, chemistry along with cosmetics and pharmaceuticals is on an increase, every year. These are deliberated as secondary metabolites which possibly play indispensable roles meant for the survival of producer by aiding transport of nutrients or by assisting microbe-host interactions or by acting as biocide agents. The other roles of biosurfactants include amassing the surface area and bioavailability of hydrophobic water-insoluble substrates, heavy metal binding, bacterial pathogenesis, quorum sensing and biofilm formation. They are also acknowledged for green synthesis of nanoparticles. The synthesis of nanoparticles using biosurfactant is an example of extracellular synthesis which offers the advantage of ease of access to the substrate, unlike the intracellular synthesis where an additional processing step for the release of nanoparticles is required. The possession of several traits outspreads their application in several fields but the altitudinous cost of biosurfactant production usually restricts their usage. Sophorolipid, for instance, the most economic and broadly obtainable microbial biosurfactant, has newly been published at \$ 34/kg active matter. Therefore, the choice of industrial wastes as substrates for biosurfactant production along with the optimization of process parameters can contribute a lot in lowering the economy of biosurfactant production process using high yielding microbial strains.

**Keywords:** Biosurfactant; Sophrolpids; Bioremediation; Agrochemicals

RF/AMT-67

## Bio efficacy of native *Trichoderma* isolates against FOC causing wilt in chickpea in Bundelkhand

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### Abstract

Present agriculture practices mainly relies on agrochemicals which having adverse effect on an environment, soil health as well as on human health. Chickpeas is a major pulse crop, widely infected by wilt disease (*Fusarium oxysporium* f. *spiciferi*). The pathogen survives in soil so management of the wilt disease is not viable through chemicals, so the best alternative is use of biocontrol agents. Biocontrol agents are cheapest, effective and eco-friendly way for the management of the disease. In present study Infected and healthy

plants of chickpea as well as soil samples were collected from Datia and Jhansi districts of Bundelkhand (using GPS), were subjected to isolation i.e., wilt pathogen FOC and native *Trichoderma*. Five isolates of *Trichoderma* were evaluated against twenty five isolates of FOC, by following dual culture method technique. Among five isolates H1T5 showed maximum inhibition of mycelial growth FOC (76.67%) against H1FOC5 isolates which was followed by R2T10 (75.56%) against R2FOC10, R2T8 (73.33%) against R2FOC8, I1T4 (72.22%) against I1FOC4 and R2T6 (71.11) against R2FOC6 respectively. Present study shows the use of native *Trichoderma* sp. for eco-friendly management of chickpea wilt disease.

**Key words:** *Trichoderma* sp; chickpea wilt; Dual culture technique; Biocontrol agent

RF/AMT-99

### Spatial distribution and identification of bacteria in stressed environments capable to weather potassium aluminosilicate mineral

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#### Abstract

In present study, we studied the distribution of silicate mineral weathering bacteria (SWB) in stressed environments that release potassium from insoluble source of mineral. Out of 973 isolates, 341 isolates were positive and mineral weathering potential ranged from 5.55% to 180.05%. Maximum abundance of SWB occurred 44.57% in saline environment followed by 23.46% in low temperature, 12.61% in moisture deficit, 12.32% in high temperature and 7.04% in acidic environment. Among all the sites, the KSB were found to be predominant in Sambhar salt lake and accounted for 16.13% of the total isolates having potential to weather silicate mineral. It was followed by Rohtang Pass (15.84%) and Jaisalmer (12.61%). The sites that showed less percentage of KSB were Vashisht & Bakreshwar each (1.47%) and Balrampur (0.88%). Among isolates, silicate mineral weathering efficiency ranged from 1.9 to 72.8  $\mu\text{g mL}^{-1}$  available K in liquid medium. Based on restriction digestion pattern of 16S rRNA gene using *AluI*, *MspI* and *HaeIII* enzymes, the number of clusters obtained varied among ecological sites and were 30 for Sambhar lake, 21 for Bhitarkanika; 19 for Rhotang Pass; 18 for Runn of Kutch, 14 for Jaisalmer; 13 for Sunderban; 12 for Leh; 7 for Chummathang, 6 for Manikaran and Kollam each; 5 for Vashisht and Bakreshwar each; and 2 for Balrampur. The phylogenetic tree of SWB (158 representative of each cluster) discriminated in three clusters viz. Firmicutes (75.95%), Proteobacteria (20.89%), and Actinobacteria (3.16%). This is the first report on SWB in stressed environments and identified 28 genera and 65 species which is not reported earlier. Among them *Bacillus* was the predominant genera (58.23%) distantly followed by *Pseudomonas* (6.96%), *Staphylococcus* (5.06%) and *Paenibacillus* (4.43%). These bacterial strains could be developed as inoculants for biological replenishment of K in stressed soils.

**Keywords:** Silicate mineral weathering bacteria (SWB); Weathering potential; Phylogenetic tree; Stressed environments; Distribution





# POSTER PRESENTATION



## Screening of Medicinal Plants for Biofabricating Zinc Oxide Nanoparticles

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### Abstract

Medicinal plants were used as major natural source from ancient time for the treatment of human diseases and drug formulations. Fabrication of nanoparticles can be done by various routes in which phyto-assisted approach has been considered as one of the most promising and innovative technology also called as green nanotechnology because it is an eco-friendly, cost-effective, easily scaled up technology as compared to physical and chemical synthesis. Zinc Oxide (ZnO) nanoparticles have broad spectrum applications such as dye degradation and treatment of industrial effluent etc. In the present study leaf extracts of different medicinal plants i.e. *Aegle marmelos*, *Terminalia belerica* has been screened for the synthesis of ZnO nanoparticles. To synthesize ZnO nanoparticles 50ml of 0.01M concentration of Zinc acetate dehydrate has been mixed with 25ml of crude aqueous leaf extracts. pH 12 of the reaction mixture has been adjusted using 20 ml NaOH (2M) and the mixture has been stirred for 3 hours. Formation of white coloured precipitate suggests the completion of reaction which was separated by centrifugation. Results suggests that out of these medicinal plants *Terminalia belerica* has been proved most significant in the synthesis of ZnO nanoparticles as large amount of ZnO nanoparticles precipitate has been observed and the size of the nanoparticles was found 68nm analysed by particle size analyser, while in case of *Aegle marmelos* leaf extracts very small amount of precipitate formation of ZnO nanoparticles has been observed and the size was found to be in the range 200-500 nm. Hence, the present work has been undertaken to screen the medicinal plants to fabricate economic and eco-friendly ZnO nanoparticles.

**Keywords:** Medicinal Plants; *Terminalia belerica*; *Aegle marmelos*; ZnO Nanoparticles; Crude Leaf Extract

## Characterization and isolation of *Pseudomonas fluorescens* from Rhizospheric Soil Samples

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### Abstract

Production of the crop is affected by deficiency of fertilizers and low number of plant growth promoting rhizobacteria in soil. At the present study, *Pseudomonas fluorescens* isolates possess a variety of promising properties which make it a good plant growth promoting traits. A total of twenty five *Pseudomonas fluorescens* isolates were obtained and characterized on the basis of their colony morphology, microscopy and biochemical test. Isolates growing *P. fluorescens* isolates on the king's B medium at 25°C to 30°C for 48 hours. The plates were exposed to UV light for 30 seconds and the isolate with pigment were exhibiting the fluorescence. All the isolates showed creamy translucent, mucoid, and circular shape colony morphology. Colonies having *Pseudomonas* like morphology were microscopically analyzed and those depicting rod shaped, gram negative bacteria were selected. All *Pseudomonas* isolates were further characterized by different biochemical test. It showed positive results in all the biochemical tests (Citrate utilization test, Methyl red test, Catalase test, Oxidase test, Nitrate reduction test, Urease test). Further, antibiotic sensitivity profiling of these isolates was done all the isolates were found resistant to

Amoxyclav and Erythromycin and all were inhibited by the Ciprofloxacin by forming a clear zone of 15mm. These *Pseudomonas* isolates were tested for physiological efficiency on different pH (6, 7, and 8). All isolates grew well on alkaline medium of pH value 8. *P. fluorescens*10 for plant growth promoting activities in green house showed a positive result. These isolates can be used as potential biofertilizers and plant growth promoter.

**Keywords:** Growth Promoter; Isolation; Characterization; *P. fluorescens*, Biofertilizer

AMT-03

### Microbial intervention to accelerate the in situ residues degradation and its effect on the soil biological properties under rice-wheat cropping system

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#### Abstract

The rice-wheat cropping sequence is the major cropping system of India, which generates large quantities of crop residues. The disposal of paddy straw becomes a major concern in the rice belt of India. A large quantum of the paddy straw is burnt on farm to clear the field for succeeding wheat crop. Incorporation of the residues in soil is not feasible because of insufficient time between harvesting of the rice and sowing of wheat as well as immobilization of the nitrogen because of wide C: N ratio of straw, which causes a reduction in the subsequent crop yield. Microbial interventions to accelerate the degradation may be a viable option for the effective management of farm residues. In this study, a field experiment including five treatments: T1: Straw removed, T2: Straw retained @ 3tha<sup>-1</sup>, T3: Straw retained + fungal consortium @ 3kg ha<sup>-1</sup> + Urea @ 30 kg ha<sup>-1</sup>, T4: Straw retained + Urea @ 30 kg ha<sup>-1</sup>, T5: Straw retained + fungal consortium @ 3 kg ha<sup>-1</sup> was undertaken at the farm of Indian Agricultural Research Institute, New Delhi during 2018-19. Application of fungal consortium (*Coprinopsis cinerea* LA2 and *Cyathus sterocoreus* ITCC 3745) along with straw incorporation (T3) resulted in a significant increase in the population of bacteria, fungi, actinobacteria, and cellulose degraders in comparisons with other treatments. Higher activities of dehydrogenase, carboxymethyl cellulase, xylanase, FDA hydrolase and alkaline phosphatase enzymes were recorded in the soil of fungal consortium treated plot. Phospholipid fatty acid biomass was also found significantly higher in T3 treatment as compared to other treatments. Microbial intervention in residues managements increased the CO<sub>2</sub> emission two to three fold within 15 days of straw incorporation. There was reduction in CO<sub>2</sub> emission at the 30 days which confirm completion of degradation within a month, as well as indicates the role of inoculation in hastening the degradation process. This study validates the hypothesis that microbial intervention can be a suitable option for *in situ* management of paddy straw.

**Keywords:** *Coprinopsis cinerea*; *Cyathus sterocoreus*; Carboxymethyl Cellulase; Xylanase

AMT-05

### To promote the growth of pulses in arid regions by using microorganisms

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### Abstract

A larger part of the world is arid, yet millions of people live in such regions. To meet the food demands sustainable agriculture is an effective way. We can improve local agriculture in arid zones by using sustainable agriculture. Pulses can utilize limited soil moisture and nutrients than cereal crops. They provide essential nutrients and can withstand adverse conditions up to a level. For this reason, they can be grown in the arid region. But things like abiotic stresses and nutrient deficiency can still hinder their growth. So, inoculating them with PGPR microbes can enhance their growth. Plant growth promoting hormones have a momentous influence on soil physiological and structural properties. They can be very effective and are potential microbes for enriching soil fertility and boost the agriculture yield. PGPR help to replace chemical fertilizer for sustainable agriculture by producing growth promoting substances. Among the PGPR group azotobacter are omnipresent, free-living, aerobic bacteria. The colonies formed by azotobacter on Ashby's media were identified as *Azotobacter chroococcum* through certain biochemical test which was later inoculated with seedlings of pulse plants and was given arid region conditions. Two groups of pots were established where the first remained without inoculation and the second was inoculated with *A.chroococcum*. In research *A.chroococcum* as inoculants on the pulse has strikingly enhanced the growth in arid region. They are free-living, nitrogen fixer diazotroph, azotobacteria genus that can synthesize phytohormones, provide all the necessary primary and secondary metabolites and can significantly increase the length, weight of root and shoot and stimulates rhizospheric microbes, protects the plants from phyto-pathogens, improves nutrient uptake and ultimately boosts up biological nitrogen fixation also tends to improve soil fertility. Thus, they helped the pulse to grow effortlessly in the arid region by providing the necessary nutrients and protecting pulse crops from abiotic stresses.

**Keywords:** Sustainable agriculture; Pulses; Arid, Semi-arid region; PGPR; *Azotobacter chroococcum*; Abiotic stresses

AMT-06

### Study of Plant Growth Promoting (PGP) Activities of Endophytic Bacteria Associated with Ethnomedicinal Plants of Manipur

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### Abstract

As Manipur falls in the Indo- Burma Biodiversity Hotspot, there is a great potential for exploring microbial biodiversity in the unique and underexplored biotopes and there is an urgent need for survey of indigenous strains suited to local conditions for sustainable production of agricultural crops especially rice. Endophytic microorganisms are able to promote plant growth through various mechanisms, such as production of plant hormones and antimicrobial substances, as well as to provide the soil with nutrients, for instance, inorganic phosphate. In this study, a total of 94 endophytic bacteria were isolated from 8 ethnomedicinal plants of Manipur in our lab namely *Celtis timorensis*, *Plectranthus ternifolius*, *Phlogocanthus jenkensii*, *Goniothalamus sesqeupedalis*, *Plantago asiatica*, *Oroxylum indicum*, *Eclipta alba* and *Tinospora cordifolia*. 76 putative endophytic isolates were screened for plant growth promoting (PGP) activities such as IAA production, Siderophore production, Ammonia production and Phosphate solubilisation. Three strains showed positive results in all the PGP traits tested. Some isolates could produce significant amounts of IAA and also solubilize significant amounts of inorganic P with concomitant decrease in the pH of the medium. The presence of bacteria inside the plant confers many advantages to host plants such as the production of phytohormones and siderophores. The metabolites produced by endophytic bacteria may enhance the fitness and growth of the host and indirectly affect the harmful microbial population. Further

optimization studies of these bioactive strains by changing different cultural conditions will be explored in future that may lead to biotechnological exploitation of these candidate endophytic bacteria.

**Keywords:** Endophytic microorganisms; *Celtis timorensis*; *Plectranthus ternifolius*; *Phlogocanthus jenkinsii*; *Goniothalamus sesquipedalis*; *Plantago asiatica*; *Oroxylum indicum*; *Eclipta alba*; *Tinospora cordifolia*; Siderophore production.

AMT-07

### Optimization of IAA production with physicochemical parameters by Endophytic bacteria

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#### Abstract

Endophytes are known to produce a class of phytohormones known as auxins. These auxins directly affect plant growth and development. Indole-3-acetic acid is the most commonly produced auxin by endophytic bacteria. It influences the growth of plant by having its impact on root initiation, cell division and cell differentiation so its production by endophytic bacteria is very important. It has been reported that there is tryptophan dependent as well as tryptophan independent pathways for IAA biosynthesis. In the present study endophytic bacteria CPHN 2 isolated from nodules of Chickpea (*Cicer arietinum*) plants grown in the fields of Hisar district, Haryana (India). It possessed multiple plant growth-promoting traits and produced significant amount of IAA. IAA production by CPHN 2 was optimized using various physicochemical parameters. IAA production was observed only in a media supplemented with tryptophan revealing that CPHN 2 uses tryptophan dependent pathway for IAA production. Various physicochemical parameters for IAA production were optimized. CPHN2 produced 945.81 µg /ml with mannitol as carbon source, yeast extract as nitrogen source, 600 µg/ml tryptophan concentration at pH 9, 30°C temperature with agitation speed of 150 rpm after 72 hours. An increase of around six folds in IAA production was obtained at optimized conditions. IAA was partially purified and confirmed with thin layer chromatography.

**Keywords:** Indole-3-Acetic Acid; Optimization; TLC; Physicochemical; *Cicer arietinum*

AMT-08

### Antimicrobial and Plant Growth Promoting Potential of Endophytic Bacteria Associated with Medicinal Plants of Manipur

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#### Abstract

Of 167 endophytic bacteria obtained from 9 ethno botanically important medicinal plants of Manipur, 66 showed antibacterial activity against one or more bacterial test pathogens (*Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and 48 showed antifungal activity against one or

more rice fungal pathogens (*Curvularia oryzae*, *Rhizoctonia solani*, *Aspergillus niger*, *Bipolaris oryzae* and *Fusarium oxysporium*). Of 11 endophytic bacteria screened for anti-mycobacterial activity, 5 showed anti-mycobacterial activity against *Mycobacterium smegmatis*. Of 5 endophytic isolates screened by MTT assay against attenuated *Mycobacterium bovis* strain and pathogenic strain of *Mycobacterium tuberculosis* (H37Rv), 4 isolates showed IC<sub>50</sub> value less than 100 µg/ml. 29 endophytic isolates were found to show good Plant Growth Promoting activities. 14 enhanced seedling growth and showed higher vigour indices over the control. Four isolates (AcRz21, PtL11, TgIB5 and TgIB12) exhibiting highest vigour indices were further assayed for rice plant growth promotion under *R. solani* challenged conditions. Isolates AcRz21, PtL11 and TgIB5

Significantly increased the growth of rice plants and decreased the disease lesions cause by *R. solani*.

**Keywords:** Medicinal plants, Endophytic bacteria, Bacterial test pathogens, Rice fungal pathogens, Anti-mycobacterial activity

AMT-09

### *In vitro* antimicrobial screening and plant growth promoting activity of endophytic bacteria from *Acorus calamus*

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#### Abstract

38 endophytic bacteria (21 from the rhizome, 13 from leaves and 4 from roots) were obtained from *Acorus calamus* (Local Name- O-hidak), an ethnomedicinal plant of Manipur. These 38 endophytic isolates were screened for antimicrobial (antibacterial and antifungal) activities. 8 of the isolates showed antibacterial activities against one or more bacterial test pathogens (*Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and 14 of the isolates showed antifungal activities against one or more rice fungal pathogens (*Curvularia oryzae*, *Rhizoctonia solani*, *Aspergillus niger*, *Bipolaris oryzae* and *Fusarium oxysporium*). The isolates were further screened for plant growth promoting activities (production of IAA, siderophore, ammonia, ACC deaminase, inorganic phosphate solubilization, etc.). 6 endophytic isolates were found to show good Plant Growth Promoting activities. 2 endophytic isolates (AcRz18, AcRz21) could enhance rice seedling growth and showed higher vigor indices over the control.

**Keywords:** Endophytic bacteria; *Acorus calamus*; Plant growth promoting activities; Seedling growth, Vigor indices

AMT-10

### Isolation, antimicrobial screening and plant growth promoting potential of endophytic bacteria from *Solanum xanthocarpum*

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### Abstract

47 endophytic bacteria were isolated from ethnobotanically important medicinal plant of Manipur. Out of the 47 endophytic bacterial isolates obtained, 11 isolates showed antibacterial activity against one or more bacterial test pathogens (*Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli* and *Pseudomonas aeruginosa*), 12 isolates showed antifungal activity against one or more rice fungal pathogens (*Curvularia oryzae*, *Rhizoctonia solani*, *Aspergillus niger*, *Bipolaris oryzae* and *Fusarium oxysporium*). 9 most promising endophytic isolates were further screened for anti-mycobacterial activity, of which 3 isolates showed activity against *Mycobacterium smegmatis*. 7 endophytic isolates showed good plant growth promoting activities. 2 enhanced seedling growth and showed higher vigor indices over the control.

**Keywords:** Endophytic bacteria; Bacterial test pathogens; Rice fungal pathogens; Anti-mycobacterial; Plant growth promoting activity

AMT-11

### Characterization of Endophytic Bacteria from Ethnomedicinal Plant *Perilla frutescens* Britton for Antimicrobial and Plant Growth Promoting Properties

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### Abstract

The applications of bacteria in biotechnology are increasing with the needs of an increased population. Microbes are major source of bioactive molecules with antimicrobial activities, and have the ability to promote plant growth. As the terrestrial sources are getting overexploited, considering endophytic microorganisms inhabiting plants especially medicinal plants is exemplary. Therefore, 43 endophytic bacterial isolates from an ethnomedicinal plant *Perilla frutescens* Britton. Were screened for antimicrobial and plant growth-promoting (PGP) properties. All the endophytic isolates were tested for antibacterial activity against 3 test organisms *Micrococcus luteus* (MTCC 106), *Bacillus subtilis* (MTCC 121), and *Escherichia coli* (MTCC 739), where antifungal activity were tested against 4 major fungal pathogens *Rhizoctonia solani* (MTCC 4633), *Pyricularia oryzae* (MTCC 1477), *Fusarium oxysporum* (MTCC 1755) and *Helminthosporium oryzae* (MTCC 3717). Among the 28 endophytic isolates 4 isolates viz. KSR7, KSM2, KRG2, and KRG4 showed potent antibacterial activity, while 2 isolates KRR9 and KSG5 showed potent antifungal activity. 3 out of the 28 isolated endophytes produced indole acetic acid (IAA), and in addition to siderophore production, they showed potential for phosphate solubilization. All potent endophytic isolates were subjected to biochemical characterization and could be further studied for use as bio inoculants for sustainable crop production.

**Keywords:** Antibacterial Activity; Antifungal Activity; Endophytic Bacteria; Ethno Medicinal Plant; Plant Growth Promoting Property

AMT-12

### Isolation and antimicrobial activities of bacteria from *Chakhao* rice plant (black scented rice varieties of Manipur) rhizospheres

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### Abstract

Black rice is the latest “Superfood”, containing high amount of antioxidants, dietary fiber, vitamins and minerals. Bacteria that can grow in the rhizosphere are ideal for use as biocontrol agents as they provide the front line defense for protection against plant pathogens. In this study, 95 rhizospheric bacteria were isolated from different varieties of Chakahao rice plants viz. *Chakhao Poreiton Rice* (CPR), *Chakhao Angoubi Rice* (CAR) *Chakhao Amubi rice* (CAmR) and *Chakhao Sempak rice*(CSR) rhizospheres. All these isolates were tested for antifungal bioassay against two major fungal pathogens, *Rhizoctonia solani* (RS) and *Bipolaris oryzae* (BO). Among these isolates, 5 rhizospheric bacteria viz. ChSl01, ChA27, ChA31, ChAmRz28 and ChSpRz42 showed antifungal activities against both the pathogens. Further antibacterial activity test were performed against 3 test organisms MTCC106- *Micrococcus luteus*, MTCC121- *Bacillus subtilis*, MTCC739- *Escherichia coli*. 2 isolates, ChA 28 and ChA 47 from *Chakhao Angoubi Rice* (CAR) exhibit antibacterial activity against all test organisms in primary screening. But in secondary screening only ChA 47 has shown potential antibacterial activity against all test organism having highest clearing zone against MTCC106 (18 mm). Biochemical characterization of all the potent isolates having antimicrobial activities was performed. These potent isolates were selected for further studies; pot trial and limited field trial against pathogen challenged condition and could be used as good biocontrol agents for rice plant diseases caused by fungal and bacterial pathogens.

**Keywords:** *Bipolaris oryzae*; Black rice; Biochemical Characterization; Rhizospheric bacteria; *Rhizoctonia solani*;

AMT-13

### Plant Growth Promoting and Biocontrol Activity of Endophytic bacterial Isolates from *Drum Phou* and *Chakhao Poreiton Rice* Plants of Manipur

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### Abstract

Endophytes living asymptotically within plant tissues have been found in almost all plants. They play major role in physiological activities of host plants influencing enhancement of stress, insect, nematode and disease resistance besides these endophytes play a key role in promotion of plant growth (PGP). In this context, *Drum Phou* and *Chakhao poreiton* rice found in Manipur, India are considered; to study bacterial endophytes for their plant growth promoting traits. 40 endophytes isolated from various part of the rice plants such as leaves, stem and root were purified and then screen for the plant growth promoting activity. The isolates, ChR48, ChL11, DphR5 and DphR17 shows promoting IAA production activity. DphL12, ChR1 and ChR1 give maximum ACC deaminase production. A number of isolates such as ChL11, ChL38, ChL34, ChL55, ChR01, ChR2, ChR48, ChS4, DphR5, DphR17 and Dph30 can solubilised phosphate. Of 40 isolates, 6 isolates viz. ChR01, ChL34, ChL38, ChL55, ChS4 and DphR30 were found to have antagonistic activity against the test fungal pathogens. The data obtained from these experiments suggest a further research and field application of these endophytes could give a good insight for improvement in agricultural productivity as well as the development of bio fertilizers and bio pesticides which can subsequently replace synthetic products.

**Keywords:** Antimicrobial activity; *Chakhao Poreiton*; *Drum Phou*; Endophyte; Plant growth promoting trait (PGPT)

## Plant Growth Promoting Traits of Phosphate Solubilizing and Nitrogen Fixing bacteria isolated from rhizospheric and bulk soil samples of *Oryza sativa* and *Avena sativa*

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### Abstract

Nitrogen (N) and Phosphorus (P) are essential macronutrients for plant growth. Plants require nitrogen in fixed form which is a limiting nutrient in soil. P although abundant in soil, in both organic and inorganic forms, is unavailable to plants as it occurs in insoluble forms. Commercially available chemical fertilizers have a negative effect on the environment, besides posing significant health hazards. Many soil microorganisms belonging to the group of PGPR (Plant growth promoting rhizobacteria) are able to fix nitrogen and solubilize this unavailable P through their metabolic activities by exuding organic acids, which directly dissolves the complex rock phosphate, or by chelation of calcium ions that release P.

The main aim of this study was to isolate PGPR from soil samples (rhizospheric and bulk) of Rice (*Oryza sativa*) and Oats (*Avena sativa*) growing in Jaspur and Choriwad village of Gujarat and then screen the recovered isolates for their ability to solubilize phosphorus and fix nitrogen. A total of 14 bacterial isolates were found to possess both properties. They were identified based on biochemical and molecular characteristics. Furthermore, they were checked for various PGP properties like IAA production, HCN production, Siderophore production and Pi release by Ames method.

Three bacterial isolates possessing good P solubilization properties beside other plant growth promotion properties were identified to be *Mesorhizobium loti* Rb2, *Rhizobium leguminosarum* Rr5 and *Sinorhizobium meliloti* Rr6 based on 16S rDNA sequencing. They were found to produce a good amount of IAA viz.,  $159.4 \pm 0.01$   $\mu\text{g/ml}$ ,  $165.9 \pm 0.05$   $\mu\text{g/ml}$  and  $120.4 \pm 0.01$   $\mu\text{g/ml}$ , respectively. HCN production was almost equal in Rr5 and Rr6 while Rb2 showed least HCN production. Rb2 was not observed to be siderophore producer while Rr5 and Rr6 showed 9.55% and 26.13% siderophore production, respectively. The maximum phosphorus solubilization was exhibited by Rb2 with a value of  $266.1 \pm 0.41$   $\mu\text{g/ml}$  while Rr5 and Rr6 showed similar values i.e.  $226.8 \pm 0.18$   $\mu\text{g/ml}$ . These isolates exhibited the potency to be used as biofertilizer after in vivo plant growth promotion analysis.

**Keywords:** PGPR; Nitrogen; Phosphorus; Rice; Oats; Biofertilizer

## Exploration of Cu-accumulating Bacteria from Copper Mines of East Singhbhum, Jharkhand, India & its Impact on Agriculture

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### Abstract

Environmental pollutions arising day by day due to the toxic substances and heavy metals because of mining, metallurgic processes, and other chemical industries. This pollution affecting human health as well as crop productivity worldwide. To overcome this major issue, bioremediation of heavy metals using microorganisms could be a better way to improve crop productivity of the heavy metal affected areas.

In this study soil and water samples were collected from ICC-HCL and different copper mine under HCL, located in East Singhbhum District of Jharkhand, India. ICP-AES, XRD and AAS was performed of the copper reached soil and water samples to understand physio-chemical characters. Presence of high percentile ferrous and copper was detected. 8 different microbial species were isolated and identified their phylogenetic relationships using 16S rDNA gene sequencing methods. Microbial isolates showed maximum similarities with *Ralstoniasyzygii*, *Bacillus paraflexus*, *Bacillus velezensis*, *Sphingobium scionense*, *Pseudomonas oleovorans* and *Micrococcus aloeverae*. Morphological and biochemical characterizations of those isolates were performed including Gram staining, microscopy, antibiotic sensitivity, extracellular enzyme production and carbohydrate assay. Copper and ferrous salts tolerance or accumulating capacity of those isolates were screened. Production of plant growth stimulators by those isolates such as GA3, IAA, Pro and SA in presence of copper and ferrous salts was also detected. All strains are capable to produce plant growth stimulators in presence and absence of copper and ferrous salts. Growth curve of that highest copper tolerant isolate was obtained in presence and absence of copper and ferrous salts or both to check whether presence of ferrous salt affecting the growth or not. Ferrous salts may play synergistic effect in copper containing media as the growth rate increased in the conditions that contains both iron and copper. Further *in vivo* mechanisms of copper accumulation and its application to increase crop productivity can be done.

**Keywords:** Copper mines; Copper tolerance; Plant growth stimulators; Crop productivity

AMT-16

## Single Step Consolidated Bioprocessing of Corn Stover to Polyhydroxyalkanoates: A New Strategy

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### Abstract

To be competitive with synthetic plastics, the production costs of polyhydroxyalkanoates (PHAs) have to be minimized. PHA production in bacteria being an aerobic process, on an average only 50% of the carbon, nitrogen source and/or additional precursors end up in the product wanted. Use of these costly raw materials, currently, impedes PHA's commercialization due to the high production cost. As an alternative, ligno cellulosic biomass is inexpensive (US \$2-4/GJ or US \$39-60/dry ton biomass) but its pre-treatment cost is high (US \$15-25/GJ), in addition to being technologically demanding. The present study involves screening for and isolating a thermophilic *Geobacillus* sp. with the dual capability of (1) utilizing one or more types of unprocessed (without pretreatment) lignocellulosic biomass as sole carbon source, and (2) producing PHA. This strain with SSBP (Single-Step Biomass-to-Polyhydroxyalkanoate) property, is found to accumulate 35-40% of a medium chain length PHA (mcl-PHA) semi-crystalline PHA composed of 3-hydroxybutyrate and one another monomer (3-hydroxyvalerate and/or 3-hydroxyhexanoate) in corn stover containing medium, over a growth period of 72 h, at 60°C, pH 6.5. Mainly composed of 3-hydroxybutyrate along with other monomers including 3-hydroxyvalerate and extending up to 3-hydroxyhexanoate, the produced polymer shows exceptional thermal stability, together with a relatively high final melting point of 161°C and is capable of thermally induced crystallization. All these biologically mediated transformations (simultaneous pre treatment, hydrolysis, and fermentation of corn stover to PHA) are occurring in a single step process configuration called a novel form of consolidated bio processing (NFCBP), which is distinguished from other less highly integrated configurations in that NFCBP does not involve a dedicated process step for cellulase and/or hemicellulose production. In long term, this research is important to improve the structural properties of PHA and decrease

the production cost, thereby taking this technology towards commercialization, environmental conservation, and real time application to the mankind.

**Keywords:** Bioprocessing, Consolidated, Corn Stover, Thermophile.

AMT-17

### Studies on salt tolerant rhizobium in Soybean

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#### Abstract

The present study was carried out in the laboratory of Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri. Basic aim of study was to isolate and categorized salt tolerant Rhizobium from root nodule of soybean grown in saline soil and to see its effect on growth, nutrient uptake and yield of soybean crop under field condition. Total of 33 salt tolerant isolates were obtained from the root nodules of soybean grown in saline soils of Western Maharashtra. Out of 33 salt tolerant isolates, 20 isolates were categorized under extremely salt tolerant, (more than 5.4 % salt tolerance limit). Four isolates were tolerant to NaCl concentration (2.10- 3.6 % salt). Nine isolates were moderately tolerant to NaCl concentration (0.09-1.50 % salt). Classification of salt tolerant Rhizobium was done on the basis of salt tolerance limit. The salt tolerant Rhizobium isolates were designated as STR. Based on the morphological, cultural, physiological and biochemical tests, the selected Rhizobium were identified at genus level as *Bradyrhizobium sp.* Three efficient salt tolerant Rhizobium were selected (STR-4, STR-14 and STR-18) to develop the liquid consortium on the basis of screening of salt tolerant Rhizobium for IAA, siderophore production and biocontrol activity. The developed liquid formulation was evaluated for their potential under field conditions. All the growth parameters of soybean were influenced by the treatment T<sub>7</sub> (Liquid consortium + 100% N) was significantly superior which was at par with treatment T<sub>6</sub> (Liquid consortium + 75 % N) other inoculated treatments in respect of their influence on growth attributing characters at flowering and harvesting stages along with yield. The consortium of efficient strains of salt tolerant Rhizobium could reduce 'N' fertilizer application by 25 % in saline condition. Moreover, the salt tolerant Rhizobium isolates (tolerance limit 5.4 %) helped to increase nutrient uptake and resulted in increasing grain yield of soybean crop.

**Keywords:** Salt tolerant *Rhizobium*; NaCl concentration; Soybean; Saline condition; Nutrient uptake

## ***Bacillus amyloliquefaciens* UL01 as a Potential Bio-control against *Corynespora torulosaca* causing Leaf Spot Disease of Cotton**

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### **Abstract**

Leaf spot disease is one of the major destructive diseases of cotton caused by a fungus *Corynespora torulosa*. The use of antagonist micro organisms against fungal plant pathogen is an attractive alternative to the use of chemical pesticides. Present study reports the isolation, characterization and identification of bacterium *Bacillus amyloliquefaciens* UL01 from rhizospheric soil of groundnut field. This bacterium showed the significant antagonistic potential against fungal phytopathogen *C. torulosa*. The antagonistic activity of *B. amyloliquefaciens* UL01 was confirmed in vitro using well diffusion method. The antagonist, *B. amyloliquefaciens* UL01 was found to inhibit more than 60% of the mycelial growth of *C. torulosa*. This is the first report of the antagonistic effect of *B. amyloliquefaciens* against phytopathogen *C. torulosa*. Considering more than 60% reduction in growth of phytopathogen, various formulations were prepared by using *B. amyloliquefaciens* as an active ingredient. The viability assessment of *B. amyloliquefaciens* in prepared formulation and efficiency of prepared formulations in controlling the leaf spot was also evaluated.

**Keywords:** Antagonist activity; Bio-control; *Corynespora torulosa*; Phytopathogen; *Bacillus amyloliquefaciens*

## **Designing of microbial consortia for stabilization of rhizosphere and soil fertility**

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### **Abstract**

Due to rapid industrialization and huge population growth raised concern to the environment. Particularly, Soil erosion is a serious threat to the environment. Crop production is dependent on soil quality in order to feed this whopping population. Restoration of both physiological as well as biological quality of soil is highly requested for the good health of soil. To improve the biological quality of soil microbial consortium approach is emerged as a promising methodology for restoration soil quality. Hence, there is an urgent need to characterize isolates for designing a microbial consortium for stabilizing rhizosphere community in order to increase soil productivity. The present study deals with seed treatment with PGPR strains for seed germination, seedling vigor, and seedling emergency. Soil samples were collected from the Banana rhizosphere, Hingoli, Maharashtra. Bacterial isolates were screened for biological activity using oxidase test, Gelatin liquefaction, Arginine dehydrogenase. A total of 6 (six) Bacilli sp. strains were characterized. These Bacilli sp. Produced phytohormones such as Indole acetic acid (IAA), Gibberellic acid, and diverse siderophores. These bacterial isolates have higher potential to assimilate insoluble phosphate and it may suppress the growth of weeds. This study reveals that under in-vivo conditions, seed treatment with PGPR strains improved seed germination, seedling vigor, and seedling emergency. Similar growth has

observed in Wheat grown in pot treated with PGPR resulted in an increased shoot height. The positive effects of PGPR strains were confirmed by Phosphate solubilizing capacity. They also increase plants immunity by increasing production of cell wall degrading enzymes such as cellulase and protease.

**Keywords:** Microbial Consortium; Microbial Ecology; Bioremediation; Rhizosphere; PGPR

AMT-20

### **Antifungal Screening and Biochemical characterization of Bacterial strains from Soil Samples of Manipur**

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#### **Abstract**

Thirty bacterial strains were isolated from soil samples. The isolates were screened for antifungal activities against fungal pathogens *viz.*, *Rhizoctonia solani*, *Fusarium oxysporium* and *Aspergillus niger* using dual culture method. Seven strains exhibited antifungal activities against one or more of the test organisms. These active strains were further screened for biochemical test. All 7 strains were found to be positive in starch hydrolysis and Indole test. Four strains (DM1, DM5, DM6 and SM2) were found to be +ve in nitrate test. Two strains (DM2 and DM5) and 5 strains (DM1, DM6, DM8, SM1 and SM2) were found to be +ve in MR and VR tests respectively. Two strains (DM5 and DM6) and another 2 (DM2 and DM5) were found to be +ve in oxidase and catalase tests respectively. All 7 strains were found to be -ve in citrate utilization, urea hydrolysis test, glucose and sucrose fermentation. One strain, DM6 was found to be +ve in fructose and mannose fermentation tests, 2 strains (DM2 and SM1) were positive in maltose fermentation test, 2 strains (DM2 and DM6) and 3 strains (DM1, DM5 and DM6) were found to be +ve in mannitol and lactose fermentation tests respectively. All the 7 strains were found to show protease activity, out of which, 3 strains (DM5, DM2 and SM1) were potent and promising. DM5 was found to be the most potent and it holds promise for further development as a biocontrol agent.

**Keywords:** Antifungal; Soil, Bacterial; Manipur; MBRL; Fungal Pathogens

AMT-21

### **Endophytic bacteria isolated from finger millet seeds promote seedling growth and development**

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#### **Abstract**

Endophytes are the microbes which reside in the healthy plant tissue including leaf, stem, root, fruits and seeds without causing any harmful effects to the plant. Bacteria which reside in side seeds are called as seed endophytes which may play crucial role in early development of plant seedlings. Finger millet (*Eleusine coracana* (L.) Gaertn) is an annual herbaceous plant belonging to poaceae family which is commonly known as ragi. The aim of present study is to evaluate plant growth promoting features of seed

endophytes of finger millet and its re-inoculation effect on seedling development. Total six endophytic bacteria (EC1-EC6) were isolated from surface sterilized seeds of finger millet and identified using 16S rDNA sequencing. All the bacterial isolates were evaluated for their plant growth promotion activities. Out of six, four endophytic bacterial isolates including EC2, EC3, EC5 and EC6 were produced auxin in nutrient broth. Four endophytic bacterial isolates including EC2, EC3, EC5, and EC6 showed both phosphate solubilization and potassium solubilization activity. Most of the endophytic bacteria showed antifungal activity against plant pathogens. In re-inoculation experiment, we found that presence of endophytic bacteria promotes seedling development in finger millet by increasing root and shoot length growth and root hair formation.

**Keywords:** Seed endophytic bacteria, Plant growth promotion, Seedlings development, Finger millet

AMT-22

### **Production and characterization of GA<sub>3</sub> by endophytic fungi isolated from *Oryza sativa* L.**

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#### **Abstract**

The rice (*Oryza sativa*) plant known to harbor endophytic microorganisms which enhance the plant growth by producing several organic substances similar to its growth hormones and antimicrobial compounds. Among such endophytes the role of bacteria and actinomycetes have been confirmed to promote plant growth, nonetheless unlike the others the fungal endophytes colonizing plant have still remain an unexplored part. On the other hand, endophytic fungi are the group of micro organisms which reside inside the healthy plant tissues without causing the negative effect to the host plant. These endophytes proffer several benefits to the host plant, particularly growth promotion and protection from pathogen. Growth promotion is achieved either through direct mechanisms by producing phytohormones such as auxins, gibberellic acid, and cytokinins or indirectly by production of antibiotics, siderophores and HCN against the pathogen. Among phytohormones Gibberellic acid is the most important plant growth hormone controlling the growth and development. In the present investigation, an attempt was made to isolate endophytic fungi from the rice plant. A total 48 fungal isolates were isolated from 288 segments of 4 rice varieties (Gitanjali, Hiranyamayee, Khandagiri and Lalat) of *Oryza sativa* L. Leaf harbored the highest endophytic fungal colonization (16.87%) followed by stem (16.40%). These isolates were screened for GA<sub>3</sub> production by qualitatively and quantitatively under submerged fermentation condition. Out of 48 fungal isolates OSLST-4 AND OSSL-4 showing maximum GA<sub>3</sub> yield were chosen to study the effect of various physical and nutritional parameters on GA<sub>3</sub> production like different media, incubation period (1-15) days, pH (4-12), incubation temperature (25<sup>o</sup>-45<sup>o</sup>c), salt concentration (1%-10%) and different carbon and nitrogen sources. Optimal condition for maximum GA<sub>3</sub> production by oslst-4 was found to be at day 8 at 30<sup>o</sup>c and pH 8, salt concentration 5% with fructose and sodium nitrate as good carbon and nitrogen source respectively whereas, the optimal condition for maximum GA production by osll-4 was found to be at day 10 at 25<sup>o</sup>c and pH 6, salt concentration 3% with starch and urea as good carbon and nitrogen source respectively. The GA<sub>3</sub> production was confirmed partially by thin layer chromatography (TLC), Fourier transformation infrared (FTIR) and High performance liquid chromatography (HPLC) analysis. These isolates will be further characterized for their ability to influence the PGP activities for Sustainable agriculture.

**Keywords:** *Oryza sativa*; Thin layer chromatography; Fourier Transformation Infrared; High performance liquid chromatography (HPLC)

## Evaluation of potential biocontrol agents towards *verticillium* wilt of cotton

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### Abstract

12 fungal isolates were obtained from organic manures and seven endophytic bacteria isolated from healthy cotton plants grown under natural conditions. Biocontrol potential of antagonistic isolates was assessed on following criteria: (1) *in vitro* inhibition of *Verticillium dahliae*, (2) activities of cell wall degrading enzymes including protease, cellulase and chitinase. To explore the biocontrol potential of antagonistic microbial strains, a screening strategy has been developed. Out of 7, *Pseudomonas sp.* and *Bacillus subtilis* have shown 61.12% and 57.64% *in vitro* antagonistic activity respectively by "Dual Culture Assay". Whereas 6 out of 11, antagonistic fungi viz. *Aspergillus niger*, *Aspergillus nidulans*, *Cladosporium sp.*, *Glicocladium species*, *Aspergillus terreus* and *Trichoderma harzianum* shown 63.20%, 61.81%, 6.95%, 61.12%, 61.81%, 72.91% have achieved positive *in vitro* antagonism respectively towards *V. dahliae*. Antagonistic isolates which have shown positive antagonism in previous study were further evaluated for *in vitro* protease, cellulase and chitinase activity. In Cellulase Activity, *Aspergillus niger*, *Aspergillus nidulans*, *Glicocladium species*, *Aspergillus terreus*, *Trichoderma harzianum* and *Cladosporium species* have produced 10.4, 7.5, 10.83, 9.83, 8.5, 0.0 mm yellow-opaque inhibition zones, 9.5, 8.1, 9.06, 9.33, 6.46, 3.46 mm clear zones in Protease Activity and in chitinase activity 9.66, 8.5, 8.43, 8.5, 8.1 and 3.2 mm respectively purple coloured halo was recorded. *Pseudomonas sp.* have shown inhibition zones 8.56, 8.70, 7.4mm and *Bacillus subtilis* produced 5.23, 6.66, 6.23mm clear zones in Cellulase, Protease and Chitinase activity respectively.

**Keywords:** Biocontrol; Antagonistic; *V. dahlia*; Cellulase; Protease

## Influence of abiotic stress on the growth of Plant Growth Promoting Bacteria isolated from agricultural soil of Jharkhand

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### Abstract

Abiotic stresses are unfavourable environmental conditions that may cause threat to the living organisms. Water, nutrients, temperature, salt and pH are among the most challenging abiotic components of agricultural ecosystem and affect the growth of plants and ultimately lead to impairment of the crop yield under stress condition. Soil comprises of heterogeneous group of beneficial bacteria which resides in the rhizosphere of plants called Plant Growth Promoting Rhizobacteria (PGPR) which directly or indirectly improves the plant growth by helping nutrition and ameliorating the abiotic stress for better survival. However, the growth and performance of these PGPR are also affected by such abiotic factors. Therefore, present investigation was carried out to study the different environmental factors such as pH, salinity and temperature on the growth response of PGPR isolates. To achieve this, five best isolates (T-1,



T-2, E-2, O-2, R-1) were selected which showed all the four plant growth promoting activities such as nitrogen fixation, phosphate solubilisation, siderophore production and IAA production in the previous study. These isolates were further screened to test the effect of different environmental factors such as pH, temperature, salt on their growth response. T-2 and E-2 isolates were found to grow in a wide range of pH 3.5- 13 and salinity range 0- 8% but the growth was slightly decreased at higher salt concentration beyond 6% NaCl. T-1 isolate showed growth in the pH range of 3.5- 10.5 and salinity range of 0- 8%. O-2 isolate was found to grow in the pH range of 7.0-9.5 and salinity range of 0-6% whereas R-1 isolate showed growth in the pH range of 7-12.5 and salinity range of 0-3%. T-1, T-2 and E-2 isolates showed tolerance to temperature range from 37-C- 44.8-C whereas O-2 and R-1 showed tolerance to temperature range from 37-C- 41.4-C and 37-C – 38.7-C respectively. So, these isolates can be used in agricultural fields in their respective environmental conditions. T-1 and T-2 may find wide range of applicability as biofertilizers and biostimulants to enhance the plant growth.

**Keywords:** PGPR; Biofertilizers; Biostimulants; pH; Salinity; NaCl

AMT-25

### To evaluate the capabilities of *Lactobacillus* strain to reduce aflatoxin production by *Aspergillus* sp. in peanuts

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#### Abstract

Aflatoxins are a group of mycotoxins, commonly contaminating maize and groundnuts, and are categorized as class 1 A human carcinogens by the International Agency for Research on Cancer (IARC, 2002). Probiotic lactic acid bacteria have been identified as a safe means to reduce availability of aflatoxins in vitro. *Lactobacillus* spp. isolated from Goat milk using MRS agar media. Antagonistic activity checked *in vitro* between *A. flavus* and *Lactobacillus* spp. Biological control assay performed up to 19 days between different combination of *Lactobacillus* spp., *A. flavus* and Seed. Different combinations like Control, S+B, S+F, S+B+F were taken. *In vivo* pot trials done in a container, with natural soil, treated seed and fungus up to 19 days. *Lactobacillus* showed antifungal activity by inhibiting the growth of fungus. Seed germination observed in presence of control, S+B, S+B+F and P+B+F. Root length, 8.3cm, 6.8cm and 9.8cm against control 9.5, 9 and 5cm measured and shoot length 7.8cm, 4.8cm and 4.5cm against control 8, 4.5 and 5cm observed. Bacteria did not produce harmful effect based on root and shoot length of S+B. In S+B+F plant survived against production of secondary metabolite by fungus due to seed treated with bacteria. In P+B+F root length indicates plant have developed more immunity during maturation, so not affected by fungal infection. Protection of Plant through treated seed with bacteria indicates *Lactobacillus* work as a protective barrier against *Aspergillus* infection and work as endophytes.

**Keywords:** Aflatoxin; *Lactobacillus* spp.; Antagonistic; *A. flavus*; Endophytes

AMT-26

### Characterization of endophytic bacteria associated with *Ocimum sanctum* linn. for their plant growth promoting potential and antagonistic effects against *Fusarium oxysporum*

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### Abstract

The use of conventional agrochemicals in agriculture adversely deteriorates plant health, soil microbiome and fertility, thereby limiting crop performance and agronomic yield. Therefore, the present study was intended to evaluate plant growth promoting and biocontrol efficacy of bacterial endophyte OC1, isolated from *Ocimum sanctum* leaves. Based on 16s rRNA gene sequence analysis and phylogenetic studies the isolate was found to be *Azospirillum* sp. strain SG5 (MN318324). The strain was positive for solubilization of phosphate inorganic  $\text{Ca}_3(\text{PO}_4)_2$  complexes (90 mg/L), production of phytohormone such as Indole acetic acid (45 $\mu\text{g/ml}$ ), stress tolerance enzyme such as ACC deaminase (850 nmol of  $\alpha$ -ketobutyrate  $\text{mg protein}^{-1} \text{h}^{-1}$ ), qualitative synthesis of siderophore, ammonia and HCN. Apart from PGP traits, the isolate indicated anti-fungal activity against phytopathogenic fungi, *Fusarium oxysporum* using dual culture assay. The isolate also has the potential for the production of fungal cell wall degrading enzymes such as chitinase,  $\beta$ -1,3, glucanases, protease which confer its potential to induce systemic resistance in plants. The pot trial experiments showed significant ( $P < 0.05$ ) effect on the growth of tomato (*Solanum lycopersicum*) plant primed with OC-1 as bio-inoculant under pathogen challenged conditions. This can be conferred due to enhancement in germination percentage and various growth parameters such as root/shoot length, root/shoot fresh and dry weight, activity of antioxidative defensive enzymes such as Superoxide dismutase, catalase, peroxidase,  $\beta$ -1,3, glucanases, Phenylalanine Ammonia Lyase, Polyphenol oxidase in comparison to non-treated plants. Hence, these results indicate that the bacterium OC-1 enhances the plant growth, induces systemic resistance and has the potential to control *Fusarium oxysporum* associated vascular wilt disease.

**Keywords:** Endophytes; Induced systemic resistance; *Fusarium*; Wilting; PGPR

ATM-27

### Accelerating the Decomposition of Different Bio-wastes using Thermophilic Ligno-cellulolytic Microbes

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### Abstract

The rise in the municipal, agricultural and industrial waste is posing a threat to the environment by polluting land, water and air. However, emission of gases from waste contributes to global warming and hence, scientific and systematic waste management is utmost needed. Composting is the best suitable way to dispose different bio-wastes, as on an average approximately 40% waste consists of organic matter which is biodegradable in most of the municipal solid waste (MSW) in India. High lignin content in the waste restricts the enzymatic and microbial access to the cellulose in waste material. Thus, combination of lignin and cellulose degrading microbes may hasten the biodegradation of crop residues, vegetable waste, leaf litters etc. In view of above importance, the present study is aimed to screen potential thermophilic ligno-cellulolytic microbes from various MSW and farm waste. All the isolates were screened for production of enzymes viz. laccase, lignin peroxidase (LiP) and Manganese Peroxidase (MnP) and cellulase. Compatibility test of all potential fungi, bacteria and actinomycetes was done in order to develop suitable microbial consortia. Among all the isolates, 4 fungi, 4 bacteria and 2 actinomycetes showed no antagonistic activity thus present work was carried out using these selected microbes. To check the efficacy of the developed consortia, different bio-wastes were selected viz. kitchen, horticulture and vegetable waste. All the bacterial and actinomycetal cultures were identified on the basis of 16s rRNA sequencing and

fungal identification was done using 18s rRNA sequencing. Maturity parameters viz. C/N, CEC/TOC, degree of humification, L/C ratio and selected enzymes reached much earlier in vegetable waste compost (20 days) followed by kitchen (25 days) and horticulture waste (35 days). Shifting of microbial diversity during every stage was studied using PLFA. The study concluded that using microbial consortia fasten the decomposition process and matured compost formed within 20-45 days.

**Keywords:** Bio-waste; Ligno-cellulolytic; Compost maturity; Microbial diversity

ATM-28

### **Biocontrol potential of marine chitinolytic bacteria towards fungal phytopathogens**

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#### **Abstract**

Biological control of phyto-pathogenic fungi, harmful insects and nematodes using chitinolytic microorganisms and their derived chitinase has received much attention due to its wide range of biotechnological applications, especially in agriculture. Therefore, pharmaceutical, agriculture, water treatment plants and various other industries greatly demand the isolation of high yielding chitinase producing microorganisms with improved properties. In the present study, based on chitin hydrolysis zone two marine chitinolytic bacterial strains were isolated out of 25 screened strains from Paradeep port area (water and soil). The effects of isolated marine bacteria on phytopathogenic fungi and production of chitinase were studied. The maximum chitinase production was obtained at 35-C and pH 7.5 after 24-48 h of incubation by APW2; and at 37 -C and pH 7 after 48 h incubation by APW7. Among the substrates, colloidal chitin was the best for both the strains. Furthermore, the strain APW7 suppressed the fungal infections when applied to the leaves of *Oryza sativa*. Hence, our results revealed the ability of APW7 and its produced chitinase as a possible biocontrol agent against fungal phyto-pathogens.

**Keywords:** Biocontrol; Chitinolytic Bacteria; Chitinase; Phytopathogens; Marine source

AMT-29

### **Effect of succinate on Mineral Phosphate Solubilization Phenotype of Multitrait Plant Growth Promoting Soil Isolate *Acinetobacter***

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#### **Abstract**

Plant growth promotion can be improved by rhizosphere-dwelling bacteria by a numerous methods. One method is by promoting nutrient acquirement from soil. Phosphorus (P) is an essential nutrient that

plants obtain from soil, but in several cases it is immobilized in forms that are not available for plant uptake. Bacteria can solubilize insoluble soil phosphates by secreting organic acid and are known as phosphate solubilizing bacteria (PSBs). But these bacteria fail as PSBs in field conditions due to various reasons. One such reason is reverse carbon catabolite repression (rCCR) in which the presence of organic acids in root exudates and rhizosphere may inhibit uptake and utilization of sugars and other carbon sources which are important for the plant growth promotion. In this study, plant growth promoting rhizobacteria isolated from the rhizosphere of *Vigna radiata* was used which was identified as *Acinetobacter* sp. SK2. It solubilized 682 µg/ml of tri-calcium phosphate (TCP) and 86 µg/ml of rock phosphate (RP) with simultaneous decrease in pH up to 4 due to the production of gluconate. The biochemical basis of the mineral phosphate solubilization (MPS) suggested that the gluconate production was mediated by mGDH and soluble glucose dehydrogenase (sGDH) enzymes. The role of succinate in repression of P solubilization via suppression of mGDH and sGDH activity was explored, which was correlated with repression of expression of respective genes (*gdhA* and *gdhB*). Furthermore, the expression of *crc* in succinate and glucose+succinate led us to hypothesize the role of *Crc* in succinate mediated repression of MPS phenotype. Isolate SK2 was found to be a multi-train PGP as it produced IAA, siderophore, HCN, ammonia and solubilized minerals of Zn and K.

**Keywords:** Plant growth promoting rhizobacteria (PGPR); Mineral phosphate solubilization (MPS); Succinate mediated repression; mGDH; sGDH

AMT-31

### Prospecting of Multitasking strain of Plant Growth Promoting Rhizobacteria with regards to Biofertilization, Biostimulation and Biological control of Plant Disease

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#### Abstract

Plant growth promoting rhizobacteria have the vital role in agriculture as they enhance the plant growth, biomass, yield, micronutrient content in grains and suppress the phytopathogens. A total 108 bacterial morphotypes were isolated from samples collected from Samastipur, Muzaffarpur, Hasanpur and Pusa regions of Bihar. These morphotypes were screened for various PGPR activities like-Nitrogen fixation, phosphorus solubilization, Zn solubilization, Siderophore production, HCN production, Ammonia production, IAA production,  $\beta$ -1,3glucanase and chitinase activities. Out of these about 20 cultures were showed nitrogen fixation and IAA production ability, 25 cultures showed phosphorus solubilization ability and 21 cultures showed Zn solubilization ability, 10 cultures showed siderophore production ability and 15 morphotypes showed HCN and Ammonia production ability and 8 cultures showed  $\beta$ -1,3 glucanase and chitinase activities. Only 5 morphotypes (AK-16, AK35, AK66, AK72, and AK 98) were such types which showed all above mention PGPR activities. Thus these selected multitasking strains may be used for deciphering their potential in different agro ecological zone with regards to biofertilization, biostimulation and biological control of phytopathogens.

**Keywords:** Rhizobacteria; Screening; PGPR activities; Multitasking strain; Biofertilization; Biostimulation; Biocontrol

## Prospecting of Rhizobium and others Rhizobacterial isolated from Tal region of Bihar

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### Abstract

Tal region of Bihar is the major challenging environment in terms of soil microbial diversity because of limiting chemical fertilizers application. Plant growth promoting Rhizobacteria and Rhizobium habitat in such type of soil ecosystem may be more tolerant to adverse environmental conditions. A total number of 10 Rhizobial morphotypes were isolated from lentil root nodule and 97 Rhizobacterial morphotypes were isolated from soil samples collected from Tal region of Mokama (Bihar). The bacterial isolates were screened for various PGPR activities like-Nitrogen fixation, Phosphorus solubilization, Zn solubilization, Siderophore production, HCN production, Ammonia production, IAA production. Only 5 Rhizobial isolates showed phosphorus solubilization, Zn solubilization and IAA production ability and only two isolates showed ammonia production ability. Siderophore production ability and HCN production ability was absent in all Rhizobial isolates. In case of Rhizobacteria, about 15 cultures showed nitrogen fixation, 23 cultures showed phosphorus solubilization ability, 16 cultures showed Zn solubilization ability, 12 cultures showed siderophore production ability, 20 cultures showed IAA production ability, and 11 cultures showed HCN and Ammonia production ability. Catabolic diversity of all selected microbial isolates was determined by using carbon profiling. Two Rhizobial isolates (DPR-3, DPR-7) and three Rhizobacterial isolates (DP-36, DP-57, DP-83) were selected for further study on the basis of catabolic diversity in different types of soil ecosystem (calcareous, diara, tal, non-calcareous).

**Keywords:** Tal Region ; Lentil ; Diversity; Screening ; Calcareous ; Diara

ATM-33

## Exploitation of microbial antagonists for pathogenic fungi management in pigeon pea (*Cajanus cajan* L.)

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### Abstract

The most common, devastating problem in agriculture is plant (pathogenic) diseases and abiotic conditions which have a profound effect on growth and yield of the plant resulting in heavy losses. In order to prevent losses, different chemicals are used indiscriminately, which in turn lead to environmental pollution due to their persistence and toxicity yet employed to meet consumer demand. Exploitation of beneficial rhizosphere microbial functions such as use of biofertilizer, biopesticide, plant growth-promoting rhizobacteria, etc. help in achieving sustainable goal and alleviating adverse impact on environment. 21% of the isolated bacteria inhibited the growth of all the three pathogenic fungi i.e., *Fusarium oxysporum*, *Alternaria tenuissima* and *Rhizoctonia bataticola*. PGPB may help plants to overcome abiotic stresses by providing the plant with IAA (indole3 acetic acid) that directly stimulates plant growth, even in the presence of otherwise inhibitory compounds. The bacteria that most effectively protect plants against a

wide range of different stresses produce both IAA and ACC deaminase. The present study was undertaken for analysis of these bacterial isolates for in vitro indole acetic acid and ACC deaminase enzyme production and studying the effect of these bacteria on *Cajanuscajan*. The selected isolates produced IAA in the range of 12.3 to 26.31 µg/ mL. The results confirmed that the application of these PGPR strains not only controlled the disease but also promoted the growth of the pigeon pea plants. These rhizobacterial isolates could further be assessed for disease control and their plant growth promotion potential under field conditions for their subsequent use as efficient biofertilizer and biocontrol agents.

**Keywords:** Antagonistic Rhizobacteria; Pathogenic Fungi; IAA; ACC; Sustainable Development

AMT-34

### **Sugar dependent mineral phosphate solubilization and its succinate mediated catabolite repression in fast and slow growing Rhizobia**

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#### **Abstract**

The most efficient mineral phosphate solubilization (MPS) in gram negative bacteria occurs through solubilization of insoluble inorganic compounds like tricalcium phosphate, dicalcium phosphate and rock phosphate. Phosphate solubilization by rhizobia may prove to be of dual advantage as it can solubilize P as well as fix N<sub>2</sub> for the plants, two of the most important macronutrients vital for plant growth. Gluconic acid production in rhizobia through external PQQ supplementation which results from extracellular oxidation of glucose via the glucose dehydrogenase (Gcd) to gluconic acid. Majority of the rhizobacteria reported so far have been shown to solubilize P only when grown on glucose. Other rhizospheric sugars have received little or no attention in this regard. After glucose, arabinose and xylose are the most abundant sugars present in the soil that can be utilized. L-arabinose is a superior C-source for the growth of rhizobia and is catabolised by different pathways that distinguish rhizobia into slow and fast growing rhizobia. Role of a pentose such as arabinose, on PGP traits of bacteria especially P solubilization has not been explored. Arabinose is not only utilized and metabolized by rhizobia but also linked with TCA cycle and produce organic acids like oxalate, malate etc. *Rhizobium* sp. RM and SE were unique as they could produce different organic acids using glucose and arabinose as C-source which leads to P solubilization. However, P solubilization and organic acid production was repressed in the presence of succinate resembling the phenomenon of catabolite repression. This is perhaps the first report on mechanism of P solubilization in rhizobia through utilization of two different sugars, glucose and arabinose and its repression by succinate. This study will certainly help to understand one of the factors of failure of efficient PSMs in field condition and possibly help in drawing a strategy to overcome it.

**Keywords:** Arabinose; Mineral phosphate solubilisation; Glucose dehydrogenase; Arabinose dehydrogenase; Glyoxylate oxidase

## Characterization of newly isolated plant growth promoting drought stress tolerant *Beijerinckia fluminensis*

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### Abstract

Drought is the major abiotic stress factor affecting the agricultural productivity. It can affect microorganisms and plant growth since water becomes a limiting factor which further affects the plant-water relations at cellular level. Drought tolerant rhizobacteria have capability as plant growth promoter through various adaptive mechanisms. This study describes isolation and characterization of novel drought resistant rhizobacteria isolated from quartzite soil associated with grass rhizosphere (pH 5.7) from Aurangabad, Maharashtra, India. Based on morphological, cultural, biochemical and 16S rRNA sequence analysis, the strain is identified as *Beijerinckia fluminensis*. The strain forms raised and slimy colonies on nitrogen-free glucose mineral agar. The strain has astonishing feature to grow at a water potential of with -2.0 MPa. The plant growth promoting attributes like secretion of indole acetic acid, siderophore, ammonia production, phosphate solubilization and nitrogen fixation were also analyzed. The strain can be further tested under field conditions and may be developed as bio-inoculant for rain-fed crops.

**Keywords:** *Beijerinckia fluminensis*; Drought tolerant; rhizobacteria

## Volatile Organic Compounds (VOCs) in Plant Holobiont: An Emerging Area for Plant Stress Management

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### Abstract

Plants have evolved 'crying for help' strategy under stress condition, whereby they release volatile organic compounds (VOCs) from root or leaf exudates to attract beneficial microbes in their holobiont. These microbes help to overcome the stress and facilitate plant adaptation in stress environment. VOCs can diffuse across long distances in soils and thus attract remote microbes. In addition, these signalling compounds inform healthy neighbouring plants also to accumulate beneficial bacteria in their vicinity. For example, root VOCs like (+)-enantiomers of carvone, limonene, sesquiterpene (E)- $\beta$ -caryophyllene, and borneol have been shown to promote bacterial quorum sensing. On the contrary, beneficial microbes also emit VOCs in a given range of scales that play a key role in signalling and regulate several physiological processes in plant health. Microbial VOCs are reported to directly inhibit growth of phytopathogens. Some identified compounds, such as hexanol, nitroguaiacol, benzothiazole, butylated hydroxyl toluene, 3-hydroxy-2-butanone (acetoin), 2, 3-butanediol, 2-pentylfuran, or dimethylhexadecylamine have shown their ability to elicit plant growth and disease resistance against fungal phytopathogens. Moreover, microbial antagonists produce several antifungal VOCs which play important role in inhibition of fruit spoilage pathogens such as *Alternaria*, *Aspergillus*, *Botrytis*, *Fusarium*, *Geotrichum*, *Gloeosporium*, *Monilinia*, *Penicillium*, *Mucor* and *Rhizopus*. Some attempts have also been made to improve preharvest

and postharvest disease management through microbial volatiles. The role of VOCs in plant holobiont as allelochemicals has begun to be recognized recently. Applications of microbial VOCs in different area are still unexplored. In addition, exploiting underlying mechanisms of VOCs may foster future microbial strategies to control crop pests and diseases. Some case studies on microbial VOCs will be presented at conference and future direction will be provided for application of microbial VOCs as technological innovation in agriculture.

**Keywords:** Agriculture; Microbes; Plants; Stress; VOCs

AMT-38

**Role of seed priming with melatonin on growth and enzyme activity in wheat (*Triticum aestivum* L.) under salt stress**

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**Abstract**

Wheat (*Triticum aestivum*) is the most important food crop in India which belongs to Gramineae family. In India, wheat is cultivated in various states like Uttar Pradesh, Madhya Pradesh, Haryana, Rajasthan, Bihar, Maharashtra, Gujarat and Punjab. India is at the second position in production of wheat after china. Wheat production is affected by many biotic and abiotic factors. Among the abiotic stresses, salinity is a major problem. Under the salinity stress, there is a considerable decrease in all the parameters like photosynthetic activity, yield and growth of wheat plants. There are many reports where melatonin, an antioxidant played a important role under many biotic and abiotic stresses. So, the present study was carried out to study the effects of different concentration of melatonin on different parameters like germination of seeds, shoot length, number of root, root length of wheat and antioxidant enzyme under salt stress. After the experiment, we observed that Melatonin pretreatment at 2.0mM and 3.0mM increased the number of seed germination, shoot length, number of root, root length of wheat under salt stress. On the basis of the experiment, it was proved that pre-treatment of wheat seeds with different concentration of melatonin increased the activity of different oxidative enzymes like catalase which is very important under the salt stress. In this study, melatonin was proved as a bio-stimulating hormone for plants which enhances various physiological and enzymatic activities of the plant helping it to cope with salt stress and improve the production of wheat.

**Keywords:** *Triticum aestivum*; salinity stress; melatonin; catalase

ATM-39

**Dissimilatory Nitrate Reduction to Ammonium (DNRA) Activity in Bacteria Isolated from Leh and Ladakh: Conservation of N-NH<sub>4</sub> in Wetland Ecosystem**

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**Abstract**

The N-cycle is operating through four main pathways *viz.* nitrification, denitrification, anaerobic ammonium oxidation and dissimilatory nitrate reduction to ammonium (DNRA). The DNRA process



'a short circuit of nitrogen cycle' is directly convert ( $\text{NO}_3$ ) in to ammonium ( $(\text{NH}_4)$  via nitrite ( $(\text{NO}_2)$  in microaerophilic conditions by microorganisms having a complex nitrite reductase enzyme complex. Considering this fact, in present study, forty two bacterial isolates were isolated from Pangong Lake and Nubra Valley of Leh and Ladakh, UT and further subjected to screening for DNRA activity by inoculating bacteria in minimal broth (without N-source) supplemented either with sodium nitrate or sodium nitrite and overlaid with sterile mineral oil followed by incubation at  $28 \pm 2$  °C for 15 days in standstill condition. After completion of incubation, the formation of ammonium was detected by using Nessler's reagent and production of brick colour was positive for ammonium production from nitrate by DNRA process. The production of nitrite in minimal broth supplemented with sodium nitrate was detected by nitrite detection kit and production of ammonium in minimal broth supplemented with sodium nitrite was detected too by Nessler's reagent. Around 76.1 % bacterial isolates were found positive for ammonium production, involving production of nitrite as an intermediate, suggesting operation of DNRA process. For example, some bacterial strains namely *Kocuria palustris* NS-4 (NAIMCC-B-02268), *Bacillus safensis* NS-5 (NAIMCC-B-02277), *Kocuria palustris* NS-8 (NAIMCC-B-02282), *Achromobacter insuavis* NS-12 (NAIMCC-B-02269), *Achromobacter insuavis* NS-13 (NAIMCC-B-02270), and *Bacillus aryabhatai* PL-15 (NAIMCC-B-02273) were found performing DNRA activity. The production of ammonium with intermediary of nitrite formation confirm occurrence of DNRA activity under microaerophilic condition. This is a simple and low cost method to detect DNRA process in bacteria instead of hazardous, costly and complex method where radioactive labelled substrate is required for detection of DNRA process.

**Keywords:** Dissimilatory Nitrate Reduction to Ammonium (DNRA); *Kocuria palustris*; *Bacillus safensis*; *Achromobacter insuavis*; *Bacillus aryabhatai*

AMT-40

### Microbial Inoculation Improves Carbon Sequestration and Soil Health

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#### Abstract

The widespread deterioration of India's soils and poor health of agricultural soils is a major concern. Soil organic matter (SOM) is the mainstay of soil health. Low availability of organic manures and reduced recycling of agricultural wastes are the principal reasons for low SOM content in Indian soils. The role of microbes in maintaining soil health is well known, however their role in sequestering carbon and formation of SOM is poorly understood. We studied the formation of melanin and other organic compounds by bacteria-*Microbacterium testaceum* (AR3), *Arthrobacter* sp. (AR7), *Streptomyces* spp. (A6, A10, A17), *Lysinibacillus* sp. (AR10) and *Bacillus subtilis* (P10) *in vitro* in culture media; as well as *in vivo* in soil microcosms for formation of SOM and improvement of soil health attributes. Spectrophotometric measurements showed that melanin production was highest in total consortium of all cultures (TC) and in *Arthrobacter* spp. An increase in aromaticity reflected in decrease of A365/A550 and A465/A665 ratios, the lowest was observed in *Arthrobacter* sp., AR7, *Arthrobacter* mixture AR3+AR7 and TC. In soil microcosms, TC inoculation increased C mineralization in plant residue amended soils (PRA) (wheat straw + legume residue) by 14.3 % over uninoculated soils (at 120 DOI), extracellular protein content by 35% (180 DOI); and showed 0.05% higher organic C and 0.003% higher labile C (absolute values) along with 13.3% higher soil dehydrogenase activity (270 DOI). The improvement of soil organic matter content and soil biological properties in the inoculated soils was consistent over the entire study period of nine months. We conclude that bioinoculants and plant residues can be used as a good alternative

to build up SOM and improve soil health given the widespread shortage and low availability of bulky organic manures.

**Keywords:** *Arthrobacter*; Bioinoculants; *Streptomyces*; Soil organic matter

AMT-41

### Detection of Tryptophan-Independent pathway operation for IAA Production in bacterial gene Pool Isolated from Tripura State

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#### Abstract

Plant growth promoting bacterium namely *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Erwinia*, *Enterobacter*, *Flavobacterium*, *Mesorhizobium*, *Pantoea*, *Pseudomonas*, *Rhizobium* and *Serratia* have been reported as IAA producers utilizing L-tryptophan via different tryptophan dependent pathways. However, evidence for operation of tryptophan independent pathway using radioisotopic method has been reported recently in *Azospirillum brasilense* only. Hence, in this study, a new and simple method was employed for detection of operation of tryptophan independent pathway in bacteria isolated from Tripura state. More than 200 diverse bacterial isolates were grown in tris minimal broth containing either casein hydrolysate or acid hydrolysed casein (lacking tryptophan) followed by inoculation with each bacterium and IAA production was detected by Salkowski's reagent. The results obtained categorised bacteria in to four categories such as bacterial isolates with operation of (1) both tryptophan -dependent and -independent pathways for IAA production, (eg. isolate TRSK-B:12.27  $\mu\text{g ml}^{-1}$  IAA in casein hydrolysate and 11.40  $\mu\text{g ml}^{-1}$  IAA in casein acid hydrolysate), (2) tryptophan dependent pathways for IAA production (eg. Isolate TAD-66:23.23  $\mu\text{g ml}^{-1}$  in casein hydrolysate), (3) tryptophan independent pathway for IAA production (eg. isolate TRPV-8: 22.16  $\mu\text{g ml}^{-1}$  in casein acid hydrolysate), (4) no IAA production (eg. isolate TRSK-100). It was further noted that most bacterial isolates predominantly operate tryptophan dependent pathways, whereas a few of them TRPV-8, TRSK-71 and TRSK-76 operate tryptophan independent pathway as a major pathway. The isolates TAD-66, TRGD-5, TRSK-43 produced IAA maximally by tryptophan dependent pathway, whereas isolates TRSK-71, TRSK-76, TAD-80, produced IAA by tryptophan independent pathway. The study also suggests that there is a great possibility of operation of tryptophan independent pathway in many bacteria isolated from different niches that emphasize the need for more research to decipher route of tryptophan independent pathway.

**Keywords:** *Azospirillum*; *Azotobacter*; *Burkholderia*; *Erwinia*; *Enterobacter*; *Flavobacterium*; *Mesorhizobium*; L-tryptophan; IAA

AMT-42

### Characterization and Preservation of *Chromobacterium violaceum* TRFM-24 isolated from Tripura

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## Abstract

*Chromobacterium violaceum* has been reported to possess many functions such as antibacterial, antifungal, anti-viral, anti-trypanosomal activities and a few attributes of agricultural importance. The objective of this study was (i) to characterize and identify the pigmented bacterial isolate designated as TRFM-24 recovered from rhizospheric soils of rice from Tripura and (ii) to develop a low cost method to preserve it using natural gums at room temperature. The bacterium was characterized using polyphasic approach and natural gums were used for its preservation under room temperature since this bacterium lost viability in a few days after its isolation. The colony of bacterium on TSA was violet, circular, smooth, entire, convex, size 1.0 mm and cells were Gram negative. The isolate was positive for catalase, oxidase, arginine dihydrolysis, citrate utilization, TSI, MR, VP, and hydrolysis of starch, cellulose and gelatine but negative for urea hydrolysis, indole production and nitrate reduction. It utilized sugar such as mannose and fructose, inulin, cellobiose and xylose. *C. violaceum* was found sensitive to kanamycin, chromaphenicol, rifampicin, streptomycin, tetracycline, nalidixic acid and resistant to penicillin and ampicillin. The 16S rRNA gene sequence of isolate TRFM-24 showed similarity to *Chromobacterium violaceum* ATCC 12472. Based on morphological, biochemical characters and sequencing homology, the TRFM-24 was identified as *Chromobacterium violaceum* (NAIMCC-B-02276). To avert immediate death of strain TRFM-24, four natural gums of babul (*Acacia nilotica*), palash (*Butea monosperma*) and dhavda (*Anogeissus latifolia*) and kondu salai (*Boswellia serrata*) were used for its preservation under room temperature. It was found that the bacterium retained its viability in gums of babul, dhavda and kondu salai up to 6 months at room temperature. This is the first report of isolation of *C. violaceum* from Tripura as well as its preservation at room temperature utilizing natural gums of Indian origin.

**Keywords:** *Chromobacterium violaceum*; TRFM-24; *Acacia nilotica*; *Butea monosperma*; *Anogeissus latifolia*; *Boswellia serrata*

AMT-43

## Zinc solubilizing bacteria and their PGPR activities

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## Abstract

Micronutrient deficiency is a global health issue and a special problem in India. Where more than one third of all Indian children are suffering from zinc deficiency, causing a range of health issues including impaired development or mental disability. Several strategies are conceived to solve the problem, including addition of zinc to final products or development of GM crops with an increased zinc uptake and use of Zn fertilizers. All these strategies suffer however important drawbacks preventing their implementation at a large scale. Use of Zn fertilizers besides being costly also has inherent problems of using a chemical fertilizer. Furthermore, part of the added fertilizers becomes locked in colloidal soil again depriving the plants of available Zn. Microbial solubilization of Zn is an alternative, economic and ecofriendly approach and can solve at least 2/3rd of the problem. A total of six soil samples were used for the isolation of Zn solubilizing bacteria. A total of 19 strains were isolated from these samples exhibiting varying degree of Zn solubilization. These strains were able to solubilize both ZnO and ZnCO<sub>3</sub>, probably through the production of organic acids. From the colony morphology it is clear that these strains belong to diverse bacterial groups, including Actinobacteria, Firmicutes (*Bacillus*) and other groups of bacteria. To the best of our knowledge there is only one report on the solubilization of Zn by *Streptomyces*. Interestingly, the Actinobacteria like strains were found to be most effective for Zn solubilization. Some strains formed *Bacillus* like colonies and from cell shape, Gram staining and biochemical tests also it is evident that these

strains are Bacillus. The diameter of zone of solubilization shows that these strains were the second most efficient Zn solubilizing strains. Interestingly, four of these strains were found to solubilize insoluble phosphorus forming solubilization zones around their colonies on Pikovskaya's medium. When, these strains were tested for other PGPR activities, these strains were found to produce siderophores and IAA, each strain was producing IAA in different concentrations. And mineralization of starch and other polymers. These traits besides the capability to solubilize Zn and P make these strains ideal for their use as biofertilizers catering to diverse nutritional requirements of the plants. And can help in developing sustainable strategies for intensive agriculture to meet the quality food demand of an ever-growing population should be prioritized. Zn being a limiting factor for major staple food is one the major issue.

**Keywords:** Micronutrient deficiency; Plant-growth-promoting bacteria; Plant-microbe interactions; Sustainable agriculture

AMT-44

### In- vitro and in -vivo estimations of *Archis hypongea* plant extracts against Some pathogenic bacterial strains

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#### Abstract

A larger number of medicinal plants and their purified constituents have been shown beneficial therapeutic potentials. In view of the increasing incidence of gram-negative infections, studies were initiated to evaluate the effect of herbal extracts on *Escherichia coli*-induced peritonitis and sepsis. We present here the protective effects of an Indian medicinal plant *Archis hypongea* as compared to Ofloxacin in *E. coli* induced peritonitis. It is the fourth important oilseed crop of the world in production after soybean, cottonseed and rapeseed. Chemical constituents present in the plant are acids, arachin, lecithin protein, flavonoids, beta-carotene, amino acids, minerals, fat, carbohydrates etc. It has varied pharmacological activities like antimicrobial, antifungal, antiviral, antioxidant, anticancer, antihypertensive, neuro-protective, anti-mutagenic, ant proliferative, anti-inflammatory. Rats were pre-treated with 200 mg/kg and 400 mg/kg/bwt dose for 3 days and fourth day with *E. coli* ( $1 \times 10^8$  CFU/ml) strain and consecutively 3 days treatment. Mortality was monitored for 14 days. After the death of rats or completion of the experiment rats were sacrifice and kidney were used for our protocol. Colonies were count and statically analysis was done. Results showed a significant depression of phagocytes activity occurred in *E. coli*-infected mice as compared with control mice that were not exposed to the bacterial challenge. Pre-treatment of mice with *Archis hypongea* improved bacterial clearance as well as improved phagocytes and intracellular bactericidal capacities of neutrophils. In the Ofloxacin treated mice although bacterial clearance was rapid, polymorph phagocytosis was depressed. These data denote that the intraperitoneal administration of *Archis hypongea* extract significantly modifies the course of *E. coli*-induced peritonitis and bacteraemia due, in part, to extract-induced enhancement of macrophage function and strong presence of flavonoids, phenols, alkaloids and tannins in methanol and ethyl acetate extracts. In the present study, bacterial isolates showed increased resistance to commonly used antibiotics. The *Archis hypongea* skin extracts showed promising antibacterial activity against the resistant bacterial strains.

**Key words:** *Archis hypongea*; Ofloxacin; *Escherichia coli*; Flavonoids

## Alleviation of drought stress in rice through ascorbic acid-mediated *Azotobacter chroococcum* Avi2

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### Abstract

*Azotobacter chroococcum* Avi2 (MCC 3432) is a potent endophytic plant-growth promoting diazotroph, isolated from rice root (*Var.* Swarna) and having capability to supplement around 25% of chemical nitrogen fertilizer in rice. The efficacy of Avi2 under oxidative stress has already been proved and documented through ascorbic acid (AA) formulation. In the present study, a pot experiment was done to analyze the plant-growth promoting ability of AA mediated-Avi2 under drought stress by using drought tolerant (Ankit, Satyabhama) and susceptible (Naveen and IR64) rice cultivars. Treatments designed for the present study were flooded control (FC), moisture stress (MS), MS+Avi2 inoculation (Avi2), MS+ AA and MS+ Avi2 +AA. Results indicated that stress indicator enzymes (superoxide dismutase, catalase, ascorbate peroxidase and proline), electrolytic leakage, chl a, chl b and total chlorophyll contents were drastically increased in plant cells under MS compared to FC, whereas Avi2+ AA treated plant under MS showed these values *at par* with FC. Fluorescence chlorophyll imaging technique was used to observe the photosynthetic efficiency of respective cultivars imposed with different treatments. Avi2+AA-treated rice cultivars imposed with MS showed the higher PS II quantum yield, photochemical (qP) and non-photochemical quenching (qN) compared to FC. Copy numbers of *nifH* (specific gene for *Azotobacter chroococcum*) in rice roots of Satyabhama, Ankit, IR64 and Naveen were significantly increased by 39.50%, 37.24%, 24.49% and 25.54%, respectively in Avi2+ AA treatment under MS compared to FC. Interestingly, Avi2+ AA-treated Naveen and IR64 showed the higher grain yield under MS compared to MS alone. Overall, we conclude that AA-based Avi2 formulation could be used as a novel and effective to alleviate drought stress in rice.

**Keywords:** Drought; Ascorbic acid; *Azotobacter chroococcum* Avi2; Fluorescence chlorophyll imaging; Rice

## Effect of different nutrient management practices on microbial population and carbon sequestration under different cropping systems

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### Abstract

Field experiment was carried out at main research farm of ICAR-Indian Institute of Farming Systems Research, Modipuram since 2004 to evaluate the effect of different nutrient management options on soil microbial population and carbon sequestration. Six different nutrient management treatments were followed i.e. T1; 100% organic, T2; 75% organic + 25% inorganic, T3; 50% organic + 50% inorganic, T4; 25% organic + 75% inorganic T5; 100% inorganic and T6 State recommendation with four different cropping systems i.e. CS1 (Basmati rice- wheat- Sesbania), CS2 (Rice- Barley- Green Gram) CS3 [Maize (popcorn) - Potato- Okra] and CS4 [Maize (sweet corn)-Mustard-Sesbania Green Manure]. Among the different treatments, the maximum bacterial population ( $2.24 \times 10^7$  CFU/g dry soil) and actinomycetes population

( $8.8 \times 10^6$  CFU/g dry soil) was observed in the 100% organic plot under CS1 (Basmati rice- wheat- Sesbania). The highest fungal population ( $7.2 \times 10^4$  CFU/g dry soil) was observed in the 100% organic plot under CS2 (Rice- Barley- Green Gram). A continuous decrease in the microbial population was observed with increasing proportion of chemical fertilizers. The minimum population of bacteria, fungi and actinomycetes was observed in 100% inorganic plots. Highest alkaline phosphatase activity was found significantly higher in 100% organic plots. Mycorrhizal fungi form a mutualistic association with plants receiving photosynthate from plants. Glomalin which is a glycoprotein secreted by arbuscular mycorrhizal fungi is directly linked to soil aggregation and positively correlated with soil aggregate stability. Abundance of arbuscular mycorrhizal fungal hyphae and soil aggregation are also positively correlated with C and N sequestration. Among the different nutrient management treatments, highest glomalin was extracted from the 100% organic plots of all four different cropping systems. The maximum glomalin content ( $652.74 \pm 7.97$  mg/kg soil) was found in 100 % organic plot of CS2 (Rice- Barley- Green Gram). With the increasing proportion of chemical fertilizers, decrease in the glomalin content was observed which indicate that chemical fertilizers negatively affect the colonization of arbuscular mycorrhizal fungi. The lowest amount of glomalin was extracted from the soil of 100% inorganic plots.

**Keywords:** Carbon Sequestration; Glomalin; Arbuscular Mycorrhizal fungi

AMT-47

## Rhizosphere Engineering and Modern Agriculture

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### Abstract

An approach that merits consideration is the use of rhizosphere microorganisms in the control of weeds. In a field trial, the efficacy of a strain of *Pseudomonas* spp. from the rhizosphere in controlling the growth of downy brome weed has been demonstrated in the USA. This approach needs to be followed up with other weeds of many important crop plants. All components of the rhizosphere can be engineered to promote plant health and growth, two features that strongly depend upon the interactions of living organisms with their environment. Increase in global population, drastic changes in the environment, soil degradation and decrease in quality and quantity of agricultural productivity warranted us to adapt sustainable farming practices. For instance, plants can be engineered to modify the rhizosphere pH or to release compounds that improve nutrient availability, protect against biotic and abiotic stresses, or encourage the proliferation of beneficial microorganisms. Rhizobacteria that promote plant growth have been engineered to interfere with the synthesis of stress-induced hormones such as ethylene, which retards root growth, and to produce antibiotics and lytic enzymes active against soil borne root pathogens. Rhizosphere engineering also can involve the selection by plants of beneficial microbial populations. The fitness of root-associated bacterial communities also can be enhanced by soil amendment, a process that has allowed the selection of bacterial consortia that can interfere with bacterial pathogens. Plants also can be engineered specifically to influence their associated bacteria, as exemplified by quorum quenching strategies that suppress the virulence of pathogens of the genus *Pectobacterium*. New molecular tools and powerful biotechnological advances will continue to provide a more complete knowledge of the complex chemical and biological interactions that occur in the rhizosphere, ensuring that strategies to engineer the rhizosphere are safe, beneficial to productivity, and substantially improve the sustainability of agricultural process.

**Keywords:** Plant growth-promoting rhizobacteria (PGPR); Rhizosphere; Rhizosphere engineering

## Study of bacterial flora involved in button mushroom compost production

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### Abstract

Mushroom composting represents an interesting example of thermogenic, solid state fermentation process that results from succession of microbial community. These microorganisms convert and degrade ingredients into suitable substrate for cultivation of mushroom. It is a rich reservoir of microbial types, comprising of mesophilic and thermophilic bacteria which convert organic waste substrates into a stabilized material i.e., compost. So for present study, bacteria were isolated from compost which is already used for button mushroom production at Department of Microbiology, Dr. RPCAU, Pusa. For this, serial dilution ( $10^{-5}$  and  $10^{-6}$ ) was performed followed by plating and streaking methods and plates were incubated at  $45^{\circ}\text{C}$  and  $65^{\circ}\text{C}$  respectively. We got 12 pure isolated cultures (ranked from I1 to I12) which were further morphologically analysed under microscope after doing Gram's staining and endospore staining. It was found that out of 12 isolates, ten were rod shaped, Gram positive and endospore former while two (I4 and I7) were rod shaped but they were Gram negative and non-endospore former. They were further subjected to preliminary enzymatic (CMCase i.e., cellulase and xylanase) assays based on the formation of haloes on agar plates. Positive clones showed good colonial development and a visible clearing zone when transferred to CMC plates. The CMCase and xylanase activity was determined by using a calibration curve for D glucose and xylose and one unit of CMCase and xylanase activity was defined as the amount of enzyme that released  $1\ \mu\text{mol}$  of reducing sugars as glucose equivalents  $\text{min}^{-1}$  or xylose equivalents  $\text{min}^{-1}$  in case of CMCase and xylanase respectively. Except two isolates (I4 and I9), all the isolates showed considerable CMCase activity, reaching their maxima (0.5 to 0.9 unit/mL) after 72 hr of cultivation while the other two isolates, presented relatively lower activities. In case of xylanase activity, six isolates (I1, I2, I4, I8, I9 and I10) showed considerable activities with maximum values (9.0 to 12.0 unit/mL) after 72 hr of cultivation. The other four isolates (I3, I5, I6 and I7), presented lower overall activity from the beginning of cultivation. These isolates can be used for preparation of microbial inoculum for compost formation of button mushroom.

**Keywords:** Compost; Cellulose; Xylanase; CMCase; Thermogenic

AMT-49

## Microbe Based Strategy to Improve Sulphur Nutrition in Pigeon Pea

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### Abstract

Pigeon pea is the second most important pulse crop grown in India. Sulphur is one of the key elements for higher pulse production and plays an important role in the formation of proteins, vitamins and

enzymes. Sulphur is a constituent of the essential amino acids cystine, cysteine and methionine. Looking at its importance, the aim of the present study was to develop microbe based strategies to improve sulphur nutrition in pigeon pea. Sulphur is taken up by the plants in sulphate ( $\text{SO}_4^{2-}$ ) form and thereby it is necessary to engineer the rhizosphere of plants with sulphur oxidizing bacteria (SOB). An attempt was made to isolate SOB from coal mine drainage in Dhanbad and 15 isolates were obtained. They were able to reduce pH from 6.0 to 2.0, when grown on thiosulfate medium. All the isolates were tested for their ability to oxidize sulphur and could sulphate in the range of 88.50 - 205.04mg/L. The five most efficient isolates were identified as *Rhizobium pusense*, *Agrobacterium fabrum*, *Klebsiella Pneumoniae*, *Stentrophomonas pavanii* and *Stentrophomonas maltophilia* on the basis 16S rRNA sequence homology. These cultures were used for seed germination and all the cultures were found to enhance seed germination and seedling growth. Further these cultures were used in Leonard jar experiment for this sterilized seeds of pigeon pea were inoculated with broth culture of each SOB. After 30 days of sowing, SPAD index, root growth parameters like root average diameter, root volume, root surface area, root length, etc., were analyzed using root scanner. All the plants inoculated with the bacterial strains showed positive result in the plant growth parameters compared to untreated control. This demonstrates a favorable plant-bacteria interaction, which can be utilized further as an alternative to inorganic sulphur source for pigeon pea.

**Keywords:** Sulphur oxidizing bacteria; coal mine drainage; pH reduction; SPAD index; Root scanner



## Development of agri-horticultural waste based rural technology for mass production of *Spirulina*

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### Abstract

In the present world, the global food protein shortage is the greatest diet related challenging problem due to improper utilization of non-agricultural land. Hence, there is an urgent need to find other protein sources. *Spirulina* is multicellular filamentous non-nitrogen fixing blue-green algae, with high protein content, being largely used as protein supplement for humans. In the present study, a cost-effective fertilizer-based culture medium was formulated for the mass production of *Spirulina* using NPK-12:32:16 complex fertilizer and growth results were compared with standard Zarrouk's media (ZM). Four strains of *Spirulina platensis* viz. (CCC-480), (CCC-538), (CCC-540) and (CCC-539) were taken from CCUBGA (Centre for Conservation and utilization of blue-green algae), IARI, New Delhi. The formulated NPK fertilizer medium was amended with varying concentration of NPK(12:32:16) fertilizer i.e., 0.5 g/L, 0.75 g/L and 1.0 g/L, sodium bicarbonate (9.0 g/L), sodium chloride (1.0 g/L) and SSP (0.1g/L). It was found that the newly formulated fertilizer medium contains 0.75g/L of NPK(12:32:16) fertilizer showed maximum turbidity (1.203), total chlorophyll (6.82µg/mL) and protein content (0.54 mg/mL) at 21 days of incubation. It was also found that the CCC-480 strain showed better growth response in NPK fertilizer mediated media which were on par to the standard culture media, so CCC-480 were taken for further study. During further experiment, the influence of agri-horticultural wastes i.e., sugarcane bagasse powder (SBP) and banana peel powder (BPP) on growth characteristics of *Spirulina* was studied. For this, the developed modified NPK media were amended with varying concentration SBP(5-50%) and BPP(5-50%) and kept at three different locations i.e., culture room, polyhouse and outdoor condition. Growth parameters were observed at an interval of 7 days up to 21 days. Through statistical analysis, it was found that among all the treatments, irrespective of their incubation conditions, treatment containing 35-45% SB, 5-15% BP and 0.75g NPK mediated media showed better result. Therefore, we can use these treatments for mass production of *Spirulina* at rural scale.

**Keywords:** *Spirulina*; Blue-green algae; Zarrouk's media; Sugarcane bagasse powder; Banana peel powder

AMT-51

## Development of multifunctional halo - tolerant consortium biofertilizer of *Rhizobium* sp. and *Enterococcus mundtii* in summer mungbean (*Vigna radiata* L.)

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## Abstract

Present study was carried out to investigate the synergistic effect of compatible consortium of salt tolerating *Rhizobium* and rhizobacterium containing 1 - aminocyclopropane - 1 -carboxylate (ACC) deaminase for improving plant growth promoting (PGP) traits, symbiotic efficiency, soil quality and grain yield of summer mungbean (SML - 668 and SML - 832), under normal and saline soil conditions. Compatibility of 8 *Rhizobium* and 4 rhizobacterium was assessed for development of consortium biofertilizer on the basis of salt tolerance, multifunctional PGP traits, bio - control activity and presence of *nifH*, *acds*, *pqq* and *ipdc* genes. In dual inoculants high significance was observed for Indole Acetic Acid (IAA) ( $84.09 \pm 2.23 \mu\text{g mL}^{-1}$ ), P - solubilization ( $12.20 \pm 1.70 \text{ mg}100 \text{ mL}^{-1}$ ), biofilm formation ( $0.74 \pm 0.05$ ), salt tolerance ( $1.88 \pm 0.04$  at  $8 \text{ dS m}^{-1}$ ), ACCD activity ( $0.75 \pm 0.06 \text{ mM } \alpha\text{-ketobutyrate } \mu\text{g}^{-1} \text{ protein h}^{-1}$ ) and seed vigour index I & II ( $1750 \pm 37.7$  and  $89.7 \pm 3.29$ ), with LSMR - 32 + LSMRS - 3, as compared to recommended consortium biofertilizer LSMR - 1 + RB - 3. Plant growth, symbiotic and soil quality parameters were highly significant at flowering stage with dual inoculant of LSMR - 32 + LSMRS - 3 treatment over *Rhizobium* sp. alone as well as control under salt stress soil, including nodulation, leghaemoglobin content, phosphatase activities and soil dehydrogenase activity. Co - inoculation of *Rhizobium* sp. LSMR - 32 (MH644039) and *Enterococcus mundtii* LSMRS - 3 (MH644178) significantly improved grain yield by 8.8% and 7.2% for SML - 668 and SML - 832, respectively over the control. Results suggest that salt tolerant dual inoculants LSMR - 32 and LSMRS - 3 could be considered as a novel approach for improving productivity and inducing salt tolerance in summer mungbean under adverse climate conditions.

**Keywords:** ACC deaminase; *Enterococcus mundtii*; *Rhizobium*; Salt stress; Summer mungbean

AMT-52

## Elucidating C-N dynamics of cyanobacteria-maize genotypes interactions

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## Abstract

Cyanobacterial inoculation is known to enhance crop growth and improve soil structure and functioning; however, their interactions with maize genotypes in terms of making available C, N in the soil is less investigated. The interactions of two cyanobacterial inoculants- *Anabaena* - *Nostoc* consortium (BF1-4) and An-Tr (*Anabaena* based biofilm - *Trichoderma viride*) with ten maize genotypes were assessed in relation to plant growth parameters and C-N related enzymes, both under in vitro and in vivo (field) conditions. Laboratory water agar mesocosms illustrated that An-Tr (*Anabaena* based biofilm) was superior in its performance across genotypes recording significant enhancement of 4-17, 17-59 % and 1-2 fold in terms of proteins, dry weight, reserve carbohydrate mobilizing enzymes respectively, and 7.5-50% in concentration of leaf photosynthetic pigments, after six days. Field experiment undertaken with the same set of genotypes and inoculant combinations recorded interesting data on nitrogen fixation in soil (measured as acetylene reducing activity; ARA), leaf Phosphoenol pyruvate carboxylase (PEPcase) activity and total chlorophyll at flag leaf stage. In general, both cyanobacterial inoculants- An-Tr and *Anabaena* -*Nostoc* consortium (BF1-4) brought about distinct enhancement across genotypes, as evidenced by significantly higher activity, over control. Nitrogen fixation was significantly higher in treatments receiving *Anabaena*

-*Nostoc* consortium (BF1-4), while significantly higher leaf PEPcase activity was observed with An-Tr. Distinct correlation was recorded among the parameters, specifically PEP carboxylase activity with leaf chlorophyll (0.8,  $p < 0.05$ ), ARA (0.65,  $p < 0.05$ ) and catalase activity (0.74,  $p < 0.05$ ). Principal Component Analysis (PCA) illustrated that the first and second principal components accounted for 66.9%, while first three principal components represented 78.5% of the cumulative variance among the variables. Our investigation helped in elucidating the assortment of the best genotype- microorganism interactions, for enhancing crop nutrition and vigor. Such cyanobacteria based biofilm formulations may be used for enriching C-N in nutrient-poor or degraded soils.

**Keywords:** Cyanobacterial inoculants; maize genotypes; mesocosms; acetylene reducing activity; Principal Component Analysis; nutrient cycling

AMT-53

### Effect of *Moringa oleifera* extracts on the biology of *Callosobruchus maculatus*

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#### Abstract

*Moringa oleifera* is the most widely cultivated species of genus *Moringa*. The miracle plant is overflowing with vitamins such as Vitamin A, B, C, D, E and minerals such as Potassium, Calcium, Iron, Selenium and Magnesium. *Moringa* leaves are completely safe for consumption and have no side effects on human health. It is also used in hand wash and in herbal medicines. Insect pest infestation is a major problem in the production of cowpea. It causes up to 95% yield reductions depending on location, year and cultivation. Biotic factors are responsible for the losses in field as well as in the store. The main pests during growing season are the aphids while main storage pest are the bruchids. The primary insect causing losses to stored cowpeas is cowpea weevil, *Callosobruchus maculatus*. The present study was carried out to study the effect of *Moringa oleifera* plant parts on fecundity, hatching, emergence of adults, mortality and sex ratio of *Callosobruchus maculatus*. The results showed that the legume species (cowpea) can be protected against the pulse beetle with use of plant extracts. The fecundity and the hatching rate of insect varied significantly in the control and treated ones. *Moringa* leaf extracts (90% mortality at 3.5ml) has been found to have highest insecticidal activity against pulse beetle than its stem extracts (83% at 3.5ml). The results showed that 90% of the bruchid beetles treated with *moringa* leaf died within 6 days after the newly emerged insects were introduced in cowpea seeds under the effect of *moringa* plant extract. In normal conditions life span of *Callosobruchus maculatus* is nearly 15-20 days. Therefore the extract of these plants can be used as protectants for legume species from *Callosobruchus maculatus*. The significant effect of the plant part extracts is observed on the total development time period of the insect, the egg laying, hatching of eggs and adult emergence.

**Keywords:** *Moringa oleifera*; *Callosobruchus maculatus*; extracts

## Screening of *Bacillus* and *Pseudomonas* strains for biosurfactant production

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### Abstract

Biosurfactants are amphiphilic compounds produced by various bacteria which reduce surface and interfacial tension and have gained considerable interest in recent years because of their wide applications. These compounds have antimicrobial activity (against fungal pathogens) and could be considered as biocontrol agent(s) to overcome the harmful effects of chemical pesticides on agricultural crops. In this study, three strains of *Bacillus subtilis*, B1, B2 and B3 and two strains of *Pseudomonas aeruginosa*, P1 and P2 were taken from Division of Microbiology, FBS & H and were screened for biosurfactant production. The potential biosurfactant producers were initially screened on Cetyl Tri Ammonium Bromide (CTAB)-Methylene blue agar. The strains showing halo on the selective CTAB-Methylene blue agar medium were further screened by using oil spreading test and emulsification activity. All the strains, except B2, showed halos around the colonies on CTAB- Methylene blue agar medium. In case of oil spreading test, a range of 4.2 -17.52 cm<sup>2</sup> clear zone was obtained with B3 showed maximum value of 17.52cm<sup>2</sup> followed by P2 (16.50 cm<sup>2</sup>). Similar reports were observed in case of emulsification index where B3 followed by P2 showed maximum value of 31.50 and 26.74% respectively. It was found that biosurfactant production commenced within 24 hours and showed maximum values at 96 hours. Out of five strains, B3 showed maximum amount of biosurfactant production i.e., 1.19 g/L followed by P2 with value of 1.04 g/L. So, these bacteria can be utilized have biosurfactant producing ability which can be utilized as biocontrol agents.

**Keywords:** Biosurfactant; Amphiphilic; Biocontrol Agent; Emulsification

## Synergistic action of CuNPs-augmented *Calothrix elenkinii* as a biocontrol option against *Fusarium solani*

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### Abstract

Antifungal potential of copper nanoparticles (CuNPs) and cyanobacterium *Calothrix elenkinii* is documented, however, their combination as a biocontrol option has not been explored. *Fusarium solani* causes root rot, a major challenge in the vegetable production, especially in tomato, leads to 70-90% losses in crop productivity and yields. Keeping this in view, the influence of CuO NPs-augmented *Calothrix elenkinii* against *Fusarium solani* in tomato crop was investigated under greenhouse conditions. Preliminary invitro studies concluded that CuNPs at 100 mg L<sup>-1</sup> concentration was most significant in terms of elicitation of antioxidant machinery of *C. elenkinii* (upto 3.0, 3.1, and 1.8-fold increase in the

activities of PAL, PPO and PO enzyme over control, respectively) and inhibition of *F. solani* growth. Soil application of CuNPs augmented *C. elenkinii* in *F. solani* challenged tomato plants led to 63-76% reduction in the severity of *Fusarium* root rot. An increase in the peroxidase activity (46%) and higher tomato yield was recorded, which was attributed to improved root architecture, in terms of root volume, area, length and weight. CuNPs augmented *C. elenkinii* application showed 20-31% reduction in the total phenol content in *F. solani* challenged plants and the soil PLFA profiles showed 40% reduction in the concentrations of fungal PLFA marker (18:2w6, 9c), compared to *F. solani* treated alone. Application of *C. elenkinii* led to an increase in the concentration of micronutrients like Mn, Zn and Cu with a concomitant decrease in disease severity, illustrating the role of availability of micronutrients in imparting resistance against *F. solani*. This highlights the indirect mechanisms used by CuNPs-Ce in imparting resistance and enhancing vigour of the host, through modulation of enzymatic activities and inhibiting the proliferation of the pathogen. Such biocontrol options can also be tested against *Fusarium* root rot of tomato under field conditions, as a promising novel option.

**Keywords:** *Fusarium* root-rot; Copper nanoparticles; *Calothrix*; Antioxidant; Biocontrol

AMT-56

### Prospecting soil-less substrates as carriers for cyanobacteria

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#### Abstract

Cyanobacteria are photosynthetic prokaryotes, many of which exhibit diazotrophy and flourish in diverse ecologies. They are used as bioagents in agriculture, and the viability of the inocula being introduced is influenced by the type of carrier. Commonly, soil, wheat straw and multani mitti (Fuller's earth) are deployed as carriers. In the present investigation, the potential of soil-less substrates [perlite (P), cocopeat (C) and vermiculite (V), individually and in combinations (1:1) such as - perlite: cocopeat, perlite: vermiculite, cocopeat: vermiculite and perlite: cocopeat: vermiculite (1:1:1)] was explored as carriers. Two agriculturally beneficial cyanobacterial cultures - *Calothrix elenkinii* and *Anabaena laxa* were evaluated, firstly, for biochemical attributes such as pigments, phenols, and flavanoids and then inoculated into the substrates. Time course analyses after 2, 10 and 20 weeks illustrated that chlorophyll, as an index of photosynthetic biomass enhanced significantly and among the two cultures, *Calothrix elenkinii* was the better performer. P:C and C:V were identified as most promising, and augmentation with either of the cyanobacteria was promising in terms of extended shelf-life. *C. elenkinii* proved to be more promising in terms of chlorophyll accretion, IAA and phenol content of the amended mixes. Microscopy and PCR amplification using 16S cyanobacterial directed primers confirmed their colonization in these substrates. Seed germination test with maize and wheat seeds showed marginal increases as a result of augmentation, with no phytotoxicity. Such novel mixes are being tested in pot and field experiments for their promise as biofertilizers.

**Keywords:** Chlorophyll; Cyanobacterium; Soil less substrate

## Chickpea rhizospheric PGPR mediated plant-microbe interactions for controlling *Fusarium oxysporum* sp. *Ciceris*

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### Abstract

The present investigation was undertaken to develop a plant growth promoting microbial bioinoculant as biofertilizer and bioprotectant to reduce the incidence of *Fusarium* wilt in chickpea for improving crop productivity in sustainable manner. *Mesorhizobium* (15) and endophytic bacterial (5) isolates were assessed for antagonism against wilt causing *Fusarium oxysporum* sp. *ciceris*. Isolates of *Mesorhizobium* (6) and endophytic bacteria (5) were selected as potential antagonists on the basis of fungal growth inhibition. Compatible consortium of *Mesorhizobium* sp. and endophytic bacteria was developed by plate assay and broth studies, further screened for multifarious PGP traits viz. IAA, Phosphate-solubilization, siderophore, ACC-deaminase and seed germination in single and dual treatments. Maximum IAA (50.13µg/ml) and siderophore production (4.0cm) was recorded with LGR33+RB-1. Highest P-solubilization (13.93mg/100ml) and ACC-deaminase (OD<sub>600nm</sub> 1.492) was observed with LGR2+NE8. *Mesorhizobium* (LGR33 and LGR2) and all endophytic isolates were able to grow on recommended dose of captan in disc plate method. Synergistic effect of *Mesorhizobium* sp. and endophytic bacteria evaluated for biocontrol, growth, symbiotic parameters and grain yield in chickpea (*desi* PBG-7 and PBG-1) under wilt sick field (maintained from 10 years) showed significant improvement in growth and symbiotic parameters with LGR2+NE8 (69NN, 104.24mg nodule dry weight, leghaemoglobin content of 5.13µg/g nodules/plant) as compared to control. An enhanced antioxidant enzymes viz. catalase (15.04U/min/g FW), peroxidase (2.404U/min/g FW) and total phenols (109.54 tannic acid equivalents/g FW) were observed with the appearance of disease in chickpea roots neutralizing the reactive oxygen species formed during the pathogen attack. On pooled mean basis dual inoculant LGR2+LCNE8 improved the grain yield by 6.5% over control. Consortium of native LGR2 and LCNE8 can be developed further as bioinoculant in improving the chickpea productivity in an eco-friendly manner.

**Keywords:** Biocontrol; chickpea; endophytes; *Fusarium*; *Mesorhizobium*

## Optimization of process parameters for enhanced saccharification of rice straw under solid state fermentation from *Phanerochaete chrysosporium*

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### Abstract

In the concern study, rice straw biomass collected from different areas on the basis of abundance, used as a substrate for fermentable sugars with the help of potential fungal strain i.e. *Phanerochaete chrysosporium*. Different pretreatment methods were employed to rice straw, out of them the best method was employed for further study. Different process parameters for biological hydrolysis of pretreated biomass were optimized through classical approach one factor at a time (OFAT) approach. The best optimized conditions so obtained were used for biological hydrolysis of rice straw and appreciable amount of reducing sugars were observed.

**Keywords:** Bioconversion; Rice straw; *P. chrysosporium*; Saccharification

AMT-59

### Exploring the potential of aloe vera as a carrier for developing a novel cyanobacterial formulation

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### Abstract

Cyanobacterial inoculation is known to improve nutritional status of soil health, stimulate growth of plants; besides reducing the severity of diseases. Development of an eco-friendly, cheap formulation ensuring stability with simple storage and application process has been a roadblock for its large-scale adaptation. In this context, the biochemical properties of nature's gift aloe vera was utilized to develop a plant (aloe vera gel) based liquid formulation (AE) without use of any additional chemical (protectants) for stabilization. The test organism was a beneficial cyanobacterium *Anabaena laxa* (RPAN8), which was used with inoculum load of 1%, 2% and 5% in different dilutions (Pure, 1:1, 1:5, 1:10) of AE in a time course study. Results indicated that all the dilutions could support cyanobacterial growth (as evident from increase in chlorophyll content) upto 70 days. Phosphorus (P) content of AE samples declined (28%) following cyanobacterial growth, indicating that P present in aloe vera were utilized by the cyanobacterium, helping it to proliferate. Interestingly, Nitrogen (N) and Potassium (K) content of AE samples were enhanced following cyanobacterial growth, which may be linked with the N fixation and K solubilization activities of the cyanobacterium. Growth was found to be higher (1.4- 2 folds on 35th day of storage) in 1:10 dilution as compared to lower dilutions or pure sample, with all the inocula levels. Microscopy and PCR based analyses confirmed the establishment of the viable populations of the inoculated cyanobacterium in the aloe vera formulations. Most importantly, these formulations were also able to maintain the plant growth promoting trait and biocontrol efficacy up to 70 days. Significant improvement of germination of wheat and mustard seeds was recorded, as compared to untreated control. This can be a novel approach to grow, store and develop as a carrier for cyanobacteria, to retain viability over long periods of time.

**Keywords:** Aloe vera; chlorophyll; cyanobacterium; nitrogen; shelf life; viability

## Isolation and characterization of cyanobacterial strains from rhizospheric soil and plant parts of selected rice varieties from different locations of India

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### Abstract

Cyanobacteria are a multifaceted group of photosynthetic prokaryotes, with large morphological diversity including unicellular, colonial and filamentous forms. These exhibit a wide range of nutritional capabilities ranging from obligate phototrophy to heterotrophy, although majority of forms examined so far exhibit phototrophy. They flourish in rice fields and play a dominant role in sustaining the fertility of this ecosystem. Various benefits attributed to rice crop from cyanobacteria include supply of biologically fixed nitrogen, hence, increase the nitrogen economy of crops, which, in turn, reduces the requirement for nitrogenous fertilizers. They also release phytohormones like indole-3-acetic acid and cytokinins which improve plant vigor. These organisms are photosynthetic, provide oxygen to the submerged rhizosphere, improve the carbon status of soil and help in amelioration of salinity and phosphates solubilization. Present study involved characterization of cyanobacterial strains isolated from rhizospheric soil and plant parts of selected rice varieties from four locations of India *viz.*, IARI New Delhi, Punjab, Meghalaya and Kerala, in terms of growth attributes, pigment profile and plant growth promoting (PGP) parameters. Two varieties were used in the study from each location. Out of total eighty five cyanobacterial strains isolated, seventy were heterocystous and remaining fifteen were non-heterocystous. The number of strains isolated from each location was 28, 18, 16 and 23 respectively. Chlorophyll content ( $\mu\text{g ml}^{-1}$ ) varied from 0.99-9.18, 1.51-10.43, 0.88-10.89 and 0.69-7.35 for New Delhi, Punjab, Meghalaya and Kerala isolates. Similarly, total soluble proteins ( $\text{mg ml}^{-1}$ ) ranged as 0.17-0.94, 0.29-0.69, 0.21-0.94 and 0.22-0.71 and nitrogenase activity ( $\text{nmoles C}_2\text{H}_4 \text{mg}^{-1} \text{chl h}^{-1}$ ) analysed as acetylene reduction assay varied as 260.77-3168.51, 317.02-1180.65, 291.05-1743.70 and 349.71-3552.44 for the isolates from four locations. Extracellular ammonia release ( $\mu\text{mole ml}^{-1}$ ) ranged from highest of 143.80, 229.70, 570.40, 116.20 to the lowest of 9.60, 8.80, 14.30 and 10.30 amongst isolates from four locations. The cyanobacterial strains exhibited a beneficial influence on seed germination of rice (variety P-1612) in comparison to control. Germination percentage was 92 % under control, however, it ranged from 95%-98%, 93%-97%, 93%-97% and 92%-95% using specific strains with efficient PGP attributes. In view of this, the study can be useful as a prelude to their utilization as effective bioinoculants in rice crop for better nutrient management and improved crop yields.

**Keywords:** Cyanobacteria; Plant growth promoting (PGP); Phosphates solubilisation; Indole-3-acetic acid (IAA); Cytokinins

AMT-61

## Studies on endophytic and rhizospheric bacteria for biocontrol and plant growth promoting potential in rice plants

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### Abstract

A total of 1099 endophytic and rhizospheric bacteria including actinobacteria were isolated from various biotopes in Manipur. Of the 774 endophytic bacteria obtained from 36 ethnomedicinal plants of Manipur, 311 showed antifungal activities against one or more 6 major rice fungal pathogens. 56 endophytes were found to show good plant growth promoting (PGP) activities. Of these 56 PGP active isolates, 14 enhanced seedling growth and showed higher vigor indices over the control. Four isolates (AcRz21, PtL11, TgIB5 and TgIB12) exhibiting highest vigor indices were further assayed for rice PGP field trials under *R. solani* challenged conditions. Isolates AcRz21, PtL11 and TgIB5 significantly increased the growth of rice plants and decreased the disease lesions caused by *R. solani*. Of the 325 rhizospheric bacteria obtained, 52 showed antifungal activities against almost all the major rice fungal pathogens. Among these positive strains, the most promising, MBRL- 576 was further screened for PGP activities and showed good PGP activity and also could significantly increase rice root lengths and dry root weights over the control in pot trials.

**Keywords:** Endophytic; Rhizospheric bacteria; Antifungal activities; Vigor indices; Plant Growth Promotion

AMT-62

### Wheat seed harbours endophytic bacteria: dominance of plant growth promoters

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### Abstract

Seed-associated bacterial endophytes may play an important role in seed preservation and germination. Bacterial endophytes are commensal plant colonizers, acting as plant growth promoters under diverse environmental conditions. It is assumed that the diversity and performance of endophytes depend on the hosts genotype and condition, growth stage, plant tissue, soil conditions and associated microbiota. Seed endophytes are of particular interest as they are vertically transmitted to the progeny providing host-aligned potential germination promoting features for the next generation. The present study was aimed to isolate and characterize the seed-borne endophytic bacteria from diverse wheat ecotypes (*Triticum aestivum*) commercially sown in India and to look for plant growth promotion features. Forty-nine (49) wheat seed endophytic bacteria (WSEB) were isolated from seed endosperm of 6 popular wheat genotypes of 2 agroecological zones of India viz. North Western Plain Zone and Peninsular Zone. All endophytic bacterial isolates were screened for plant growth promoting activities such as solubilization of P, K, nitrogen fixation ability (ARA), production of siderophores, IAA, NH<sub>3</sub> and HCN. Majority of isolates exhibited plant growth promoting activities; 38 exhibited P solubilization, 3 solubilized K and 5, 3, 31, 29, 21 isolates produced siderophores, ARA, IAA, NH<sub>3</sub>, HCN respectively. *Pseudomonas parafulva* and *Pantoea agglomerans* had multi PGP activities for P, K solubilization, siderophore, ARA and IAA production. One out of 5 *Bacillus subtilis* and *Bacillus proteolyticus* isolates were positive for P, siderophore, ARA and IAA activities. Many WSEB exhibited tolerance to salinity (15%) and osmotic stress (15% PEG6000), indicating their possible application under stress conditions. These PGP traits can be helpful in endophytic establishment and advantageous to the host plant. Based on morphometry and PGP characteristics, 15 isolates were selected for genomic characterization using 16S rRNA gene sequence analysis, which revealed that 13 isolates belong to gram positive *Bacillus* group as *Bacillus subtilis* (5), *Bacillus megatarium* (2), *Bacillus cereus* (1), *Bacillus proteus* (1), *Bacillus aerius* (1), *Bacillus aryabhatai* (1), *Bacillus stratosphericus* (1) and *Bacillus halotolerans* (1). Only two isolates were gram negative as *Pseudomonas parafulva* and *Pantoea agglomerance*. Endophytic nature of the selected WSEB isolates was confirmed for their presence in roots of the host seedlings with the help of TTC (2,3,5-triphenyl-2H-tetrazolium chloride) and H<sub>2</sub>O<sub>2</sub> staining for inter & intra cellular movement after the germination of seeds. Thus, the WSEB identified in the present study can be explored as potential bio inputs for

improving plant growth and productivity under stressed conditions, besides helping in understanding the plant-endophyte interactions for agricultural applications, could further be transmitted during plant growth.

AMT-63

### Qualitative and quantitative assay for siderophore producing bacteria in calcareous soil

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#### Abstract

Iron although the fourth most abundant element in the soil, its deficiency is a worldwide problem and usually occurs in many crops. Iron deficiency is common to calcareous soils, which cover over 30 per cent of the Earth's land surface. Siderophores are low molecular weight compounds produced under iron-limiting conditions by microorganisms that chelates Fe<sup>3+</sup> (ferric iron) with high specific activity, which in turn make it available to the plant system. In the present study the calcareous soil samples were collected from 10 different locations and enriched with minimal medium. Thirty three different bacteria were isolated based on morphological characters and further screened to seventeen based of CAS plate assay. All the seventeen isolates were also tested for the quantity and type of siderophore. Quantification of siderophore production in CAS media ranged from 1.472 to 16.39 percent Siderophore Units (PSU). Subsequently, chemotyping of siderophore by Arnow's assay for catechol type siderophore test proved that nine isolates had the capability to produce Catechol type of siderophore. Further qualitative analysis of tetrazolium salt test for Hydroxamate type of siderophore and Vogel's chemical test for Carboxylate type of siderophore was performed. Artificial calcareousness was induced in Nutrient agar medium with KHCO<sub>3</sub> under four different concentrations viz., 10, 15, 20 and 25 mM. The maximum population (CFU) was recorded in 20 and 25 mM KHCO<sub>3</sub> concentration irrespective of bacterial isolates. The Colony Forming Units (CFU) ranges from 6.06 to 7.21 log<sub>10</sub> CFU ml<sup>-1</sup> of culture. For the plant to be healthier and more competitive, a formulation of such siderophore producing bacterial isolates can be used to increase iron supply.

**Keywords:** Siderophore, CAS, Catechol, Hydroxamate and Carboxylate.

AMT-64

### Physiological responses of the cyanobacterium *Anabaena doliolum* exposed to salinity induced by chloride and sulphate

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#### Abstract

In general the soil salinity is of mixed type where Na<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> and Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup> and HCO<sub>3</sub><sup>-</sup> are the dominant cations and anions respectively. However, most of the studies on salt toxicity are conducted

using NaCl. Therefore, in the present investigation, the effect of chloride and sulphate induced salinity on the growth and physiology of the nitrogen fixing cyanobacterium *Anabaena doliolum* was studied. It was observed that both sulphate and chloride decrease growth and cellular constituents such as protein, chlorophyll and phycobiliproteins of the test cyanobacterium. However, the sugar content was found to increase in the cyanobacterium with time and attained their maximal value at 144 hour of salt exposure. Intracellular Na<sup>+</sup> levels increased due to NaCl induced salinity whereas the cells exposed to Na<sub>2</sub>SO<sub>4</sub> accumulated less Na<sup>+</sup>. Exposure to NaCl and Na<sub>2</sub>SO<sub>4</sub> also resulted in increased intracellular K<sup>+</sup> and Ca<sup>2+</sup> content. Cells exposed to NaCl and a mixture of NaCl and Na<sub>2</sub>SO<sub>4</sub> failed to maintain favourable K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratio. On the contrary, favourable K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratio was maintained by the cells exposed to Na<sub>2</sub>SO<sub>4</sub>. The results showed that chloride was more toxic to the growth of the cyanobacterium *A. doliolum* as compared to sulphate. However, a combination of chloride and sulphate exerted further deleterious effect on the growth and physiological parameters of the cyanobacterium *A. doliolum*. The results may be useful in the evaluation and selection of appropriate cyanobacterial strains for the development of biofertilizer for salt affected soils.

**Key words:** *Anabaena*; cyanobacteria; growth; cellular constituents; salinity

AMT-65

### A new approach for dewaxation of pressmud using potent fungi

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#### Abstract

Sugarcane industry is the second largest agro-industry in the country with several co-products one of which is pressmud (produced during clarification of sugarcane juice). Pressmud is a waste for the sugar industry but contains nutrients mainly N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O, and variety of elements like Mg, S, Zn, Cu, Fe, etc. The indiscriminate application of chemical fertilizers has completely destroyed soil health. The composted pressmud is one of the alternative organic fertilizers for the improvement of soil microbial health and fertility. However, due to improper decomposition of pressmud into a value-added product along with high wax content (6.70–11.01%), it cannot be applied directly to the soil. Repeated application of undecomposed pressmud clogs the soil. The application of wax degrading fungi during biodegradation of fresh pressmud would enhance the quality of pressmud as organic manure. A small laboratory experiment was carried out using the enrichment technique for the isolation of wax degrading fungi. A total of five isolates were obtained and designated as WX1 to WX5 based on zone of hydrolysis on the Tributyrin agar plate qualitatively. Out of five isolates, the three efficient isolates were further subjected to quantitative lipase enzyme hydrolysis and finally one isolate WX2 was selected for the application for degradation of fresh pressmud along with lignocellulolytic fungal consortium in windrows after screening the compatibility of all fungal cultures. The wax content was analysed before and after the decomposition of pressmud. The identification of the wax degrading fungus is under progress.

**Keywords:** Agro-industry; Microbial health; Pressmud Compost; Organic manure

## Metabolic profile of root exudates of rice inoculated with methanotrophs and exopolysaccharide producing bacteria

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### Abstract

One of the most important factors responsible for rhizosphere effect is the great variety of organic substances available at the root region by the way of root exudates which directly or indirectly influence the quality and quantity of rhizosphere microorganisms. The metabolic profile of root exudates of rice (CO 51) under elevated CO<sub>2</sub> level (550 ppm) inoculated with microorganisms methanotroph [*Met* (NSM, PSM)] and exopolysaccharide producing strains (EPS1, EPS2) responsible for moisture stress tolerance was studied *in vitro*. Root exudates from pot cultured paddy of three different stages namely seedling, flowering and maturity stages were collected from *Met* and EPS treatments along with an uninoculated control. Metabolic profiling of rice root exudates by GC-MS analysis revealed phthallic acid and phenolic compounds as the chief compounds making up the root exudates irrespective of the inoculants and the stages of rice growth. In the uninoculated plants, adipic acid was abundantly found (12.5 %) while in the inoculated roots it was in trace levels. The amino acids proline, alanine and glycine were present lower quantities in the inoculated root exudates which was completely non detectable in control. During maturity stage, amphetamine was detected at high concentrations (32.8 %) from the roots of *Met*-EPS inoculated rice respectively. Our study showed that amphetamine was excessively secreted in the roots of rice during maturity stage due to the *Met*-EPS inoculation. Phenolic acids are also the main constituents of root exudates and they have an allelopathic effect on the microbial community in the rhizosphere. Phenolic compounds may exert their antioxidant activity in different ways. Some phenolic compounds may bind pro-oxidant. Number of root exudates compound from rice plant were significantly greater at 21 days after transplantation than at panicle initiation. The root exudates influenced upon the inoculation of functional microbes can further be exploited for microbe-microbe and plant -microbe interaction.

**Keywords:** Root exudates; Rice; *Met*; EPS; GC-MS;

## Molecular phylogeny, host-association and genome characterization of a novel marine adapted plant-beneficial *Ciceribacter* from brackish grown wild pokkali rice

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### Abstract

Several rice varieties tolerant to brackish water stress are cultivated along the coastal agro-ecosystems of India. Pokkali, a GI tagged rice crop of Kerala is known for its brackish and submerged tolerance posed

due to frequent sea water intrusions. In account of its unique geographical location, the microbiome of pokkali is assumed to be very unique that may address the challenges of sustainable agriculture in saline-stressed farm lands to some extent. Among the several potential isolates screened, L1K22 and L1K23<sup>T</sup> showed very good growth in media containing 100% natural sea water. Moreover, hydroponic based binary association studies performed under gnotobiotic conditions confirmed that these strains require at least 20% NSW to remain viable as planktonic cells followed by its subsequent colonization with the host plant, pokkali compared to non-saline conditions. 16S rRNA gene based phylogenetic analysis confirmed the strains to be a member of *Ciceribacter* genus with only 3 species described till date. Further, Multi-locus sequence analysis comparison between L1K23<sup>T</sup> and closest reference strain, *Ciceribacter lividus* MSSRFBL1<sup>T</sup> comprising six housekeeping genes (atpD, glnAII, glnA, recA, rpoB, thrC) and genomic DNA fingerprinting confirmed the strain to be a probable novel species under the genus. The 4.80 Mbp draft genome of L1K23<sup>T</sup> harboured several plant-beneficial and brackish adaptive genes. More importantly, L1K23<sup>T</sup> genome coded for flagellar motility, chemotaxis (CheA, CheD, CheY, CheB, CheW, CheR) and tight adherence (tad) operon genes assumed to play a major role in their host colonization. In addition to type I secretion system, L1K23<sup>T</sup> genome coded for type VI secretion system aiding for its competitive colonization in the rhizosphere. Additionally, genes for ectoine degradation, transport and glycine betaine uptake signifies the brackish adaptive traits of L1K23<sup>T</sup> strains. Further studies are ongoing to understand the impact of salinity in its host association and influence.

**Keywords:** Brackish; Pokkali; Natural sea water; *Ciceribacter*; Genome

AMT-69

### A potent *Burkholderia* with broad spectrum antimicrobial activity from landrace rice variety Aiswarya of saline affected area and its genome analysis

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#### Abstract

Plant-microbe interaction plays a major role for the adaptability and productivity of plants under varying environmental conditions. The study on inland plants that grow under saline affected areas largely remains unexplored. Hence, the plant probiotic studies of these ecosystems will definitely offer an opportunity to obtain novel biologically active metabolites and biostimulant that can be of agricultural benefit. During the screening for microbial cultures producing antimicrobial agents from the rhizosphere of this saline affected inland rice, we have isolated a potential culture named as Bmkn7 that showed broad spectrum antifungal and antibacterial activity against wide range of phytopathogens (*Macrophomina phaseolina*, *Alternaria alternata*, *Fusarium oxysporum*, *Escherichia coli*, *Staphylococcus aureus* etc.). The 16S rRNA gene base phylogeny and draft genome analysis confirmed the strain to be a member of *Burkholderia* genus within cenocepacia complex. The characteristic feature of this Bmkn7 is its antagonistic effect in agar but not in broth. Interestingly, Bmkn7 also elicited antimicrobial activity in presence of 20% natural seawater. Majority of the *Burkholderia* species reported till date has siderophore mediated antimicrobial activity. As expected, siderophore production was confirmed in Bmkn7 by CAS assay. However, the

siderophore inhibitory assay done by amending 100µM iron to the CAS assay also showed antimicrobial effect against phytopathogens confirming that the antagonistic action is non-siderophore mediated. This led us to sequence the draft genome of Bmkn7 where we could identify certain unknown clusters that might encode for novel compounds analysed through antiSMASH. Finally, in-planta studies with Bmkn7 confirmed that the strain doesn't have any negative effect on rice seed germination and growth. Altogether, the above findings summarise the possibility of a novel antimicrobial compound from a rice associated *Burkholderia*, for which further analytical based characterisation studies are underway.

**Keywords:** Inland rice; Burkholderia; Antimicrobial; Seawater; Novel compound

AMT-70

### Effect of crop growth stages on microbial diversity in different rice establishment methods

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#### Abstract

Rice is the staple food for more than half of the world's population. It grows in different establishment methods, like, Aerobic (AR), Alternate wetting and drying (AWD) and Flooded (NTP). In India, rice occupies one-quarter of the total cropped area and contributes about 40 to 43 per cent of total food grain production. Rice field harbour tremendous diversity of soil microbes, soil fauna and plants. The growth and colonization of soil microorganisms can be influenced by chemical, physical and biological properties of the soil and also crop growth stage, soil microbes play a vital role in providing soil nutrients specially N and P. In an experiment to study the influence of rice crop growth stage on microbial population in different rice establishment methods, samples were collected from the IIRR research farm for total microbial enumeration at different crop growth stages viz. Tillering stage, Flowering stage, harvesting stage from different rice establishment method viz, AR, AWD and NTP. Samples were analyzed using specific media, samples collected from AR method at flowering stage was found to support highest microbial population; bacteria (270 CFU/ gram of soil), actinomycetes (120 CFU/ gram of soil) and fungi (25 CFU/ gram of soil). and NTP method at harvesting stage was found with lowest microbial population; bacteria (150 CFU/ gram of soil), actinomycetes (50 CFU/ gram of soil) and fungi (12 CFU/ gram of soil). The grain yield was highest in NTP (7.3 t/ha) followed by AWD (5.9 t/ha) and AR (3.9 t/ha).

**Keywords:** Rice; Aerobic (AR); Alternate wetting and drying (AWD); Tillering

AMT-71

### Appraisal of liquid microbial inoculants in INM of fodder cowpea

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## Abstract

India is having the richest livestock population of 520 million heads, which is about 20 per cent of the world's livestock population. Livestock contributes nearly 4% to National GDP and it is source of employment to about 70% population in rural areas. Sustainable production of quality forages in ample quantities is imperative for a profitable livestock productivity. Nevertheless, forage digestibility is related to chemical compositions particularly of fiber, lignin and crude protein to some extent. Cowpea (*Vigna unguiculata* L. Walp.) has emerged out as a potential crop for meeting the requirement of high quality fodder to fast expanding cattle population. Moreover, the demand for larger amount of fertilizers and associated environmental problems have forced us to think on the urgent need of increasing good quality forage supply by adopting improved agronomic techniques. Thus, the situation accelerates the use of non-conventional approaches like use of PGPR to enhance crop growth, yield and quality. Keeping aforesaid points in view the present investigation was carried out to evaluate the role of liquid microbial inoculants in integrated nutrient management of forage cowpea (*Vigna unguiculata*). Liquid microbial inoculants of *Burkholderia seminalis*, *Burkholderia* sp. and *Bradyrhizobium* sp. were prepared using trehalose (5mM) in basal medium and showed maximum viable count upto 180 days of storage as compared to charcoal carrier based formulations at room and refrigerated temperature. The field experiment was conducted at Punjab Agricultural University, Regional Research Station, Bathinda and Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during Kharif 2018 respectively with eleven treatment combinations of liquid microbial inoculant (*Burkholderia seminalis*, *Burkholderia* sp. and *Bradyrhizobium* sp.) with 75% and 100% RDF framed in randomized complete block design and replicated thrice. It was observed that treatment T<sub>10</sub> (75% RDF + *Burkholderia* sp. + *Burkholderia seminalis*) significantly ( $p < 0.05$ ) improved vine length, stem girth, leaf length, leaf breadth, number of branches per plant, number of leaves per plant, leaf: stem ratio, green fodder yield, dry matter yield, acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein content, in vitro dry matter digestibility (IVDMD), total ash content, total chlorophyll content, total sugars content and total phenolic content. Moreover, percentage increase in green fodder yield (q/ha) with T<sub>10</sub> treatment over T<sub>1</sub> (control) was 12.86%. Therefore, it can be concluded that liquid microbial inoculants could play a predominant role in integrated nutrient management of forage cowpea for enhanced productivity and quality.

**Keywords:** *Bradyrhizobium* sp.; *Burkholderia seminalis*; *Burkholderia* sp.; Cowpea; Forage; Integrated nutrient management; Liquid Microbial inoculants

AMT-72

### Morphological and Physiochemical Variations of Sporocarp-Producing *Azolla* Strains and its Germination Under Influence of Different Combinations of Inducing Factors

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## Abstract

*Azolla* is a small free-floating aquatic fern and reproduces sexually by producing hetero-spores. Its unique interaction with nitrogen-fixing cyanobionts, flourishes inside the leaf cavity. On the basis of vegetative and reproductive morphology, several botanists described it into seven distinct species such as *A. caroliniana*, *A. filiculoides*, *A. mexicana*, *A. microphylla*, *A. rubra*, *A. pinnata* and *A. nilotica*. Though, taxonomy of *Azolla* was extensively studied in past but still arise confusion for its characterization at the level of different species. Hence, the present study was attempted to study the variations of *Azolla* strains based on distinct morphological and physiological differences of *Azolla* at NRRI, Cuttack (Odisha) condition. Results indicated that 23 strains (out of 102 strains of *Azolla*) were sporulated in the natural condition of NRRI, Cuttack (Odisha) Results also showed that among 6 known *Azolla* spp. (*A. caroliniana*,

*A. filiculoides*, *A. microphylla*, *A. mexicana*, *A. pinnata*, and *A. rubra*), only two species viz., *A. microphylla* and *A. pinnata* had shown sporulation. At morphological level, generally two types of plant body shape (star and triangular) with different leaf imbrications (high, medium and low) were found in different *Azolla* strains. *A. pinnata* showed thick root hair under root imaging technique which was different from other *Azolla* strains. Results related to relative growth rate (RGR) and chlorophyll fluorescence imaging of different *Azolla* strains revealed a clear differentiation of sporocarp-producing strains from non-sporocarp producing strains of *Azolla*. Overall, the present study describes variations between sporocarp and non-sporocarp producing *Azolla* strains due to the flexibility to climate changes. Validation of *Azolla* sporocarp induction was done by using micronutrient/trace minerals. Phosphorous (30ppm), urea (15 ppm), ammonia (7.5 ppm), nitrite (70 ppm), nitrate (15 ppm) helped partially in sporocarp germination. Hence, a combination of other factors is helpful to induced sporocarp germination of *Azolla* in the future.

**Keywords:** *Azolla*; Sporocarp production; Climatic factors; Germination; Chlorophyll imaging

AMT-74

### **Influence of high and low nitrogen-responsive genotypes of rice on structural and functional diversity of rhizospheric and endophytic microbial community**

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#### **Abstract**

A microplot field experiment was conducted to evaluate functional microbial dynamics in high (Var. Ranjeet & Pooja) and low nitrogen responsive responsive cultivars (Var. Sabita) of rice. Rhizosphere and plant samples were collected at flowering stage of rice crop from each cultivar. Population dynamics of different microbes from rhizosphere and plant parts (root, stem & leaves) were analyzed along with various chemical and biological properties of soil. Biolog ecoplates-based functional soil and root microbial community was assessed in response of high and low N-responsive cultivars treated with or without fertilizers. The average well colour development derived from Biolog revealed the significant and non-significant functional microbial community among N-responsive and non-responsive cultivars in root and soil, respectively. Shannon diversity was found non-significant while significant in case of McIntosh index. Principal component analysis revealed the varietal significance in soil due to strong interaction of available nitrogen, microbial biomass carbon and urease activity. Significantly more count of diazotrophs was observed in rhizosphere, root, stem and leaf of high N-responsive cultivar Ranjeet than low N-responsive cultivar Sabita. Altogether 20 endo- & epiphytic diazotrophs were selected among 248 isolates for further characterization and finally 9 multifunctional plant-growth promoting diazotrophs (*Paenibacillus glucanolyticus*; *Bacillus aerius*; *Microbacterium proteolyticum*; *Klebsiella pneumonia*; *Aeromonas aquatic*; *M. proteolyticum*; *P. glucanolyticus*; *B. aryabhatai*; *M. proteolyticum*) were selected and identified through 16S-rDNA sequencing.

**Keywords:** Biolog; Microbial community; Rice; Diazotrophs; Principal component analysis.



## Application of Bispyribac sodium in rice alters the functional soil microbial community in paddy soil

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### Abstract

Bispyribac sodium is frequently used herbicide in the rice field. Though it has been targeted to kill rice weeds, but its non-target effect on soil microbes in paddy soil was largely unknown. Therefore, in the present study, an attempt was made to assess the non-target effect of BS on alteration of functional variation of soil microbial community and their correlation with microbial biomass carbon and soil enzymes. A microcosm experiment set up was made comprising three treatments *viz.*, control (CON) (without application of bispyribac sodium), recommended dose of bispyribac sodium (35 g ha<sup>-1</sup>) (BS), and double the dose of bispyribac sodium (70 g ha<sup>-1</sup>) (DBS). Results indicated that the microbial biomass carbon and soil enzymes activities (dehydrogenase, alkaline phosphatase and urease) in BS and DBS-treated soil were significantly ( $p < 0.05$ ) declined from 1<sup>st</sup> to 30<sup>th</sup> day after application as compared to CON. Counts of bacteria, actinomycetes and fungal population were also decreased in BS and DBS-treated soil. The average well color development (AWCD) values derived from Biolog<sup>®</sup>eco-plates followed the order of DBS - BS - CON. Shannon index value was high ( $p \leq 0.05$ ) in CON compared to soil-treated with BS and DBS. Principal component analysis (PCA) showed a clear distinction of the cluster of treatments between CON, BS and DBS. Biplot analysis and heatmap suggested that carboxylic compounds and amino acids, showed positive response towards BS-treated soil, whereas phenolic compound showed positive correlation with DBS-treated soil. PCA analysis showed that oligotrophs was rich in BS-treated paddy soil, whereas copiotrophs and asymbiotic nitrogen fixers were richer in DBS treatment. Overall, the present study showed that application of recommended dose of BS and its double dose alter the soil microbial population, enzymes and microbial community in paddy soil.

**Keywords:** Bispyribac sodium; Biolog<sup>®</sup>; Microcosms; Paddy soil; Herbicide

## Variation of Cyanobacteria diversity under influence of long-term resource conservation technologies in direct seeded Rice-Green Gram cropping system

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### Abstract

Cyanobacteria in rice fields are important microbial members that are employed as bio-inoculants for enhancing fertility, improving structure of soils and crop yields. Hence, the aim of the present study was to know the influence of long-term (more than 6 years) resource conservation technologies under direct-seeded rice-green gram cropping system on cyanobacterial structural diversity, q-PCR-based *nifH* gene abundance, soil physico-chemical properties and soil microbial enzymes activity. Treatments comprising of T<sub>1</sub>: Conventional Direct Sowing (DS) + No N, T<sub>2</sub>: Conventional Direct Sowing + 100% RDF-N, T<sub>3</sub>: Brown Manuring (BM) + 75% RDF-N, T<sub>4</sub>: Green Manuring (GM) + 75% RDF-N, T<sub>5</sub>: Paired Row Rice

+ GM + 75% RDF-N, T<sub>6</sub>: Wet-DS (Drum Seeder) + 100% RDF-N and T<sub>7</sub>: Zero Tillage + 100% RDF-N. Results revealed that cyanobacteria and *nifH* gene abundance along with relative mineralizable carbon and microbial biomass carbon was found to be highest in T<sub>4</sub>. Soil physico-chemical parameters such as pH, EC, available nitrogen, available phosphorus and fluorescein diacetate activity were found highest under T<sub>5</sub>. On the other hand, the highest total nitrogen and urease activity was observed in T<sub>6</sub>. Other soil enzymatic activity; acid and alkaline phosphatase, Dehydrogenase, available potassium and organic carbon were found highest in treatment T<sub>7</sub>. The above findings suggest that conservation agriculture certainly have a great influence on soil health and cyanobacterial diversity. Hence, this information may help to identify and choose the suitable resource conservation practices for rice-based cropping system for enhancing its yield and productivity.

**Keywords:** Rice; Cyanobacteria; Conservation agriculture; Green gram; Green and brown manuring

AMT-77

### **Influence of elevated CO<sub>2</sub> on functional and structural variations of cyanobacteria in paddy soil**

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#### **Abstract**

The present study aimed to assess the abundance and frequency of cyanobacteria under long-term exposure of elevated (e) eCO<sub>2</sub> (550 ppm & 700 ppm) and ambient (a) CO<sub>2</sub> (400 ppm) in open top chamber (OTC) in two depths (0-15 cm and 15-30 cm) of paddy soil by using different morphological and molecular approaches and also analyzed its relation with soil properties. Results indicated that *Leptolyngbya* was dominant cyanobacterial genera irrespective of treatments (eCO<sub>2</sub>, aCO<sub>2</sub> and outside control). Moreover, Illumina MiSeq and heat map analysis through targeted cyanobacterial gene sequences also revealed the abundance of *Leptolyngbya*. The decreased cyanobacterial abundance was observed in OTC exposed with CO<sub>2</sub> continuously for 9 years through qPCR-based cyanobacterial copy numbers. The Illumina MiSeq study of OTC soil was analyzed and it was revealed that the Synechococcophycidae and Nostocophycidae were found to be abundant under eCO<sub>2</sub> (700 ppm) compared to aCO<sub>2</sub>, whereas Oscillatoriophyceae and Stigonematales were suppressed under eCO<sub>2</sub>. Interestingly, Pleurocapsales was encouraged by continuous exposure of eCO<sub>2</sub>. The dendrogram result of diazotrophic *nifH* gene under elevated CO<sub>2</sub> showed that the genus *Bradyrhizobium* was found to be dominant in eCO<sub>2</sub> (700 ppm) followed by *Azospirillum*. The present study also revealed that the soil biological properties were more in surface soil (0-15 cm) as compared to deeper surface soil (15-30 cm) and also abundance of cyanobacteria was highly influenced by soil carbon and nitrogen pools. Overall, the present study revealed the presence of *Leptolyngbya* as abundant cyanobacterial genera in paddy soil under aCO<sub>2</sub> & eCO<sub>2</sub> which could be used as one of the effective nitrogen bioinoculant for rice crop under anticipated climate change.

**Keywords:** Cyanobacteria; Elevated CO<sub>2</sub>; q-PCR; Illumina Miseq®; Rice

AMT-78

### **Evaluating the effect of substrates on the mineral content of oyster mushrooms (*Pleurotus* and *P. florida*)**

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### Abstract

Edible mushrooms are considered as good vegetables with high protein content, minerals and vitamins. Oyster mushrooms are edible and can grow well on lignocellulosic substrates and hence convert agricultural wastes into human food. In this study, the effort was taken to evaluate the effect of substrates on the mineral content of two different species of oyster mushrooms viz., *Pleurotus eous* and *Pleurotus florida*. Paddy straw, ragi straw and the combination of paddy straw and ragi straw in the ratio 1:1 were used as substrates. The mineral content (N, P, K, Ca, Mg and Fe) of the fruiting bodies of mushrooms was determined. It was found that all the minerals (N, P, Ca, Mg and Fe) except K were highest in the fruiting bodies of both *P. eous* and *P. florida* that were grown on Ragi straw, followed by combination of paddy straw:ragi straw (1:1) and paddy straw as substrates. The result showed that the substrates on which the mushrooms were cultivated may have an effect on the mineral content of the fruiting bodies.

**Keywords:** *Pleurotus eous*; *Pleurotus florida*; Lignocellulosic; Paddy straw; Ragi straw

AMT-79

### Study of root colonizing fungi of rice (*Oryza sativa* L.) of Uttar Dinajpur having bio-fungicidal and bio-fertilizer activity

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### Abstract

Uttar Dinajpur West Bengal famous for its diversity of folk rice varieties (*Oryza sativa* L.). Tulaipanji is one of the most popular folk rice known for its aroma, and cultivated mainly in the district Uttar Dinajpur and some areas of Dakshin Dinajpur. Tulaipanji rice is found to be infested by several fungal pathogens which are responsible for both loss of quantity and quality of rice. One such fungal disease, known as blast of Tulaipanji rice caused by *Pyricularia grisea* is found to be responsible for 10%-30% yield loss per year in addition to degradation of quality of aroma. Biological control is the best alternative way for plant disease management for sustainable agriculture in spite of synthetic pesticides and fungicides. In our present study, a number of root colonizing fungal isolates such as *Penicillium*, *Trichoderma* and *Aspergillus* were screened and identified from rhizosphere of Tulaipanji rice of Uttar Dinajpur through serial dilution method. Some isolates were found to play dual roles such as bio-fungicides against *Pyricularia grisea* through dual culture method and growth promotion in rice by increasing plant growth parameters such as seed germination, root and shoot length, IAA production, phosphate solubilization and so on.

**Keywords:** Blast disease; *Pyricularia grisea*; Rhizosphere; Bio-fungicides; Plant growth promotion

AMT-80

### Isolation & screening of sulphur oxidizing bacteria from different eco-niches

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### Abstract

Sulphur (S) is recognized as an essential nutrient for plant growth as its deficiency leads to potential decrease in crop productivity. Sulphur is the key element playing important role in the formation of

proteins, vitamins and enzymes as it is a constituent of amino acids such as cysteine and methionine. Plant S also plays an important role in disease protection and defense response as a component of glucosinolates and allin compounds. Sulphur is most frequently associated with improvement in oil content of oilseed crops. Plants take S from the soil in the form of sulphate, which is mainly produced by the oxidation of S available in different forms by sulphur-oxidizing bacteria (SOB). Most of the sulphur-oxidizing bacteria can be isolated from diverse habitats such as acid, neutral or alkaline environments, cold, moderate or hot habitats, as well as from crop rhizospheres and waste water. In the present study, sulphur oxidizing bacteria have been isolated from different samples such as compost, waste water, dung, rhizospheric soils & plant parts of mustard, maize, soybean and sugarcane. These collected samples were enriched in Starkey broth supplemented with elemental S for 15 days under the incubation condition. After enrichment of 3 cycles, the culture broth was used for isolation of SOB and kept at 30 °C for incubation upto three days, then isolated 13 bacterial isolates from all these samples were characterized morphologically and purified & preserved for further use. The obtained isolates were inoculated on thiosulphate agar medium containing bromocresol purple dye for confirmation of sulphur oxidization. The putative SOB isolates were able to change the color of the dye from purple to yellow which indicates the positive sulphur oxidization. All the putative SOB isolates after dye reduction test were tested for the amount of sulphate ion ( $\text{SO}_4^{2-}$ ) production during growth in thiosulphate broth using spectrophotometer. Out of 13, 5 SOB isolates were found to be efficient in sulphate production (0.03-1.08 mg/ml) after 7 days and 0.78-10.92 mg/ml after 14 days of incubation. These isolates have been tested for various biochemical and plant growth promoting activities. The potential isolates are being genotypically characterized. The source of these isolates indicate that SOBs significant populations involved in the internal sulphur cycle occurring in the wastewater, crop rhizospheric soils & plant parts and might be responsible for the oxidation of sulfide and/or elemental sulphur to sulfate under oxic conditions.

**Keywords:** Sulphur-oxidizing bacteria (SOB); Oilseed crops; Glucosinolates

AMT-81

### Advances in biology, genomic resources and management of whitefly

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#### Abstract

Whiteflies (*Bemisia tabaci*) are polyphagous invasive hemipteran insects that cause serious economic losses of several billion dollars annually worldwide by direct feeding on phloem sap, excreting honeydew and transmitting diverse pathogenic viruses of several important crops. It has emerged as a major threat to global agriculture and food security. Till date, the major problems to unravel their complex interactions with host plant and transmitting viruses are their smaller size, limited studies on physiological/biochemical processes and genomic resources. Further, their wider host adaptability, high fecundity, global invasiveness and rapid evolution of resistance towards pesticides have rendered management of this world pest very difficult. Several strategies like cultural practices, use of pesticides and biotic agents, host-plant resistance either alone or in combinations (Integrated Pest Management) have been used to control whiteflies. But none of these strategies is effective for their control. However, recently several studies have demonstrated the potential of genetic engineering approaches including RNAi for the control of whiteflies. With the advancement in Next Generation Sequencing technology, a large volume of transcriptome sequencing and genome data is available for the cryptic species of whitefly. Combining the recently developed genome sequences with transcriptome and proteome data in *B. tabaci* will provide insights into molecular mechanisms underlying global invasiveness, host-adaptation, viral

transmission capacity and insecticide resistance that assist the progress of novel tactics for effective and resilient control of whiteflies as well as the viruses they transmit for sustainable food and nutritional security.

**Keywords:** Whitefly, *Bemisia tabaci*; Biology; Genomics; Transcriptomics; Proteomics; Interactions with plants; Viruses and Endosymbionts; Pesticide resistance; Thermal tolerance; RNAi

AMT-82

### Microbes for sustainable development scope & application in agriculture

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#### Abstract

The worldwide awareness of harmful effects of chemical fertilizers, insecticides & pesticides is also making impact on Indian Agriculture. This has lead us to go for organic farming using microbial technology in agriculture. Nobbe and Hiltner in 1895 produced the first laboratory grown "Rhizobia" for nearly 17 different legumes in U.S.A. under the brand name "Nitragin". The Russian products "Azotobakterin" and "Phosphobakterin" containing the cells of *Azotobacter chroococcum* and *Bacillus megatherium* var *phosphaticum* respectively were used in U.S.S.R. and European Countries for the bacterization of different crops. Subsequently *Anabene* - Bluegreen algae and *Azolla* by China, Japan, Vietnam, India were developed for rice crop. To control plant diseases we should use natural & Microbial pesticides & Insecticides instead of chemical pesticides & Insecticides. We have excellent results with the insect specific toxin proteins produced by the Bacterium *Bacillus thuringiensis*, commonly called BT, Toxin kills the insect that eat plant. The *Trichoderma* is used for the biological control of Soil borne plant pathogen for e.g. peanut plant. Best from the waste compost preparation. A consortium of four hyperlignocellulolytic fungal cultures namely *Aspergillus nidulans*, *Trichoderma viride*, *Phanerochaete chrysosporium* and *Aspergillus awamori* has been developed on the basis of their lignocellulolytic enzyme production potential. This consortium can degrade diverse agricultural residues & waste. There are three methods of composting. Pit Method, Perforated Tank Method, Heaps Method. Biogas Production - The waste Biomass such as cattle dung, plant residues, sewage, farmyard manure etc. on fermentation in Anaerobic condition. Organic matter is degraded by *Clostridium* is very active in converting cellulose into organic acid. Methane bacteria such as *Methanococcus*, *Methanobacillus* etc. which are secondary colonizers breakdown organic acid in  $\text{CH}_4$  (Methane) &  $\text{CO}_2$ . This generated Methane gas is a Biogas which is used as fuel for domestic purpose in villages. The above microorganisms have been divided according to their application in Agriculture as following group. (1) Biofertilizer (2) Biopesticide (3) Best from the Waste Compost preparation (4) Biogas production.

**Keywords:** *Methanococcus*; *Azolla*; Biogas Production; Perforated Tank Method; *Phanerochaete chrysosporium*; *Aspergillus nidulans*; *Trichoderma viride*; *Aspergillus awamori*

AMT-83

### Proficient IAA producing rhizobacteria in weed inhibition and plant growth promotion of wheat

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## Abstract

Wheat (*Triticum aestivum* L.) is an important grain crop and source of staple food in many countries of the world. India is the fourth largest producer of wheat worldwide which covers more of the earth's surface than any other cereal crop. It accounts for 8.7% of the world's total production of wheat. Wild oat (*Avena fatua* L.) is economically harmful annual grass weed compete with crop plants for natural resources which causes 30-80% yield losses and biomass reduction in cereal crops. Application of agrochemicals and herbicides control the destructive weed community but their indiscriminate use is environmentally unsafe and uneconomic. Therefore, effective biological control is an attractive approach in which rhizobacteria or beneficial microorganisms could be used to suppress the growth of weeds. In the present study, selected rhizobacterial isolates were screened for IAA production, germination of weed on water agar plates, weed growth inhibition and plant growth promotion under pot house conditions. Rhizobacterial isolate BWA18 produced maximum amount of IAA i.e., 53.80 µg/ml and it showed root growth and shoot growth inhibition at both 5<sup>th</sup> and 10<sup>th</sup> day of seed germination of *Avena fatua* on water agar plates. At 75 days of observation under pot house conditions, inoculation with bacterial isolate BWA18 caused 4% increase in shoot dry weight of wheat and its inoculation showed 14% decrease in root dry weight of *A. fatua* in comparison to uninoculated soil treatment. BWA18 was identified as *Acinetobacter variabilis* by the 16S rRNA sequence analysis. These environment-friendly biocontrol strategies are highly compatible with the sustainable agriculture which targets to meet the food demands of the escalating global population. Therefore, there has been a considerable interest recently for identification of bioherbicidal compounds for management of weeds.

**Keywords:** *Triticum aestivum*; *Avena fatua*; *Acinetobacter variabilis*; Rhizobacterial isolate

AMT-84

### Occurrence and molecular detection of the toxigenic *Fusarium* species associated in Maize samples, India

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## Abstract

Mycotoxins are secondary metabolic produced by several species of fungi and its contamination poses a major health threat to the humans and animals. The major mycotoxins produced by *Fusarium* species are trichothecenes (TRI), zearalenone (ZEN) and fumonisin (FUM) which are agriculturally important in food safety point of view. Maize (*Zea mays* L.) is one of the commercially important cereal crop grown worldwide and it is highly cultivated grain after wheat and rice. *Fusarium*, *Penicillium* and *Aspergillus* which can infect the Maize during their pre and post-harvesting conditions. However, species of the genus *Fusarium* having phytopathogenic and toxigenic properties which affect yield, nutritive value and quality of Maize. The present work aimed to study the occurrence and detection of mycotoxigenic *Fusarium* species associated with maize. The maize samples (140) were collected from eleven states of India and enumeration of toxigenic species of *Fusarium* was done. The morphologically isolated strains were further authenticated by molecular identification using PCR by targeting translation elongation factor-1 $\alpha$  (tef-1 $\alpha$ ). A total of 75 *Fusarium* isolates were recovered in all samples and the prevalence was recorded as, *F. verticilloides* (77%), *F. fujikuroi* (7%), *F. poae* (6%), *F. euwallaceae* (2%), *F. aywarte* (6%) and *F. scirpi* (2%). In most of the Indian maize samples we observed that *F. verticilloides* is the major dominating species. The molecular detection of ZEN, NIV and FUM was carried using PCR by targeting their biosynthetic pathway genes. Furthermore, detection of toxins was performed by HPLC and LC/MS/MS and also optimized and developed the multiplex PCR assay for the simultaneous detection of FUM, ZEN and

NIV, which could be helpful for diagnosis and early detection of *Fusarium* species and their management. *Fusarium* diversity was also studied with the help of PCR-RFLP analysis of 75 *Fusarium* strains with single cutter enzyme EcoRV differentiate all *Fusarium* isolates into 4 major groups based on their banding patterns. In conclusion of the present study Indian Maize samples harboured diverse *Fusarium* species including *F. verticilloides* and the production of multiple mycotoxins in food and feed system. There is need of rapid and cost effective methods for the early detection of toxigenic *Fusarium* species and their mycotoxins in food security point of view.

**Keywords:** Maize; *Fusarium*; Mycotoxin; PCR; RFLP

AMT-85

### Screening microbiome of Little millet (*Panicum sumatrense* L.) for abiotic stress tolerance towards developing a new bio-inoculant for climate resilience

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#### Abstract

Little millet (*Panicum sumatrense* L.) is one of the important small millets indigenous to Indian subcontinent. It is cultivated both in the tropics and sub-tropics and even at an altitude of 7000 ft above MSL. Little millet is the much needed food grain among the tribal and is staple food for millions in many parts of the world. Little millet is well known for its drought tolerance and is considered as one of the least water demanding crops. Plant associated bacteria can mitigate abiotic stress through induced systemic tolerance (IST). The present study was carried out in little millet seeds of ATL -1 variety for their ability to tolerate drought by creating artificial osmotic stress using PEG 6000. Seeds were able to tolerate osmotic stress about 10 bars. Little millet plants ATL-1 were grown under stressed (S) condition by limiting field capacity. Bacterial isolates were isolated from roots, shoots and apoplast fluid of stress induced plants. A total 25 isolates were selected based on their ability to tolerate drought upto 64 bars on PEG infused agar plates. The potential microbes of strain number LM1, LM2, LM20, LM21, LM22 were further screened for P and Zn solubilization, IAA production, siderophore and exopolysaccharide production. The results concluded that little millet harbours potential microbes that tolerate abiotic stress, which can be further extrapolated into a newer bio-inoculant for abiotic stress mitigation.

**Keywords:** Abiotic stress; PEG (Polyethylene glycol); Osmotic stress; Bio-inoculant

AMT-86

### Comparative Analysis of Selected Heterocystous and Non- Heterocystous Cyanobacteria for Phyco-biliproteins Production

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#### Abstract

Cyanobacteria are oldest photosynthetic microorganisms known which inhabit almost each and every type of habitat including both man-made and natural. These considered as an alternative

source for the production of various bioactive compounds such as proteins, biofuels, carbohydrates, terpenes, flavanoids, biopigments (carotenoids, Phycobiliprotiens), and vitamins. Phycobiliproteins are basically classified into three types *viz* Phycocyanin, Allophycocyanin and Phycoerythrin on the basis of absorption maxima. These phycobiliproteins have great biotechnological potential in industries like cosmetic, food, pharmaceutical, nutraceutical and analytical etc. Application of phycobiliproteins in different aspects depend on the level of such as for food grade purity should be above 0.7 and for analytical purpose it should be more than 4.0. In the present study, two heterocystous and three non-heterocystous cyanobacteria *viz* *Nostoc commune*, *Anabaena variabilis*, *Lyngbya sp.*, *Oscillatoria sp.*, and *Phormidium sp.* were evaluated for their ability to produce phycobiliproteins. Among them highest phycocyanin content was observed in *Oscillatoria sp.* (386.94 µg/ml), followed by *Lyngbya sp.* and *Phormidium sp.* at 289.95 µg/ml and 276.75 µg/ml respectively. The highest phycoerythrin was observed in *Nostoc commune* and *Anabaena variabilis* *i.e* 223.62µg/ml and 147.20µg/ml respectively. Phycocyanin to the purity ratio ( $A_{620}/A_{280}$ ) of 2.56, 3.25 and 2.13 after dialysis from *Lyngbya sp.*, *Oscillatoria sp.*, and *Phormidium sp.* respectively. Additionally, purity of phycoerythrin achieved from *Nostoc commune* and *Anabaena variabilis* was 3.06 and 2.83 respectively. The purity ratio obtained in this study is high enough for the phycocyanin and phycoerythrin to be used in foods and feeds.

**Keywords:** Cyanobacteria; Biopigment; Phycocyanin; Phycoerythrin

AMT-87

### **Phytochemical analysis of Bioactive Natural compound from endophytes of *Citrus limon* (L.) Burm. f.**

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#### **Abstract**

Endophytic microorganisms are to be found in virtually every plant on earth. These organisms reside in the living tissues of the host plant and do so in a variety of relationships, ranging from symbiotic to slightly pathogenic. It inhabits such biotopes, namely, higher plants, which is why they are currently considered to be a wellspring of novel secondary metabolites offering the potential for medical, agricultural, and/or industrial exploitation. Planting fruit trees also has many helpful environmental benefits, from cleaner air to reduced energy costs and green jobs. *Citrus* genus is the most important fruit tree crop in the world and lemon is the third most important *Citrus* species. Several studies highlighted lemon as an important health promoting fruit rich in phenolic compounds as well as vitamins, minerals, dietary fiber, essential oils and carotenoids. The whole Lemon Plant as a sample was collected from Scientific Nursery of Department of Horticulture, AAU, Anand, India. Total 27 bacterial endophytes were isolated from leaves, stems, roots and seeds of *Citrus limon* on Nutrient agar and Tryptic soy agar medium, out of them 24 are gram positive and remaining three are gram negative bacteria which produce Flavonoids, alkaloids, tannins, Saponins and phenolic compounds as Phytochemical. These secondary metabolites as natural compound which are responsible for the desired therapeutic properties and definite physiological effects.

**Keywords:** Endophytes; Phytochemical; Secondary metabolites



## Biocontrol potential against phytopathogenic fungi and chickpea plant growth promotion due to *Bacillus* sp. strains

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### Abstract

Chickpea (*Cicer arietinum* L.) contributes 18% of the global production of grain legume and serves as an important source of dietary protein. Soil-borne pathogens like fungi affect plant growth and food production worldwide. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is the major soil-borne fungus affecting chickpeas globally. Fusarium wilt epidemics can devastate crops and cause up to 100% yield loss. *Bacillus* species are attractive due to their potential use in the biological control of fungal diseases. In the present study, the antagonistic activity of 193 bacterial isolates obtained from soil samples collected from the rhizosphere of chickpea, were tested against four fungal isolates belonging to four different genera, *Aspergillus niger*, *Neovossia indica*, *Rhizoctonia solani* and *Fusarium oxysporum*. Twenty four antagonistic *Bacillus* isolates showed significant inhibition of tested fungi. Selected antagonistic *Bacillus* isolates with *Mesorhizobium* strains on coinoculation showed significant effect on plant growth parameters like nodule number, shoot and root dry weight of chickpea at 60, 75 and 90 days after sowing. Significant gains were observed by coinoculation of chickpea with treatment KR48+SYB101 in SDW (46.1%) and RDW (105.6%) at 90 DAS. On the other hand, coinoculation of *Mesorhizobium* strain MBD26 with *Bacillus* isolates i.e., RCA3, RCA7, HCA61 and SYB101 showed significant gains (113.9, 79.2, 59.7 and 55.3%) in SDW, whereas coinoculation with these isolates caused 50.6, 35.6, 64.8 and 25.6% increase in RDW, respectively. Study of these antagonists under field condition is needed for the more evident confirmation of their activity against Fusarium wilt and plant growth promoting traits for chickpea growth. The use of molecular tools also offers great potential for chickpea improvement, specifically by identifying molecular markers closely linked to genes controlling fusarium wilt.

**Keywords:** Chickpea (*Cicer arietinum* L.); *Fusarium oxysporum*; *Aspergillus niger*; *Mesorhizobium*

## Influence of farming practices on nitrogen and phosphorus cycles' bacterial guilds in the rhizosphere of *Cajanus cajan*

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### Abstract

Soil health is one of the most vital factors for enhancing crop productivity. Intensive application of pesticides, degradation of agricultural soil by erosion, increased salinity, and loss of organic matter negatively impact the soil health. Various agricultural practices have been reported to adversely affect the soil microbial structure and function. Farming practices influence crop production by altering the functional microbial communities involved in biogeochemical cycles like nitrogen and phosphorus

cycles. Therefore, farming system needs alternative approaches that can preserve the microbiome, and decrease the susceptibility of agriculture to climate variation. So, the purpose of the study was to understand the effect of farming practices on bacterial guilds involved in nitrogen and phosphorus cycles in the rhizosphere of *Cajanus cajan*. Nine different modules were set under the three farming approaches (conservational, convention and organic agriculture), which included different types of tillage, beds, crop rotation, fertilizers dosages, and NPK. The rhizospheric bacterial dynamics was examined qualitatively and quantitatively. Qualitative analysis was performed by 16S rRNA PCR-DGGE, NGS, and quantitative analysis was done by q-PCR of various genes including N and P cycles (16S rRNA, *nifH*, *amoA*, *narG*, *nirK*, *phoD* and *gcd*). Quantitative analysis of 16S rRNA gene revealed that rhizospheric soil bacterial community dynamics was enriched in conservational agriculture, followed by organic and convention agriculture. Qualitative analysis of 16S rRNA, N and P cycle genes showed lesser microbial community diversity in conventional agriculture because of high mechanical inputs, erosion and loss of organic matter. The study goes a step further in our understanding of enhancement of crop yield by agri-management practices.

**Keywords:** Bacterial community; Agri-management practices; Crop productivity; Soil health; Biogeochemical cycles

AMT-92

### Molecular characterization of cyanobacteria isolated from different habitats and their potential for pigments production

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#### Abstract

Cyanobacteria are potential cell factories to produce variety of pigments like chlorophyll, carotenoids and phycobilins. The health issues related to synthetic or chemical dyes have resulted in enhanced preference towards natural colorants. The present work focused on identification of cyanobacterial strains isolated from different habitats (agricultural fields, garden, desert and pond) and screening them for pigment production. A total of 79 cyanobacterial strains were obtained and identified through 16S rRNA gene sequencing which revealed that the strains belonged to 18 different genera. Among them, unicellular forms included *Cyanobacterium*, *Chroococcidiopsis*, *Geminocystis*, *Chlorogloeopsis*, *Limnococcus* and *Gloeothece*, while the filamentous forms were *Desertifilum*, *Lyngbya*, *Phormidium*, *Hapalosiphon*, *Aulosira*, *Toxopsis*, *Halomicronema*, *Nostoc*, *Alkalinema*, *Leptolyngbya*, *Scytonema* and *Trichormus*. When these isolates were screened for different pigments production, 51 isolates showed high phycobilin (Phycocyanin and phycoerythrin) content. Filamentous forms were prominent pigment producer as compared to the unicellular forms. The chlorophyll and protein content of cyanobacteria were also high. These pigments can be used as natural dyes in food, cosmetics and textile industries.

**Keywords:** Cyanobacteria; Molecular Identification; Carotenoids; Phycobilin; Total Protein

AMT-93

### Patentability in Microbiological Research for Sustainable Development

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## Abstract

The patentable technology or process related to agriculture, environmental and food microbial industry has vital role to promote sustainable development with demand of society. This is the way by which we can convert our research into industrialization, commercialization in environmental friendly prospects substantially. The whole planet of earth is composed of unlimited number of good and bad microbes, we need to figure it out with their property and utilize the same for industrialization in eco-friendly approach. As we know since 14 May 2002 Microorganism are Patentable Subject matters after the second amendment bill was passed by Parliament. The role of microbiology in industrial and commercialization prospects are increasing day to day and environmental awareness made people more conscious about eco-friendly invented products.

**Keywords:** Section-3 patent act; Patentable technology; Microorganisms

AMT-94

### Compatible interaction and root colonizing efficiency of *Rhizophagus intraradices* with *Purpureocillium lilacinum* in tomato

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## Abstract

Tomato (*Lycopersicon esculentum* L.) is an important crop for its nutritional value, antioxidant content etc., and their cultivation under low level of fertilization with reduced disease, pest and especially nematode infestation is the major challenge. Towards secured yield along with reduced nematode incidence, nematophagous fungus, *P.lilacinum* is widely used as biocontrol agent against root knot nematode (*Meloidogyne* sp). On the other hand, plant root develop associations with mycorrhizae which positively influences plant physiology and helps in increased nutrient uptake, provide tolerance against various biotic and abiotic stresses. With this scenario, it is noteworthy to study the interaction between mycorrhizal fungus and nematophagous fungus. With this aim, present study was conducted to evaluate the potential root colonization effects of *R.intraradices* with *P.lilacinum* in tomato under greenhouse condition. The tomato seeds were treated with *R.intraradices* + *P.lilacinum*  $10^6$  and  $10^8$  cells  $g^{-1}$ . Based on germination and seedling growth characters the best media was selected (Vermicompost +Coir pith). Then the standardized media along with normal nursery mixture (sand + soil + FYM - 1:1:1 ratio) were used as growth medium. The seeds up to a period of 21 days were examined for mycorrhizal infection. The extent of root colonization was measured after harvest. Approximately 2 g of tomato fine roots ( $\leq 1$  mm) from each plant were transferred to separate tubes. Staining of roots by Artursson and Jansson method followed by their determination with magnified intersection method exhibited the fungal colonization. The results suggests that mycorrhizal infection is unaffected by *P. lilacinus*. This study sets platform to explore the interaction effect of both the fungus in their mechanism and beneficiary to the plants.

**Keywords:** Biotic stress; Mycorrhizae; Nematophagus fungi; Root colonization

## Bacterial colonization patterns as influenced by host genotype-*gfp* tagged studies for understanding cereal-diazotrophspecificity

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### Abstract

To study the colonization pattern rice varieties were inoculated with the *gfp* tagged diazotrophic bacterium in gnotobiotic conditions. For tagging *pjmo62* plasmid DNA was electrotransformed into the competent cells of diazotrophic bacterium. Rice seeds from two NUE contrasting varieties BPT5204 (low NUE) and Varadhan (high NUE) were surface-sterilized and treated with the bacterial suspension with  $10^9$  cfu/ml. Observation was taken at three different time points 7<sup>th</sup> day, 15<sup>th</sup> day and 30<sup>th</sup> day after inoculation using light and confocal microscopy. Confocal laser scanning imaging showed that the *gfp*-labeled bacterial cells infect both rice genotypes in a differential manner. At 7<sup>th</sup> day in (BPT5204) the surface colonization of the inoculated roots; regions in primary roots close to the points of emerging lateral roots were heavily colonized by bacteria, some bacteria were present on elongating root tips, however, in Varadhan, mainly primary roots were colonized and very less colonization appeared in lateral roots. In rice genotype BPT5204, on 15<sup>th</sup> day bacterium started moving towards the root hair tip while in Varadhan bacterium left the main root and started moving towards root hair. 30<sup>th</sup> day imaging showed bacteria accumulated in the cells near the lateral root emerging point due to cell injury and some bacteria were present on the surface of primary root close to the lateral root emergence in variety BPT5204. However, in the rice genotype Varadhan, the bacteria accumulated heavily on the lateral roots. We conclude that the colonization pattern is driven and controlled by the host genotype in rice, very much like in Legume-Rhizobium symbiosis, where the legume host is the determining factor. Low NUE genotype BPT5204 allows the bacteria to more specifically colonize the lateral root junction and root hairs. Contrastingly, in Varadhan bacteria were found colonizing the main root and lateral roots.

**Keywords:** Confocal laser scanning microscopy; Lateral root; Root hair; Primary root

AMT-96

## Isolation and characterization of *Rhizobium* strains isolated from pea, lentil, lathyrus and chickpea grown in Tal region of Bihar

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### Abstract

Biofertilizers are the major sources of organic farming because they provide essential nutrients to the plants. In addition to nutrients they may be biostimulator by producing phytohormones and biocontrol agents by producing antibiotics, siderophores, and hydrolytic enzymes. *Rhizobium/Bradyrhizobium* plays the crucial role in legume crop production. In our study, samples were collected from Mokama Tal region of Bihar in 2019. Forty eight *Rhizobium* isolates were obtained from lentil, pea, lathyrus and chickpea from Tal land. All the *Rhizobium* isolates were screened for IAA production, Phosphorus solubilization, siderophore production, Chitinase production, HCN production and ammonia production. About 40%

isolates showed the IAA production and Phosphorus solubilization ability and about 20% isolates showed the chitinase and ammonia production ability. About 05 *Rhizobium/Bradyrhizobium* isolates were selected on the basis of Plant growth promoting attributes. These selected isolates were further characterized for catabolic diversity by using Cabo-Kit. 13 carbohydrate sources utilized by RT1 & RT 27 while only 7 carbohydrate sources utilized by RT15, RT39 and RT 47. Only RT27, RT15 and RT 47 showed the ability of utilization of carboxylic acid, inulin, salicin and glycerol. Whereas RT1 gave positive test only for salicin. *Rhizobium/Bradyrhizobium* pool germplasm was further strengthening with the addition of pea, lentil, lathyrus and chickpea isolates from Taal land of Bihar.

**Key words:** *Rhizobium*; Phosphorus solubilization; IAA; HCN; Chitinase

AMT-97

### **Chitinolytic *Streptomyces* Sp. as a Biocontrol Agent**

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#### **Abstract**

Among the various natural sources of biomolecules actinomycetes hold outstanding position due to their diversity and bioprospecting aspect. An actinomycete *Streptomyces* sp. from rhizospheric soil of chilly has been evaluated for its bioprospecting. The actinomycete was competent to produce considerable yield of acidic chitinase. Thus produced chitinase is potent to control the hatching of nematode eggs. The actinobacteria is capable to control root-knot nematode infection in a medicinal plant *Coleus forskohlii* which could results in the enhanced yield of a valuable commercial compound forskolin. Hence, *Streptomyces* sp. is a reliable biocontrol agent for nematode and simultaneously it promotes the growth of plants.

**Keywords:** *Streptomyces* sp.; Forskolin; Biocontrol of root-knot nematode

AMT-98

### ***Bacillus* sp. mediated Stimulation of Mustard (*Brassica juncea* L.) seed germination under osmotic stress condition**

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#### **Abstract**

Water deficit stress is one of the principal abiotic stress affecting plant's growth and development. Rhizosphere and endophytic microorganisms play a significant role in the alleviation of water deficit stress in plants. In the present study, the effect of bacterial inoculation on various physiological and biochemical parameters of mustard seedlings under osmotic stress condition were investigated to elucidate the mechanism of bacterial mediated stimulation of germination. Surface sterilized seeds were dipped in definite density (log phase broth cultures) of bacterial cultures *Bacillus* sp. and *Bacillus*

*cosmogenesis* and allowed to grow on 0.8% agar plates with osmotic stress (20% PEG) and without stress (Control). Observations on germination percentage, seedling vigour, osmolyte accumulation, gibberellic acid and abscisic acid contents were taken at 24, 48 and 72 h of germination. Reduction in germination percentage, total length of seedlings and seedling vigour were observed in osmotic stress condition. However, inoculation with bacterial strains showed significant improvement in these parameters. *Bacillus* sp. treated seedlings showed low proline and glycine betaine content under osmotic stress condition compared to uninoculated seedlings. These results indicating that bacterial treated seedlings do not face much osmotic stress, therefore, the osmolytes accumulation is less in the presence of bacteria. Inoculated seedlings showed significant increase in gibberellic acid and abscisic acid contents compared to control under osmotic stress condition. Gibberellic acid enhance germination by stimulating cell division and cell elongation and promoting developmental phase transitions. Bacterial inoculation plays a critical role in different abiotic stresses by regulating various downstream ABA-dependent stress responses by increasing abscisic acid levels. Significant changes in glyoxylate cycle enzymes - isocitratelase, malate synthase and in lipases were also observed in seedlings upon bacterial inoculation at 48, 72 and 96 h after germination under water deficit stress condition. Hence, the results of the present study show that bacterial inoculation improves the germination of mustard seeds under water deficit stress condition probably through the alteration of various physiological and biochemical responses.

**Keywords:** *Bacillus cosmogenesis*; Gibberellic acid; Isocitratelase; Water deficit stress

AMT-100

### Select optimal Pigeon Pea Rhizobium combinations for nodulation, nitrogen fixation and grain yield

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#### Abstract

Legume-rhizobial symbiosis plays crucial role in agricultural sustainability. A study is conducted in Leonard jar assembly to evaluate sixteen strains of pigeon pea rhizobia for their efficiency in growth enhancement across different cultivars, viz., ICPL 87, ICPL 332, ICPL 14003, ICPH 2740 and Asha 87119. The following parameters, nodule number, weight of nodule, dry shoot and root weight, were observed to select efficient strains for different cultivars. For the cultivar ICPL 87 maximum number of nodules was recorded by APP150 while APP 151 recorded maximum nodule weight and shoot dry weight. Highest root dry weight was recorded by APP36. For cultivar ICPL 332 maximum number of nodules was recorded by APP149, while maximum nodule weight, shoot dry weight and root dry weight were recorded by APP84, APP149 and APP152 respectively. For the cultivar ICPL 14003 highest number of nodules and maximum nodule weight, shoot and root dry weight were recorded respectively by, APP149, APP150, APP150 and APP152. For the cultivar ICPH2740 maximum number of nodules was recorded by APP152 and highest nodule weight was recorded by APP113. APP152 recorded maximum shoot and root dry weight. For the cultivar Asha 87119 highest number of nodules was recorded by APP152 and maximum nodule weight was recorded by APP3. APP152 and APP36 recorded maximum shoot and root dry weight respectively. APP152 was found to record better parameters across the cultivars followed by APP 149. Hence these two rhizobial strains can be used for increasing production of pigeon pea across different cultivars.

**Keywords:** Rhizobium; Pigeonpea; cultivars; Plant growth; Response

## Bacterial endophytes of pigeonpea (*Cajanus cajan* L) and their potential antimicrobial activities against phytopathogens

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### Abstract

Endophytes are the microbes that live inside the plant tissues without causing any apparent harm to the host. These microbial communities are known to play a crucial role in the functioning of plants by influencing their physiology and development. Among them bacterial endophytes live inside the plant tissues and play a vital role in physiology, development of plant as well as protection from various biotic and abiotic stresses. A total of 45 endophytic bacteria were isolated from surface sterilized plant tissues (root, stem, leaf, flower and pod) of seven varieties of Pigeonpea (*Cajanus cajan* L.) cultivated in Chhattisgarh. Morpho-molecular characterization identified that 28 isolates were gram stain positive and 17 were negative. The bacterial endophytes were found in all the plant tissues studied but higher number was found in the root and leaf tissues and microbial composition varied among the tissues. On the basis 16S rDNA amplicon sequencing bacterial isolates were identified and grouped in different genera's namely *Bacillus*, *Fictibacillus*, *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Beijerinckia*, *Pantoea* and *Serretia*. The plant growth-promoting ability of bacterial isolates showed that a total of 24.5% (11), 42.3% (19), 8.9% (4) and 28.9% (13) isolates were able to show phosphate solubilization, siderophore production, indole acetic acid production, DNase activities, respectively. The antagonistic activity test showed that 42.3 % (19), 31.2% (14) and 37.8% (17) bacterial endophytic isolates were having antagonism against soil-borne fungal pathogens *Sclerotium rolfsii*, *Fusarium verticillioides* and *Rhizoctonia solani*, respectively. Promising isolates exhibiting antibacterial and antifungal activities may be used for development of biocontrol formulations for controlling multiple biotic stresses, thus finally paving a way forward for enhancing the crop productivity and farmers income.

**Keywords:** Bacterial Endophytes; Pigeonpea; Plant growth promotion; Antagonistic activities; *Fusarium*

### *Taxus Wallichiana* as a source of Plant growth-promoting Endophytes

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### Abstract

Endophytes are endosymbionts, often bacteria and fungi that live within a plant for at least part of its life cycle without causing apparent disease. They are generally ubiquitous and highly varied microorganisms. In-plant tissues, fungal and bacterial endophytes are prevalent inhabitants and have been shown to support growth and health of the plant. Endophytes act as reservoirs of novel bioactive compounds such as antidiabetic, antioxidant, antimicrobial, anticancer, enzymes and Plant growth promoting properties etc. The use of endophytes as Plant growth promoting to increase crop production is gaining importance

among environmentalists and agronomists as they would significantly reduce chemical pollution into the environment. Innovative microbial inoculation-based approaches are gaining more interest in minimizing the detrimental effects of conventional farming techniques. Most importantly, plant-microbe symbiosis affects plant health and growth, effectively improving agricultural characteristics. However, not much is reported about *Taxus wallichiana* growth-promoting endophytes (PGPE). Therefore, the purpose of this work was to isolate fungal and bacterial endophytes from *Taxus wallichiana* and to classify the properties of these endophytes which promote plant growth (PGP). Seven fungal and ten bacterial endophytes were obtained from different parts of *Taxus wallichiana*. The isolated fungal and bacterial endophytes produced ammonia and indole acetic acid (IAA) differently, and they also exhibit antimicrobial and enzymatic activities. Based on above results two fungal endophytes were selected for their possible ability to promote plant growth. The results showed that fungal endophytes isolated from *Taxus Wallichiana* played a role in increasing plant root growth and these could be used as plant growth promoters.

**Keywords:** Endophyte; Plant growth-promoting endophytes (PGPE); IAA; Antimicrobial activity; Enzymatic activity

AMT-103

### Biogenic silver nanoparticle effective contrivance for efficient antimicrobial activity than chemogenic analogue

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#### Abstract

Pathogens have always been destroying human health and agricultural production resulting in big loss on the social and economic front. Biogenic nanomaterials have attracted scientific interest as a new generation of antimicrobial agents for resistant pathogens. Biological synthesis of silver nanoparticles by enhancing antimicrobial activity, has fulfilled urgent need a search for a suitable bio-candidate. Herein we describe the enhancement of antimicrobial efficacy of biogenic silver nanoparticles by reducing and modifying the surface with antimicrobial metabolites of *Trichoderma viride* (MTCC 5661) in comparison to chemogenic silver nanoparticles. Nanoparticles were characterized by visual observations, UV-visible spectroscopy, dynamic light scattering, and transmission electron microscopy. Synthesized particles were measured 10–20 nm in size and spherical shape. Metabolites on the surface of biogenic silver nanoparticles were determined by gas chromatography–mass spectroscopy and Fourier transforms infrared spectroscopy. The antimicrobial activity of both silver nanoparticles were evaluated against *Shigella sonnei*, *Pseudomonas aeruginosa* (Gram negative bacteria), *Staphylococcus aureus* (Gram positive bacteria), *Fusarium oxysporum* and *Alternaria brassicicola* (phyto-pathogenic fungus). Particles internalization in bacterial cells were determined by flow cytometric analysis. Biogenic silver nanoparticles increase the dry weight reduction by 20 to 48.80% in *F. oxysporum* and *A. brassicicola* respectively in compared to chemogenic analogue. Higher production of reactive oxygen species and enhanced oxidative stress caused severe damage to the microbe's membrane. They have increased further uptake of particles and revoking other pathways for the complete and rapid death of pathogens. Live/dead assay was performed by dual fluorescent staining method and electron microscopic observation. Downregulation of the antioxidant mechanism was reported in fungal pathogens by RT-PCR analysis. *In-vitro* toxicity and cells migration test was carried out on the animal cell line. Detailed investigation resulted that antimicrobial efficiency of biogenic silver nanoparticles was much higher than chemogenic silver nanoparticles.

**Keywords:** Biogenic silver nanoparticles; Antimicrobial activity; Bio-candidate; Chemogenic analogue



## Effect of plant growth promoting rhizobacteria in the presence of fertilizers on soil properties during growth of Mustard Crop

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### Abstract

Plant growth promoting rhizobacteria (PGPR) play a key role in maintaining agricultural productivity. There is need to increase crop production by reducing synthetic fertilizers use. The PGPR includes naturally existing soil bacteria which boost development and productivity of plants through different growth-promoting substances. The most predominant rhizosphere colonizing bacteria belong to genera *Pseudomonas* and *Bacillus* because of their association with soil organic matter, nutritional diversity and rapid growth rate. The PGPR strains were studied to check their effect on mustard crop by performing pot experiment. Three strains of bacteria were used as bio inoculants (*Bacillus subtilis*, *Pseudomonas*, and *Brevibacillus* species). Different treatments were made using NPK fertilizers and PGPR strains. Standards were taken for comparison with different treatments. Chemical analysis of the soil samples was carried out every month until maturation of mustard crop. Soil were observed for any change in properties like pH, WHC, total Nitrogen, sodium, Potassium, TOC and microbial load. Maximum change in plant growth after bacterial inoculation along with NPK fertilizer was observed compared with controls. The soil was divided into five sets: sample A, B, C, D, E were taken as controls the first control is A containing seeds without any PGPR strain or fertilizers. B consists of seeds and nitrogen fertilizer. C consists of seeds and phosphorous fertilizer. D consists of seeds and potassium fertilizer. E consists of seeds with added NPK fertilizer. Sample A1, B1, C1, D1, E1 were inoculated with *Bacillus subtilis* with respective fertilizers used in controls. Sample 2 A2, B2, C2, D2, E2 was inoculated with *Pseudomonas* strain with respective fertilizers used in controls and sample 3 A3, B3, C3, D3, E3 inoculated with *Brevibacillus* with respective fertilizers used in controls. Soil sodium, potassium levels increased tremendously after inoculation of PGPR bacteria as compared to the controls. Organic carbon content also increased gradually from first month to 90 days of plant maturation. Microbial load also increased in the soil after treatment. In all samples treatment E showed maximum change in properties of soil after bacterial inoculation along with NPK fertilizer when compared with controls.

**Keywords:** Plant growth promoting rhizobacteria (PGPR); *Bacillus subtilis*; *Pseudomonas*; *Brevibacillus* species

AMT-105

## Functional Characterization of a High Affinity Iron Transporter (PiFTR) from an Endophytic Fungus *Piriformospora indica* and its Role in the Plant Growth and Development

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### Abstract

Iron (Fe) is an essential micronutrient required for vital cellular activities. Most iron is not readily available to plants and then to animals, causing chlorosis and anemia respectively. Biofortification

through plant-fungal co-cultivation might be a sustainable approach to increase iron content in crop plants. We have investigated the role of the high affinity iron transporter of the root endophyte *P. indica* (*PiFTR*) in plant growth and development. *PiFTR* shows 40% similarities with *FTR1* of ascomycetes, and has a length similar to that of FTR proteins in Basidiomycota. This transporter functionally complements the yeast high affinity iron transporter mutant. Kinetic analysis ( $K_m$  2.5  $\mu$ M) reveals that *PiFTR* is a high-affinity iron transporter. To understand the physiological role of *PiFTR*, knockdown (KD) transformants were prepared using electroporation and RNA interference (RNAi). KD transformants transported a significantly lower amount of Fe to the colonized plant than WT *P. indica*. Plants colonized with the WT *P. indica* were found to have more biomass and total Fe content than that of plants colonized with KD-*PiFTR*- *P. indica*. We propose that *PiFTR* will be useful to develop iron- rich crops to improve human health.

**Keywords:** Iron; Biofortification; Rice; Colonization; *Piriformospora indica*

AMT-106

### Transformation of cotton gin agricultural waste to bioethanol

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#### Abstract

Conventional crops are incapable of meeting the worldwide demand to produce bioethanol owing to their prime significance of food and feed. Lignocellulosic materials *viz.*, agricultural wastes are striking feedstocks to produce value-added products. Agronomic wastes are plentiful, renewable and cost effective. The current study emphasizes on the characterization of the chemical composition of cotton gin waste along with culturing of the desired microbes for biodelignification (pretreatment) of lignocelluloses and optimizing the pretreatment conditions for effective hydrolysis towards utmost ethanol yield. The grinded cotton gin waste used for the present study was steam treated in an autoclave prior to biological pretreatment. The composition analysis of cotton gin wastes were 6.7% moisture content, 7.4% ash-content, 8.7% ethanol extractives, 24.6% lignin, 21.4% hemicellulose and 30.9% cellulose, determined by the standard analytical procedures. The characteristics of Basidiomycetes fungi fruit bodies collected from decaying wood, decaying leaf litter were corticoid (effused), steroid (effusoreflexed), corolloid, dimidiate (shelves or brackets), cyphelloid (capulate), polyporoid, agaricoid and boletoid. Mycelial species of approximately 3 mm<sup>2</sup> of the plate cultures were used as inoculums for lignolytic activity. Kinetic model development for hydrolysis reaction will be performed. The comprehensive outcomes will be presented.

**Keywords:** Cotton-gin waste; Biodelignification; Basidiomycetes; Cellulose; Kinetic model

## Isolation and Characterization of Rhizobacteria Isolated from Hot Desert of Jaisalmer to Alleviate Drought Stress in Wheat

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### Abstract

It is predicted that world population will be increased upto 8 billion by 2030 which will create the problem of food security to the people. Drought is a major abiotic stress which affects the growth and productivity of crops all over the world. Millions of microorganisms inhabit plant root system forming a complex ecological community that influences plant growth and productivity through their metabolic activities and plant interactions. Among them, plant growth promoting rhizobacteria (PGPR) can directly and indirectly enhance growth, nutrition and ameliorate abiotic and abiotic stresses. Hence, an investigation was undertaken to isolate drought tolerant bacteria from *Argimone mexicana* and wheat crop plants growing in hot desert of Jaisalmer, Rajasthan. A large gene pool of bacterial isolates was recovered from rhizosphere soils of *Argimone mexicana* and wheat (*Triticum aestivum*) crops of four villages of Jaisalmer. The gene pool was subjected for screening to screen out drought tolerant bacteria and around 20 isolates were selected as drought tolerant bacteria which had tolerated PEG-6000 up to -4.65 Mpa (20% w/v). It was also noted that these 20 bacterial have been found to produce considerable amount of exopolysaccharides and exhibited PGP traits. All the 20 bacterial isolates were identified as *Bacillus* species based on morphological, biochemical and 16S rRNA gene sequencing. The bacterium *Bacillus subtilis* DT-76 (NAIMCC-B-02231) was found best in term of having PGP traits and enhancing growth of wheat crop by conferring drought tolerance and other functions.

**Keywords:** *Argimone Mexicana*; Plant growth promoting rhizobacteria (PGPR); *Bacillus subtilis*

## Isolation and Characterization of Salt Tolerant Potassium Solubilizing Bacteria

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### Abstract

After nitrogen and phosphorous, potassium is considered as third most important element required by plants. The amount of soluble potassium in soil is 0.1-0.2% which is very important for plant growth but it contributes very less to total available soil K content. The major pool of potassium is unavailable for plants (90-98% of total soil K content) and occurs in the form of silicate minerals such as micas. This study was carried out to isolate and characterize efficient salt tolerant potassium solubilizing bacteria (KSB) suitable for alkaline soils of semi-arid regions like Mahendergarh. The modified Alexandrov's agar medium containing potassium alumino-silicate as K source was used for isolation of K solubilizers. Out of total 32 isolates selected on the basis of polysaccharide production, 9 isolates were selected based on qualitative screening on modified Alexandrov's agar medium containing bromothymol blue as

pH indicator. The isolates were designated as SSV3, SSV4, SSV5, SSV7, SSV8, SSV10, SSV19, SSV26 and SSP. Modified Alexandrov broth with mica and potassium alumino-silicate was used for quantitative screening and isolates were selected based on amount of K solubilized from mica and potassium alumino-silicate. The isolate SSV4 (9.85ppm) showed maximum solubilization of potassium alumino-silicate while the maximum solubilization of mica was exhibited by isolate SSV5 (11.75ppm). Isolates were also checked for other plant growth promotion activities (PGP) like HCN, antibacterial activity and ammonia production. Salt tolerance ability of isolate SSV4 was found to be maximum. The organic acids were identified using HPLC analysis and organic acids succinic acid, malic acid, citric acid, formic acid and oxalic acid were identified from different cultures. The isolate SSV3 was tentatively classified under genus *Bacillus* while the isolate SSV4 was classified under genus *Pseudomonas*. The use of potassium solubilizing bacteria in agriculture can be helpful in increasing the amount of soluble potassium for plant uptake. The efficacy of the isolated potassium solubilizing bacteria needs to be tested under different environmental conditions in agricultural fields.

**Keywords:** Alexandrov medium; KSB; Organic acid; *Bacillus*

AMT109

### Eco-friendly commercial utilization of sugarcane bagasse

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#### Abstract

Generally, sugarcane bagasse (SBg) is considered as an environmental hazard due to the emission of greenhouse gases during its burning either in agriculture fields or in power plant. The present study aims to demonstrate the potential application of sugarcane bagasse as an excellent field emitter, carbon source for diamond growth and sensing element for detection of alcohol. Field emission property of as-pyrolyzed (p-SBg) and plasma treated sugarcane bagasse has been investigated. It has been observed that electronic nature of p-SBg transformed from semiconducting to metallic after plasma treatment. Furthermore, high-resolution transmission electron microscopy (HRTEM) study was performed and it has been observed that various carbonaceous structures such as 3D nanographeneoxide (3D-NGO), graphite nanodots (GNDs), carbon nanotubes (CNTs), and carbon onions are present in both pre-treated and plasma-treated p-SBg. The p-SBg was used as sensing element for alcohols detection. It has been successfully demonstrated that by using sugarcane bagasse as a carbon precursor, highly crystalline diamond films possessing H3 [N-V-N] and [SiV]<sup>-</sup> optical center can be grown on Si (100) substrates using a thermal-CVD growth. In the present work we will also demonstrate the potential applications of sugarcane bagasse for alcohols sensing.

**Keywords:** Diamond; Field emission; Alcohol sensor ; Graphene ; Activated carbon

# BIOENERGY



# ORAL PRESENTATION





## Immobilization of $\beta$ -glucosidase from *Bacillus subtilis* PS onto magnetically recyclable nanoparticles: A recoverable nano-biocatalyst and evaluation of its application in cellobiose hydrolysis

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### Abstract

Immobilized enzymes (IMEs) are used commercially because of their improved product quality and cost-effectiveness. In recent years, substantial efforts have been made to establish nano-biotechnological entrepreneurship for the sustainable bioenergy production based on nano-biocatalytic systems. However, nanoconjugated enzyme-catalyzed bioenergy production processes are still in their infancy. The present study was carried out with an objective to improve the catalytic efficiency of  $\beta$ -glucosidase through covalent immobilization on magnetic nanoparticles for potential application in bioenergy production.  $\beta$ -Glucosidase (BGL) of Glycosyl hydrolase (GH) family 1 from *Bacillus subtilis* PS was immobilized on carboxyl activated magnetic nanoparticles (MNPs) by covalent binding. The immobilization efficiency and yield of enzyme was found to be 89.78% and 84.80%, respectively. Immobilized and free BGL were characterized using Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), Fourier Transform Infrared Spectroscopy (FTIR) and Circular Dichroism (CD) spectroscopy techniques. After immobilization of  $\beta$ -glucosidase on magnetic nanoparticles, optimum pH remained 6.0 but optimum temperature shifted to 70°C. The immobilized  $\beta$ -glucosidase retained 77% of its initial activity after 60 min incubation at 70°C while free  $\beta$ -glucosidase had only 40% of its initial activity after exposure to 70°C for 60 min.  $K_m$  value for free and immobilized  $\beta$ -glucosidase were 0.8196 mM and 0.9411 mM, respectively. The  $V_{max}$  for  $\beta$ -glucosidase increased from 54.46 to 57.67  $\mu\text{mole}/\text{min}/\text{mg}$  after immobilization.  $\beta$ -glucosidase retained about 85% activity upto 10th cycle of reuse. Cellobiose hydrolysis potential of the immobilized enzyme was analysed using High Performance Liquid chromatography (HPLC) analysis. Covalent immobilization of  $\beta$ -glucosidase led to enhance thermal and pH stability, reusability and higher rate of reaction ( $V_{max}$ ) without affecting its secondary structure. This study demonstrates that the  $\beta$ -glucosidase immobilized onto magnetically recyclable nanoparticles has promising prospect in sustainable industrial applications.

**Keywords:** Immobilized enzymes; Magnetically recyclable nanoparticles; Nanobiocatalyst;  $\beta$ -Glucosidase

## Molecular Approach for Enhanced and Economic Bio-fuel Production for Future

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### Abstract

Continuous efforts have been made in the past few decades for conversion of lignocellulosic biomass into bioethanol with the help of fermentative yeasts. Preferred yeasts include *Saccharomyces*, *Candida*, *Kluyveromyces*, *Pachysolen*, *Scheffersomyces*, *Brettanomyces*, *Schizosaccharomyces* etc. However, *Saccharomyces cerevisiae* is the yeast of choice for most fermentation exploiting hexose sugars, but its incapability to ferment pentose sugars present in lignocellulosics, led an urge to look for other alternative solutions. On the other hand, among all the pentose fermenting microbes, *Scheffersomyces sp.* offers highest productivity; however, it has low osmo and ethanol tolerance. Researchers have hypothesized that by developing genetic engineered microbes, the problem of lignocellulosic fermentation could be solved. D-Xylose is the main pentose sugar present in lignocellulosic biomass, could be utilized by *S. cerevisiae* by either incorporating Xylose reductase (XR) and Xylose dehydrogenase (XDH) enzymes from pentose fermenting yeast, which can convert D-Xylose first into Xylitol and then D-Xylulose, or by incorporating genes for Xylose Isomerase (XI), which can facilitate the direct conversion of D-Xylose into its isomer D-Xylulose. This D-Xylulose can subsequently be utilized by *S. cerevisiae* by the action of Xylulo-kinase (XKS). However, for a cost effective lignocellulosic fermentation process, simultaneous conversion of both pentose and hexose sugars is a prerequisite. Since, naturally, the yeast utilize the same transporter genes for pentose and hexose sugars, which arises the problem of catabolite repression, and to solve the problem, dedicated pentose transporter are required such as AUT1 (Arabinose transporter) of *Scheffersomyces sp.* In the present study, we are trying to improve fermentation efficiency of yeast by genetically modifying *S. cerevisiae* in such a way that it can metabolize pentoses along with hexose sugars. Another possibility is to modify *Scheffersomyces sp.* or any other pentose fermenting yeast in order to enhance their ethanol tolerance. These strategies may help in the development of yeast strains capable of co-fermenting pentoses and hexoses for the cost-effective and economic production of bioethanol in order to combat increasing fuel demand in future.

**Keywords:** Lignocellulose; Recombinant; *Saccharomyces*; *Scheffersomyces*

# POSTER PRESENTATION



## Substituting fed batch fermentation process through polymeric matrix support towards bioethanol production from surface pine needle

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### Abstract

The use of non-renewable petroleum products is adversely affecting the environment and economy of the world. Many countries are using first generation biofuels as an alternative but they are causing competition between food and fuel. So, second generation biofuels produced from lignocellulosic biomass can be potentially used for cost effective biofuel (ethanol) production. Fallen pine needles are abundant in nature and are major cause of forest fire and illness of animals when consumed. These needles having high conversion efficiency of pentose and hexose sugar to ethanol (85 and 92% respectively) through fermentation can be used as a good source for bioethanol production. Various enzymes (hydrolases) produced by native soil microbes digests the lignocellulosic feedstock but the process is very slow. Enhancing the activity of these enzymes through genetic manipulation can enhance the conversion of biomass into sugar. Produced sugar will be fermented for ethanol production using genetically modified fermenting microbes for high yield. A polymeric matrix support for the heterologous expressed enzyme conjugates can be prepared which will not only enhance the activity of enzyme but will also help in multiple usage of the same enzyme. The overall process being biological will be cost effective and no harmful waste product will be generated.

**Keywords:** Polymeric matrix support; Fed batch fermentation; Biofuel; Lignocellulosic biomass; Biological methods

## Enhanced hydrogen production in SRB based bio-electrolysis system by inhibiting of methanogens

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### Abstract

Hydrogen is environmentally clean fuel as it generates only water vapours after combustion. Bio-electrolysis system (BES) is a new and promising approach for hydrogen production. Undesired methane ( $\text{CH}_4$ ) production is a serious challenge faced in BES. Methanogens can consume  $\text{H}_2$  to produce  $\text{CH}_4$  in BES, which has led to a drop of  $\text{H}_2$  yield. This research was done to investigate, the effect of pre-treatment of anaerobic sludge enriched with sulfate reducing bacteria at applied voltage to enhance the hydrogen production by inhibition of methanogens activity in two phase using ferrous and sulfate concentration (optimum condition obtain from RSM experiment) in alkaline media pH 7.5. Results show that higher  $\text{H}_2$  production ( $7.713 \pm 1.508 \text{ mM/ M COD}$ ) with minimum  $\text{CH}_4$  production ( $1.631 \pm 1.436 \text{ mM/ M COD}$ ) was observed in Phase II in reactor without electrolysis and pretreated at  $40 \pm 5.0 \text{ mV}$  by adjusting the pH after phase-I with maximum yield of cumulative VFA ( $115.701 \pm 3.33 \text{ mM/L}$ ) produced by major concentration of (butyric acid > isobutyric acid > acetic acid > formic acid). This pretreatment strategy would be benefited for enhance hydrogen production by inhibition of methanogens with a small external voltage in alkaline medium where methanogens activity is found dominant.

**Keywords:** Bio-electrolysis system (BES); Methanogens; Sulfate reducing bacteria; Hydrogen production

## Differential enrichment and mining of lignin and hemicellulose degrading genotypes during anaerobic digestion of agricultural residue

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### Abstract

Anaerobic Digestion (AD) seems to be an ideal and sustainable option for managing large quantum of agricultural waste generated in developing countries. However, the major hindrance in biofuel production from agricultural residue is the high lignin content bound to cellulose and hemicellulose fractions which reduce the bioavailability of fermentable sugars. In this study, a scenario was created for enrichment and selection of key hemicellulolytic and lignolytic enzyme producing microbial communities in presence of rice straw and cotton crop waste. The shift in genotypes was compared with that of initial anaerobic inoculum used in the AD process. Metagenomic analyses revealed varying relative abundances of the lignin & hemicellulose degraders. Members of hemicellulolytic genera viz., *Bacteroides*, *Clostridium*, *Prevotella*, *Thermotoga* showed higher abundance in rice straw acclimatized metagenome whereas the genus *Cytophaga* was more abundant in cotton crop waste acclimatized metagenome. Amongst the lignocellulose degraders, genera *Bacillus*, *Geobacter*, *Flavobacterium*, *Spirocheta* were predominant in rice straw metagenome and genera *Klebsiella*, *Escherichia*, *Pseudomonas*, *Burkholderia* were predominant in cotton crop waste metagenome. Analysis of the methanogenic communities revealed the enrichment of hydrogenotropic genera of phylum Euryarchaeota, i.e. *Methanospirillum*, *Methanothermobacter*, *Methanoregula*, *Methanosphaerula*, *Methanobrevibacter* in presence of rice straw while in the cotton waste digesting community, acetoclastic genera *Methanosaeta*, *Methanosarcina* in addition to hydrogenotropic genus *Methanoculleus* were found to be more prevalent. Fold change in abundance pattern of genes for various Carbohydrate Active Enzymes (CAZymes) along with anaerobic aromatic compound degrading genes in both the acclimatized metagenomes in comparison the metagenome from initial inoculum supports the findings. The metagenomic data was validated by evaluation of the biochemical methane potential for these substrates where anaerobic digestion of rice straw and cotton crop waste resulted in a biogas yield of 622 mL/g VS and 414 mL/g VS of biogas respectively.

**Keywords:** Lignin; Hemicellulose; Anaerobic digestion; BMP assay; Metagenomics

## Assessment of Inhibitory Effects of Benzalkonium Chloride on *Chlamydomonas reinhardtii* and Control of Grazers in Open Pond Cultivation

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### Abstract

Algae serves as a third-generation feed stock for production of biofuels. Production of algal biomass needs sustainable algal cultivation. The major threat in outdoor open pond mass cultivation of algae is the

culture crash by invading grazers. *Chlamydomonas reinhardtii* is the model green algae widely characterized in the lab and many genetic expressions were studied before implementing in other green algal cultures. However outdoor pond cultivation of this strain is a challenging task due to its sensitivity to varied environmental conditions and lack of suitable grazer control chemicals. In this study the sensitivity of *Chlamydomonas reinhardtii* to various concentrations (1.5, 2.5, 3.5 & 4.5 ppm) of benzalkonium chloride was studied at pond level under greenhouse conditions in autotrophic growth mode. In the sensitivity study, till 2.5 ppm no inhibition was observed whereas, 4.5 ppm was determined as minimum inhibitory concentration which had inhibited > 50 % of growth (MIC-50). At MIC-50 concentration optical density (OD), ash free dry biomass (AFDW) and maximum quantum yield (Fv/Fm) were inhibited by 56%, 68% and 54% respectively as compared to control. The *Chlamydomonas reinhardtii* was cultivated in batch mode for 7 days in 1m<sup>2</sup> ponds and invading ciliates were controlled by applying the optimum concentration of 2.5 ppm benzalkonium chloride which was determined during the sensitivity study. With the applied crop control measures, culture had shown OD jump of 0.23±0.01 per day, final AFDW concentration of 0.624 ±0.028 g/L and volumetric productivity of 89.22±4.12 mg/L/day whereas the control ponds have shown decreasing OD and AFDW with final culture crash at the end of batch. This study suggests that benzalkonium chloride above certain concentration is inhibitory to *Chlamydomonas* and affects the growth and photosynthetic performance. The dosage of biocide for grazer control could be fixed based on inhibitory concentration.

**Keywords:** Model algae; *Chlamydomonas*; Benzalkonium chloride; Crop control; Grazers; MIC-50

BE-7

### **Comparative pretreatment using mild alkali and organosolv method for improving enzymatic digestibility of Sugarcane bagasse using cocktail of Chimera (CtGH1-L1-CtGH5-F194A) and Cellobiohydrolase (CBH5A) for bioethanol production**

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#### **Abstract**

There is a great incentive for the development of bio-based economy i.e. using of non-fossil feedstock, in order to address economic, strategic and environmental problems. Lignocellulosic biomass clearly represents a sustainable and low cost renewable resources for the production of biofuels and chemicals on large scales. Efficient utilization of lignocellulosic biomass for bio-refinery required an effective fractionation of biomass into its constituent components which can be achieved by effective pretreatment stages. In the present study, comparative pretreatment strategies using mild alkali and organosolv consisting of phosphoric acid- acetone has been performed using sugarcane bagasse as a target lignocellulosic biomass. The raw biomass was subjected to mild alkaline soaking using 1%(w/v) NaOH for different time interval at 50°C. The pre-soaked biomass was further treated using organosolv pretreatment. This pretreated biomass was then subjected to enzymatic saccharification using cocktail of cellulases i.e. Chimera ( $\beta$ -glucosidase and endo  $\beta$ -1,4 glucanase) and cellobiohydrolase. The TRS yield obtained from enzymatic saccharification at 30°C for 96h was 185 mg/g of pretreated biomass. The quantitative estimation of the hydrolysed sample obtained after 96h of enzymatic saccharification gives a maximum glucose yield of 112 mg/g of pretreated biomass. The efficacy of the recombinant enzymes was obtained on the dual pretreated biomass by mild alkali soaking for 2h at 50°C followed by

organosolv pretreatment. The effectiveness of the pretreatment processes was confirmed using FESEM and FT-IR analysis. Further, the reducing sugars obtained were fermented for bioethanol production using *Saccharomyces cerevisiae*.

**Keywords:** Lignocellulosic biomass; Sugarcane bagasse; Pretreatment; Enzymatic saccharification; Bioethanol

BE-8

### Single cell oil production by a novel yeast *Trichosporon mycotoxinivorans* from mixed sugars derived from lignocellulosic biomass

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#### Abstract

Oleaginous yeasts capable of utilizing low cost substrates like lignocellulosic biomass serve as a potential feedstock for sustainable biodiesel production. A mixture of glucose and xylose is obtained following saccharification of pretreated lignocellulosic biomass. Exploration of oleaginous yeast isolates growing on both glucose and xylose is necessary for the complete utilization of substrate. Therefore in this study, efforts were made to isolate xylose utilizing yeasts from soil and different rotten fruits. A total of eleven isolates were obtained which were identified and screened for their ability to utilize various carbohydrates as well as exhibit lipogenesis. One promising yeast isolate *Trichosporon mycotoxinivorans* S2 was selected for further research based on its capability to use a mixture of both glucose and xylose and produce 44.86±4.03% lipids by dry weight. Since this yeast does not produce cellulolytic enzymes, 1% alkali pretreated paddy straw was saccharified with commercial cellulase (15FPU/gds) for 48 hours resulting in 8.206g L<sup>-1</sup> sugars. The selected yeast strain was found to be tolerant for inhibitors generated during saccharification, the non-detoxified hydrolysate containing 34 g L<sup>-1</sup> of reducing sugars, was used after supplementation of 0.05% yeast extract, 0.18% peptone and 0.04% MgSO<sub>4</sub> which yielded 5.17g L<sup>-1</sup> lipids. Further optimization was carried out by Box Behnken design which helped to optimize the supplementation level of yeast extract, peptone and MgSO<sub>4</sub> to maximize the lipid yield. The optimized conditions proposed by the model predicted lipid yield of about 6.98g L<sup>-1</sup>. FAME analysis of single cell oil revealed oleic acid (69.72%) and palmitic acid (15.78%) as major fatty acids which illustrates the potential of the oil produced by *T.mycotoxinivorans* S2 as a feedstock for biodiesel. In future, metabolic engineering strategies may help to improve the lipid yield to develop commercially viable process of SCO production from lignocellulosic biomass.

**Keywords:** Inhibitor tolerance; Lipogenesis; Fermentation inhibitors; Lignocellulosic hydrolysate; Biodiesel



## Alkaline hydrogen peroxide pretreatment of sugarcane bagasse for production of bioethanol using thermotolerant yeast strain

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### Abstract

Increasing energy demands and shifting to renewable sources have paved a way for sustainable carbon neutral biofuels such as bioethanol to play a table turning role in transportation sector. Paradigm has been shifted to lignocellulosic material as feedstock due to its availability in abundance and even distribution on earth. Although complexity of lignocellulosic materials thrives for pretreatment step which results in more effective enzymatic hydrolysis but increases process cost. Chemical pretreatment is suggested as one of the effective pretreatments. Hydrogen peroxide at alkaline pH (pH=11.5) readily produces superoxide radicals which breaks bonds within lignin, hemicellulose and cellulose. The present study deals with optimization of sugarcane bagasse pretreatment using alkaline hydrogen peroxide, to solubilize lignin and expose cellulose and hemicellulose for enzymatic hydrolysis and fermentation. Response surface methodology approach using software Design Expert version 8.07.1 was used by varying four parameters H<sub>2</sub>O<sub>2</sub> concentration (1-7 %), solid loading (5-20 %), time (15-90 min), and temperature (60-100 °C). The optimized conditions were found to be 6.67 % H<sub>2</sub>O<sub>2</sub> concentration, 5 % solid loading at 91 °C in 47 min. Optimized pretreated biomass upon enzymatic saccharification using Cellic<sup>®</sup> Ctec2 enzyme yielded 75.64±3.69 g/L of reducing sugar, which accounts for 64.69 % of total reducing sugar content present in sugarcane bagasse after 72 h. Furthermore, bioethanol production was carried out using thermotolerant yeast strain *K. marxianus* NIRE K3.2. The fermentation was carried out at 45 °C for 16 h with an initial sugar concentration of 40.826 g/L, total volume was made to be 20 mL, which resulted in ethanol concentration of 15.015 g/L corresponding to 191.06 g bioethanol/kg of dry biomass. The ethanol productivity was calculated as 0.938 g/L/h, while ethanol yield on fermentable sugars basis was 46.43 %.

**Keywords:** Lignocellulosic material; Thermotolerant; Sugarcane Bagasse pretreatment

BE-10

## Thermophilic biocatalysts from hot springs of Himachal Pradesh for commercial applications

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### Abstract

Microbes has thrived extreme environments like hot springs, hydrothermal vents, deserts, deep seas etc. and has given clues for the existence of life in other planets. Thermophiles are of interest to researchers to study microbial adaptation, evolution and unique enzymes it possess to withstand extreme physical and chemical conditions. Novel microbes have been discovered from these habitats and has been a potential source of useful industrial enzymes. India have several geothermal hot springs and Manikaran is one of the hottest among the springs with temperatures as high as 106°C. In the present study, thermophilic microbes were selectively enriched at Manikaran Hot springs of Himachal using various natural polymeric substrates rich in cellulose, starch, lignin etc. Thermophilic microbes that grow at 50°C were isolated and screened for various commercial enzymes. Among 40 thermophilic isolates screened by plate assay, 13

morphologically distinct isolates produced enzymes including cellulase, amylase, protease and lipase. Some of these isolates were also capable of dye degradation which is an indicator of lignolytic activity. Interestingly, few isolates were showing temperature tolerance up to 80°C and could grow from pH 4 to 12. The isolates were identified using 16S rDNA sequencing and phylogenetic profiling as most of them belonging to *Brevibacillus borstelensis* and *Bacillus subtilis*. The study has led to the identification of thermophilic microbes and enzymes with potential for industrial applications.

**Keywords:** Thermophiles; Hot springs; Enzymes; *Bacillus*; *Brevibacillus*

BE-11

### Waste to wealth: Kitchen waste to bioethanol

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#### Abstract

Food production and wastages are serious issue for global economy and environment. One third of food produced for human consumption is getting disposed as food waste. The demand for biofuels *viz.*, bioethanol and biodiesel is emerging rapidly. Development of sustainable biofuel production from food waste is the need of the hour. As kitchen waste (vegetable peels, food waste, etc) encompasses significant amount of carbohydrates, the current work focuses on employing the waste to produce bioethanol. The organic-rich kitchen waste collected from university canteen was washed and subsequently oven-dried. Lactic acid bacteria (LAB) grown on yeast extract peptone dextrose (YPD) media was spread on the surface of kitchen waste to keep them fresh. They were thereby pretreated with 0.6 M NaOH. The ideal simultaneous saccharification and fermentation (SSF) is to keep reducing sugar at a low level; the sugar produced could promptly be exploited by the microorganism. Kitchen waste based SSF trials employing *Saccharomyces cerevisiae* in fed-batch mode witnessed 0.41 g g<sup>-1</sup> ethanol yield. Estimation of novel approaches to obtain substantial bioethanol from kitchen waste as well as to control waste management leading to green environment is underway. The detailed outcomes will be exhibited.

**Keywords:** Kitchen waste; Lactic acid bacteria; SSF; *Saccharomyces cerevisiae*; Bioethanol

BE-12

### Screening of oleaginous fungi from Molasses Distillery Spent Wash (MDSW) for biodiesel production: a waste to wealth approach

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#### Abstract

Sugar industries discharge an enormous amount of untreated molasses distillery spent wash (MDSW) effluent annually into the environment causing serious soil, water and air pollution. The MDSW contains high concentration of COD, BOD, nitrate and phosphate which can be utilized for lipid production by

oleaginous microbes for the production of next generation biofuel. Bio-trap based enrichment screening of oleaginous microorganisms resulted in 44 fungi, 59 yeasts and 7 microalgae were isolated from the soil and distillery effluent samples collected from Sakthi Sugars Pvt. Ltd, Tamil Nadu. These isolated fungi, yeast and microalgae were screened for biomass, lipid yield and lipid content in the standard glucose containing lipid production medium for a period of 7 days of incubation. The fungal isolate DESF1 was found to have maximum lipid content of 33.75% followed by DELF3 with 28.77%. Also the biomass and lipid yield of DESF1 was 4.59 and 1.55 g L<sup>-1</sup> respectively. The oleaginous fungi (22), yeasts (23) and microalgae (7) which were positive for lipid production in the glucose containing media were again screened in the molasses distillery spent wash effluent medium for biomass, lipid yield and lipid content. Among these 22 fungi, 23 yeasts and 7 microalgae, the fungal isolate DESF1 showed maximum lipid content (20.62%) and lipid yield (1.95 g L<sup>-1</sup>) with biomass of 10 g L<sup>-1</sup> in the MDSW medium. The DESF1 was identified as *Aspergillus terreus* through molecular identification based on amplification of ITS region. Further research regarding, increasing the lipid yield and potential nutrient removal by *A. terreus* DESF1 in MDSW effluent is under progress.

**Keywords:** Oleaginous; Biodiesel production; *Aspergillus terreus*; Sugar industry

BE-13

### Characterizing gamma irradiated mutants and wild type of *Chlorella* sp. for their growth, biochemical composition and lipid productivity for biofuel production

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#### Abstract

With increase in global energy demand, shortage of fossil fuels and global climate change, alternative fuels are receiving considerable attention. Oleaginous microalgae have become promising approaches and alternate energy resources for biodiesel production. *Chlorella* strains have a great potential to be a resource for biodiesel production due to its faster growth and easier cultivation. Improving physiological properties of microalgae is often required to meet the production at industrial level. One of the classical and ecofriendly approaches for strain improvement is mutagenesis. In this study, we have subjected one of the *Chlorella* sp. Isolated from Nilgiri Biosphere Reserve to gamma radiation and obtained 12 stable mutants (M<sub>1</sub> to M<sub>12</sub>). Growth and physiological characteristics *viz.*, biomass productivity, generation time, specific growth rate chlorophyll content, macromolecular composition (carbohydrate, protein and lipid) of the mutants and wild type were evaluated under batch culture for a period of 21 days with 5 days interval. The biomass productivity of the mutants ranged from 33 mg L<sup>-1</sup> d<sup>-1</sup> to 45.33 mg L<sup>-1</sup> d<sup>-1</sup>. Certain mutants accumulated more lipids (27 to 43%). Some mutants contained higher carbohydrates and protein, which can valorize biofuel based byproducts. Among the mutants, M<sub>3</sub> & M<sub>5</sub> showed maximum macromolecules (95% & 90%) composition. Hence, these results conclude that this novel strategy of using gamma radiation for strain improvement in terms of biomass, macromolecular composition and lipid accumulation capacity will meet the future requirement of industrial biofuel production.

**Keywords:** Microalgae; *Chlorella*; Gamma irradiation; Mutant; Biofuel

## Biorefinery approach to food waste generating value-added products

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### Abstract

In the light of rapidly rising costs combined with energy supply and waste disposal and increasing public worries with environmental quality, the alteration of food wastes to energy is promptly becoming an environmentally benign and economically attractive practice. Food waste is one of the most promulgated organic biowastes around the world. Superfluous management of kitchen wastes viz., indulgent dumping leads to polluting surface and also enhances the breeding of disease bearing vectors. The organic wastes like kitchen waste can be converted into bioproducts and bioenergy, creating economic value, generating benefits for the society. Biorefinery approach leading to various value-added products along with zero-waste production is a prerequisite of the biofuel sector. In the current research, kitchen waste was crushed and mixed with 0.5 M NaOH solution along with subsequent acid-hydrolysis process with the help of concentrated H<sub>2</sub>SO<sub>4</sub> (0.3 M) leading to subsequent lignin removal. The acid-treated kitchen waste was then washed with distilled water repeatedly until the pH became neutral. The food waste was then dried for 2 h at 60°C followed by batch yeast fermentation trials, provided ethanol yield of ~0.34 g/g. The left-over kitchen waste was then mixed along with appropriate cow dung inoculum in 3 experimental set-ups of 1L, 2L and 5L bottles. A prominent increment in biomethane production was witnessed in comparison to the conventional biogas production process. Experiments on the usage of slurry produced during biogas production process as biofertilizer in agricultural areas is underway.

**Keywords:** Food waste; Biorefinery; Bioethanol; Biogas; Biofertilizer

## Attempts in formulating efficient enzyme cocktails for saccharification of hemicellulose of lignocellulosic residues for bioethanol production

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### Abstract

Increase in energy demands, depletion of fossil fuels and adverse environmental effects have drawn significant attention towards biofuels as cleaner alternative energy source. Lignocellulosic biomass (LC) is the most abundant and ubiquitous renewable bioresource in the world that makes LC bioconversion very attractive option over other alternative energies. LC contains mainly cellulose (30-50%), hemicelluloses (20-40%), and lignin (20-30%). Of the three components, cellulose and hemicelluloses can be converted into fermentable sugars which can be fermented to bioethanol and other value-added products. There are several ways for deconstructing biomass (thermochemical, chemical, biological). The biological route using enzymes is the most popular one. Microbial cellulases and xylanases are useful in the bioconversion of biomass into monomeric sugars, which can be fermented to ethanol and other value-added products. One key objective of our research is selection of a suitable pretreatment method for LC, sugarcane bagasse

and paddy straw. In this regard various physiochemical methods of pretreatment were employed to assess their utility in retaining maximum amount of hemicellulose in combination with cellulose. 1% NaOH + 2% Na<sub>2</sub>SO<sub>3</sub> + 0.5% V/V formaldehyde at 900W microwave for 20 minutes was selected for paddy straw as this pretreatment yields five-fold increase in reducing sugars due to hydrolysis. The complexity of different feed stocks, however, brings the need of customized enzyme proportions, specific for each of them for attaining maximum saccharification. This investigation is aimed at formulating tailor-made enzyme mixtures targeted for hydrolyzing the selected crop residues. In this regard, crude enzymes from *Myceliophthora thermophila* and recombinant metagenomic xylanase and β-xylosidase from *Bacillus halodurans* are produced. Hemicellulase genes from *Myceliophthora thermophila* were cloned and expression optimization is going on in *E. coli*. For cocktail purpose, metagenomic xylanase and *Bacillus halodurans*'s β-xylosidase were produced and cocktail preparation is in progress.

**Keywords:** Lignocellulose; Biofuel; Hemicellulose; Pre-treatment; Cocktail



# **ENVIRONMENTAL AND MICROBIAL TECHNOLOGY**





# ORAL PRESENTATION



## Reclamation of diesel contaminated site using integrated bio- and phytoremedial approaches

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### Abstract

Hydrocarbon contaminated oil spilled areas and oil products have caused serious harm with increasing attention for the development implementation and removal of these contaminants. Bacterial diversity and selective population on succession at the petroleum hydrocarbon contaminated environment can give answer to the problem. Such land have serious problems since they are totally barren or with very rare plantation. Bacteria can thereby be exploited for the mitigation and mobilization of hydrocarbon to enhance the nutrient availability for vegetation. The present study involves the utilization a consortium of three bacteria for diesel contaminated site reclamation. Diesel contaminated soil samples were collected from different sites of Rajasthan and around (n=30) with hydrocarbon load in the range 78.28% to 99.57%. During bacterial screening, bacteria were encountered possessing  $62.058 \pm 0.022$  % to  $92.579 \pm 0.017$  %. Bacterial sample initially tried to analyse its impact on phyto-cum bio-degradation with all the 5 selected plant species (wheat 4037, IPM-02-3, mustard RH-407, maize IP4M-1 and sorghum CSV-11) respectively. For the reclamation of hydrocarbon two methods were used in SMB (with bacterial biopreparation watering and seed coating). Out of the two methods watering perform better in SBM. Alternatively in pot trial experimentation two methods were used (with bacterial biopreparation watering and mixing with husk). The method with bacterial biopreparation with husk performed better statistically in pot experimentation. The product Bio-Reclaim is designed from the combination of bacterial population impregnated in husk to mitigate diesel from soil and restore the nutrient cycle in presence of plants. Since such sites are encountered with very low or no vegetation, this study will certainly give a possible solution to the prevailing issue and helps in the reconstruction of nutrient pool in those sites.

**Keywords:** Site restoration; Diesel contaminated site; Bio-Reclaim; Microbial consortium; Bioremediation; Phytoremediation

OP/EMT-05

## Biocatalytic- Bioplates containing entrapped biomass and its kinetics with RSM models for the resourceful removal of a noxious OP-Methylparathion from wastewater

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### Abstract

The theory of immobilization of biocatalysts (microorganisms and enzymes) includes bioaugmentation, enzyme technology, rhizoremediation and bioelectrochemical remediation process. Immobilization-based technologies are applied as engineered ex-situ treatment systems as well as in-situ for the bioremediation of groundwater, wastewater and soil. Developing biodegradation kinetics models were inturn results in the advancement in the performance of technologies. The bioplates containing entrapped biomass were used in the organophosphate (methyparathion with 2g/l) removal in a batch reactor.

Influent and effluent samples were taken at regular intervals and analysed for the parameters estimation which includes COD, phosphate and nitrate along with the parent compound (methylparathion). The batch reactor process readily promotes a reaction with aeration. The qualitative chromatographic and quantitative end product analysis revealed that the bioplates system facilitated over 93% of bioremoval of methylparathion at high concentration with optimum pH 6.5, temperature 30°C and rpm of 120 rpm. The bioremoval was the result of a mutual effect among the activity of the biocatalyst, the cling capacity and the sorption nature on the support material. Further studies are needed to ensure the processes in detail about the degradation pathways. The kinetics with models facilitates the process in a relatively efficient manner in terms of cost and technology execution.

**Keywords:** Bioplates; Entrapped biomass; Kinetic Models; Bioremoval; Aqueous methylparathion

OP/EMT-14

### Bioremediation of carcinogenic metals by Thermophilic Bacteria isolated from hot Springs of Garhwal Himalaya

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#### Abstract

The rapidly increasing urbanization and industrialization have led to the discharge of huge amount of contaminants in the environment which besides causing disaster in the environment may also cause chronic diseases in human beings. The different types of physical and chemical approaches used for metal removal are cost-ineffective and sometime generate a large amount of toxic by-products. The biological means of metal remediation are cost-effective and eco-friendly. The aim of this study was to screen the thermophilic bacteria to find an effective mean for metal removal. Fifty thermophilic bacterial isolates were screened for tolerance to four carcinogenic metals ( $\text{Cr}^{3+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{As}^{3+}$ ). The live and dead cell biomass was then immobilized on corn cob to increase the biosorption potential of bacterial isolate. The various reaction conditions *viz.*, pH, temperature, adsorbent dosage, contact time and agitation were studied to determine the optimized conditions for metal biosorption. The adsorption kinetics of metals were studied using Langmuir and Freundlich isotherm at different concentrations. TKD 5 isolate was found to adsorb more concentration of metal (89.54% of  $\text{As}^{3+}$ , 84.55% of  $\text{Cd}^{2+}$ , 84.93% of  $\text{Cr}^{3+}$  and 89.77% of  $\text{Ni}^{2+}$ ). The dead cell biomass immobilized on corn cob adsorb more metal concentration as compared to the living cell biomass. The maximum absorption of all metals was found at 24h, 50°C, pH 6 to 7, 550 mg dosage of adsorbent and 200 rpm agitation. Both adsorption models describe a better absorption process as indicated by the value of  $R^2$ . TKD5 was identified as *Bacillus licheniformis* based on morphological characteristics, biochemical characteristics and 16SrRNA sequencing. The present study has revealed promising levels of metal biosorption by a thermophilic bacterial isolate. This work can be further extended towards the development of a technology for the metal remediation from the contaminated sites.

**Keywords:** Thermophilic bacteria; Carcinogenic metals; *Bacillus licheniformis*; Biosorption; Metal remediation

**Bioconversion of chicken feather waste into bioactive Keratin hydrolysate by a newly purified keratinase from *Bacillus* sp. RCM-SSR-102**

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**Abstract**

The present study deals with the purification of a keratinase from *Bacillus* sp. RCM-SSR-102 and its application in preparation of bioactive keratin hydrolysate from chicken feather waste. At first, keratinase production was optimized by Response Surface Methodology using Design Expert 6 software. Then the keratinase was purified by gel filtration chromatography. Feather keratin hydrolysate (FKH) was produced from chicken feather waste by using purified keratinase. Antioxidant and antityrosinase activity of the FKH was determined. Statistical optimization resulted in 3 folds increase in keratinase production over unoptimized conditions. The purified KER102 keratinase was found to be a serine-metalloprotease having molecular weight of 30 kDa with optimum pH and temperature of 10 and 50°C respectively. The keratinase was quite stable at high pH (retained 98% activity at 10 pH) and high salt concentrations (retained 55% activity at 3.4 M or 20% salt). Moreover, the KER102 keratinase was found to be highly stable in presence of oxidizing agents, surfactants and organic solvents. The keratinase could also hydrolyze both soluble and insoluble complex protein substrates. The KER102 keratinase can hydrolyze up to 5% (w/v) feather concentration releasing 8.58±0.5 mg protein. The feather keratin hydrolysate (FKH) thus produced has both antioxidant and antityrosinase activity. The IC<sub>50</sub> value of FKH in 2, 2-diphenyl 1-picrylhydrazyl (DPPH) scavenging activity (1.02±0.01 mg/ml), 2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) scavenging activity (20±40.04 μg / ml) and antityrosinase activity (1.2±0.22 mg / ml) was observed. Antioxidant and antityrosinase compounds have potential applications in pharmaceutical and cosmetic industry. Hence, the purified keratinase can be a potential candidate for production of antioxidant and antityrosinase compounds from chicken feather waste.

**Keywords:** Keratinase; *Bacillus* sp. RCM-SSR-102; Feather keratin hydrolysate; Antioxidant; Antityrosinase

OP/EMT-56

**Microbiome analysis of Winogradsky columns established with polluted site sediments and enriched with LDPE**

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**Abstract**

Plastics are defined as the light weight polymers (solid materials) which on heating become mobile and can be cast into moulds. The word plastic comes from the Greek word "Plastikos" which means able to be molded into different shapes. Plastics are non-biodegradable, strong, light-weight, moisture resistant and durable materials and they have replaced natural resources, such as stones and metals. Discarded plastics, besides being highly visible are rapidly increasing percentage of solid waste in landfills, resistant

to biodegradation leading to pollution and also harmful to the natural environment. Low-density polyethylene can be degraded in various methods as follows: chemical degradation, photodegradation and biological degradation (Da Luz et al. 2014). The microbial species associated with the degrading polymers were identified as bacteria (*Pseudomonas*, *Streptococcus*, *Staphylococcus*, *Micrococcus*), fungus (*Aspergillus niger*, *Aspergillus glaucus* and *Trichoderma*). The Winogradsky column is a useful model microbial ecosystem to study environmental influences on microbial community structure and dynamics. This complex community can be maintained or manipulated under carefully controlled laboratory conditions with the addition of compounds in the column. In the present study, Amlakhadi polluted soil sediment and water samples were used to build Winogradsky column along with normal and LDPE enriched condition. After maturation, various microbial community was visibly observed in the column. After eight weeks of incubation columns were broken and upper- aerobic zone and lower- anaerobic zone were separated to extract the DNA from each zone of each column. The DNA was subjected to quality checking followed by 16s rRNA sequencing using Illumina Miseq Platform. The data were analyzed by Kraken and One Codex and compared using Megan and STAMP. Community analysis revealed rich microbial diversity and comparative analysis demonstrated fluctuation in community composition with the reference tool. Presence of microorganisms with high proportion was also compared to have LDPE biodegradation with literature review. Six species were reported to have significant difference and *Arthrospira* spp. was found in abundance in our metagenome. The study will end towards exploring the polluted site for the removal of pollution. Microbial community stimulated by Winogradsky column can be used for LDPE depolymerisation.

**Keywords:** Metagenomes; Microbiome Winogradsky column; Polluted site; LDPE biodegradation

OP/EMT-70

### Removal of benzoate and 4-hydroxybenzoate by metabolically engineered *Acinetobacter baylyi* ADP1

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#### Abstract

Lignocellulosic biomass is the most abundant and underutilized biomass on planet earth with composition- cellulose, hemicelluloses and lignin as main constituents. The biomass can be converted into value added- products like liquid biofuel (ethanol, butanol) by bioconversion process which includes pretreatment, hydrolysis (acid/enzymatic) and fermentation. The pretreatment is unavoidable step in bioconversion process which leads to production of organic acids, furan derivatives and phenolic compounds. These compounds produced during pretreatment act as inhibitors for microbial growth, decrease ethanol yield and productivity. Selective removal of inhibitors with bio detoxification of hydrolysate is a promising approach where microorganisms selectively consume inhibitors without affecting sugars. In the present study, *Acinetobacter baylyi* ADP1 and constructed strains were used for selective removal of benzoate and 4-hydroxybenzoate, phenolic compounds produced during pretreatment of lignocellulosic biomass. The *Acinetobacter baylyi* ADP1 strain was not consuming xylose and arabinose, and engineered strain was shown incapable of consuming the main sugar i.e. glucose, but consuming benzoate and 4-hydroxybenzoate. The consortium of engineered strains of *Acinetobacter baylyi* ADP1 is able to degrade benzoate and 4-hydroxybenzoate simultaneously under batch and continuous conditions in the presence of sugars.

**Keywords:** Biomass; Benzoate; 4-hydroxybenzoate; Inhibitors

**Role of toxin antitoxin system in regulating virulence of *Salmonella entericaserovar Typhimurium*****Prajita Paul, Paritosh Patel, Bikash R Sahu, Mrutyunjay Suar<sup>a\*</sup>***School of Biotechnology, Kalinga Institute of Industrial Technology (KIIT), Bhubaneswar, Odisha, 751024, India*

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**Abstract**

Response to stress in bacteria is regulated by several factors associated with these organisms, of which toxin-antitoxin (TA) modules are known widely to play a pivotal role. Toxin-Antitoxin systems (TA) are small modules encoding two elements; an intracellular toxin and its labile antitoxin that neutralizes the toxic effect of its cognate toxin resulting in tight co-transcription and co-translation. The TA systems of *Salmonella* plays important role in virulence, intracellular survival and persistence. Presence of toxin-antitoxin modules in bacteria is known to be significantly associated with higher pathogenicity. In our present study we investigated Hha and TomB form a bonafide TA system where Hha serves as a toxin while TomB functions as an antitoxin. Deletion mutant of both TA genes showed reduced uptake by macrophages as compared to wild type strain. However, there was a significant reduction in % uptake for  $\Delta$ Hha as compared to Wild type. Since *S. Typhimurium* resides in host macrophages and undergo replication, we further examined the difference in replication among three different strains (Wild type,  $\Delta$ Hha and  $\Delta$ TomB) inside macrophages. The results showed a significant reduction in replication of  $\Delta$ Hha strain inside macrophages as compared to wild type. Our *in vivo* experiment showed that  $\Delta$ Hha showed limiting colonization in the gut of C57BL/6 mice and unable to induce acute inflammation at 3 days post infection (p.i.). Our study revealed the possible role of Hha toxin in contributing to virulence of *Salmonella*.

**Keywords:** Toxin-Antitoxin; *Salmonella*; Persistence; Virulence; Macrophages

RF/EMT-71

**Application of electron microscopy in validating bioremoval of  $Pb^{+2}$  by *Chlorella pyrenoidosa*****Chandrashekharaiyah P.S<sup>a, b\*</sup>, Avishek Banik<sup>c</sup>, Purbasha Sarkar<sup>d</sup>, Debanjan Sanyal<sup>b</sup>, Santanu Dasgupta<sup>d</sup>**<sup>a</sup>*Department of Microbiology, RK. University, Rajkot, Gujarat, 360020, India*<sup>b</sup>*Reliance Industries Ltd, Jamnagar, Gujarat, 361140, India*<sup>c</sup>*School of Biotechnology, Department of Life Sciences, Presidency University, Canarabank Rd, DGBlock, Action Area 1D, Newtown, Kolkata, West Bengal, 70015, India*<sup>d</sup>*Reliance Industries Ltd, Ghansoli, NaviMumbai, 400701, India*

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**Abstract**

In bioremediation process, microbes can remove heavy metals through various mechanisms. In addition to bulk elemental analysis methods, microscopy based semi-quantitative analysis can provide high-resolution spatial information about element distribution in microbial cultures for a comprehensive and accurate understanding of the metal removal processes. In this study the green alga, *Chlorella pyrenoidosa* procured from NCIM, Pune, India, was characterized for its bioremoval capacity of  $Pb^{+2}$ . In 8 days, incubation study, *C. pyrenoidosa* was found to remove 32.46 %  $Pb^{+2}$  from an initial concentration of 200 ppm. The fractionate analysis of residual  $Pb^{+2}$  in the media and bioaccumulated  $Pb^{+2}$  in the cell pellet by inductively coupled plasma optical emission spectroscopy indicates that ~99.22% bioremoval was through biosorption and ~0.78% was by bioaccumulation. The surface accumulation or biosorption of  $Pb^{+2}$  was confirmed by performing scanning electron microscopy and energy dispersive spectroscopy

(SEM-EDS). The SEM images also confirmed the changes in cell structure and deformation which has occurred during biosorption. The  $Pb^{+2}$  which was bioaccumulated inside the cell and surface adsorbed outside the cell, was visualized and confirmed by high angle annular dark field scanning transmission electron microscopy and energy dispersive spectroscopy(HAADF-S/TEM-EDS). Overall, the advanced electron microscopic techniques could be effectively used to study the topographical changes,surface and intracellular elemental ( $Pb^{+2}$ ) mapping and to confirm the heavy metal removal mechanisms by microorganisms.

**Keywords:** Algae; Electron microscopy; SEM-EDS; HAADF-S/TEM-EDS; Bioremoval



# POSTER PRESENTATION



## Biogenic Silver Nanoparticles (Ag NPs) inhibit the growth of Methicillin Resistant *Staphylococcus aureus* (MRSA) through Induction of ROS

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### Abstract

Microbial infection in public health is considered as major problem due to development of antimicrobial resistant (AMR) strains. To overcome these problems, nanotechnology based strategies has been applied and biogenic nanoparticles (NPs) gives eco-friendly NPs, which are nontoxic and mono dispersed. In the present report, we have synthesized the silver nanoparticles (Ag NPs) through biological route followed by different parameters of synthesis. After synthesis, Ag NPs have been characterized using UV-visible spectrophotometer, Dynamic Light Scattering (DLS) and Transmission electron microscopy (TEM) techniques. Antibacterial activity of Ag NPs is investigated against *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus* (MRSA). Controlling of infection caused by MRSA is difficult owing to its antibiotic resistance properties. We checked the growth of susceptible *S. aureus* and MRSA in the presence of Ag NPs and observed that growth of both bacteria has been down regulated with low concentration (10 and 25µg/mL) of NPs. Biofilm formation by the above pathogens is tough to eliminate due to secretion of extracellular matrix. In this regards we have examined the anti-biofilm activity of the metal NPs against both the test pathogens. Significant decrease in the mature biofilm of *S. aureus* and MRSA has been observed after treatment with Ag NPs. Possible antibacterial mechanism has been investigated through the measurement of Reactive oxygen species (ROS) and obtained data suggest that Ag NPs has induced intracellular ROS, which might inhibit the growth of bacteria. Our investigation suggests that as synthesized Ag NPs have shown antibacterial activity in ROS dependent manner.

**Keywords:** Antibacterial; Biofilm; Silver; *Staphylococcus aureus*; Methicillin Resistant *Staphylococcus aureus*

## Doped ZnO Nanoparticles Assisted Sonophotocatalytic Disinfection of *Salmonella* sp.

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### Abstract

Gastrointestinal diseases originating via microbial contamination of water sources poses huge threat to the well-being and economy of developing countries. The situation is worrisome considering the evidences where microbes evolve and abscond he existing water treatment techniques. In this context, we have developed an efficient disinfection process using Iron (Fe) doped ZnO nanoparticles (ZnO:Fe NPs) under the exposure of sunlight, called Sonophotocatalysis (SPC), to eliminate different serovars of *Salmonella* sp. SPC was found to be superior in disinfecting *S. Typhimurium* among the other disinfection processes

tested. Moreover, the efficiency of ZnO:Fe NPs were higher to TiO<sub>2</sub>, a commonly used photocatalyst. Role of reactive oxygen species (ROS) were elucidated using ROS scavenger assay and it was found that •OH and O<sub>2</sub>• were the key radicals involved in SPC. Microbial membrane perturbation caused by SPC was confirmed by Live/Dead fluorescent microscopic imaging and electron microscopy. Interference of SPC in hindering the metabolic functions of *S.Typhimurium* was established using resazurin assay. Alterations in fatty acid composition post SPC were also observed, suggesting the stress caused by the disinfection process. Most importantly, solar-SPC was successful in disinfecting *S.Typhimurium* spiked in various natural water samples such as river, pond, lake and municipal tap. Also, SPC treated water was found to have positive effect on *Zea mays* seed germination compared to *S.Typhimurium* infected setup, implicating its use in various agricultural purposes. Hence, solar-SPC could be considered to have potential real world applications considering its overall efficiency in eliminating *S.Typhimurium*.

**Keywords:** Water; Microbial contamination; Sunlight; ZnO:Fe NPs; Sonophotocatalysis

EMT-06

## Hexavalent Chromium: Toxicity, Health Threats and Bioremediation

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### Abstract

Heavy metals are potentially toxic with high density and are natural constituents of the environment. Chromium (Cr) is one of the heavy metal whose prolonged use and release by various industries into environment have deleterious effects on human life and aquatic biota. Though, it exists in many oxidation states ranging from -2 to +6 due to its electronic structure, Cr(III) and Cr(VI) are the stable oxidation states having different physicochemical and biochemical properties. Cr(VI) is the most toxic form of Cr due to its strong oxidation potential, higher solubility in water, rapid permeability through biological membranes. Cr(VI) has been classified as one of the most toxic chemicals by the Agency for Toxic Substances and Diseases Registry (ATSDR) and listed as grade 'A' human carcinogen by the United States Environmental Protection Agency (U.S.EPA). Cr(VI) is used in a variety of industrial applications such as leather tanning, metallurgy, electroplating, petroleum refining, textile dyeing, paints, pigment production, steel making and thermonuclear weapons manufacturing. Similar to some organic pollutants, Cr tend not to be degraded and gets accumulated into the environment, Cr compounds may enter the food chain and can cause different effects like accumulation in the placenta, impairing fetal development, skin allergies, vomiting, diarrhea, brain damage and premature death in mammals. Some chemical methods are used to decrease the toxicity of Cr(VI) like adding lime, coagulation, ion exchange, membrane separation. However, these include high costs, low efficiencies, and the generation of toxic waste that require careful disposal. Usually, the biological species used in the bioremediation process are indigenous species. Bioremediation is an attractive strategy that is cost-effective, safe and produces no secondary by-products. Bioremediation of Cr(VI) by microorganisms is considered one of the most useful methods for reducing Cr(VI) to less toxic Cr(III), being an economical and environmental friendly compared to chemical methods.

**Keywords:** Hexavalent chromium; Heavy metal; Bioremediation; Toxicity

## Efficient arsenic biotransformation mediated by a novel *Bacillus* sp. from an arsenic-contaminated site

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### Abstract

Arsenic is a naturally occurring toxic element found in environment. It comes from natural process and anthropogenic activities such as mining smelting and wood preservative, industrial process, pesticides and fertilizers. Due to high arsenic concentration there are serious health risks such as bladder cancer, diarrhoea, neurological disorders and vascular disease. Arsenic founds in four oxidation states (+5, +3, 0, -3) but mainly arsenic (III) and arsenic (V) are common. In present scenario bioremediation techniques offering the effective method for conversion of toxic arsenic to non-toxic forms. In this present study, a novel rod shaped bacteria was isolated from Delhi NCR. This isolate could survive in a medium containing sodium arsenate concentration up to 5000ppm and 1500ppm of Sodium arsenite concentration. Morphological, biochemical and molecular identification was done. The optimum pH and temperature were found to be at 7 and 37°C, respectively. The growth pattern of the isolates in the absence and presence of arsenic was also observed. The bacterial strain was resistant to recommended doses (30mcg) of some antibiotics like Kanamycin, Neomycin, Tetracycline, Vancomycin, Nalidixic acid, Chloramphenicol, Doxycycline and was sensitive for Amoxycylav. The arsenic extrusion from the cells results in reduced accumulation of arsenic inside the cells as confirmed by SEM-EDX analysis. This strain has significant potential to transform arsenate to arsenite. Hence, this strain is useful for the development of effective bioremediation strategies in the detoxification of arsenic from polluted environments.

**Keywords:** Arsenic; Arsenite; Arsenic resistant bacteria; Bioremediation

## Improved cultivation of *Clarias batrachus* (Linn) through application of probiotic organism and study of growth performance, carcass quality and haematological parameters

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### Abstract

*Lysinibacillus sphaericus* PKA17, *Bacillus cereus* PKA18 and *Bacillus thuringiensis* PKA19, isolated from the intestine of adult *Clarias batrachus*, were evaluated to be used as candidate probiotics. *C. batrachus* fingerlings (4.01 ± 0.01) g were cultivated in rearing tanks with probiotic-supplemented experimental feed and control feed. The T1 set has displayed considerably higher specific growth rate, protein efficiency ratio and significantly (P≤0.05) lower feed conversion ratio (1.23±0.01) than the other groups. The carcass protein content was significantly (P≤0.05) higher (164.9±0.23g kg<sup>-1</sup>) in T3 fed fish followed

by T1 and T2. The T1-fed fish produced considerably higher amount of carbohydrate ( $2.8 \pm 0.06 \text{ g kg}^{-1}$ ) and iron ( $2.21 \pm 0.01 \text{ mg } 100\text{g}^{-1}$ ) and fat-soluble vitamins than the other groups. The amino acid analysis of fish displayed significantly higher ( $P \leq 0.05$ ) content of glycine, arginine, aspartic acid, glutamic acid, histidine, isoleucine, lysine, methionine, proline, threonine, tryptophan, tyrosine and valine in T1 fed fish than the others. Fish with probiotic-supplemented feed showed higher amount of HDL cholesterol and calcium than the control fed fish. The results suggested that feed incorporated with these putative probiotics can safely be used for the sustainable development of *C. batrachus* that can restore the species from further deterioration.

**Keywords:** *Lysinibacillus sphaericus*; *Bacillus thuringiensis*; *Clarias batrachus*; HDL cholesterol

EMT-09

### Development of HPLC Method for the Detection of Ciprofloxacin, Tetracycline and Cefixime

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#### Abstract

Antibiotics are the most commonly used pharmaceutical compound for prevention of microbial infections in humans as well as animals and continuously discharged in the environment through discharge of hospital & pharmaceutical plants, human and animal excretion and sewage & municipal wastewater in pure form or as a metabolized form. So, the presence of antibiotics residues in water may harm the non-targeted population critical for the balance of aquatic life. HPLC is a well-known analytical technique for the detection of antibiotics in water samples. So HPLC method was developed after standardization of mobile phase, flow rate and column temperature. The developed method includes water and acetonitrile both with 0.1 % formic acid for positive ionization as mobile phase with a flow rate of 1.0 ml/min. at 40°C column temperature in a gradient run of 23 min. with a retention time of 01:47.1 min for the detection of ciprofloxacin; water and acetonitrile both with 0.1 % formic acid as mobile phase with a flow rate of 1.0 ml/min. at 20°C column temperature in a gradient run of 16 min with a retention time of 01:30.7 min for the detection of tetracycline and 1% phosphoric acid (pH 5) and acetonitrile as mobile phase (40:50) with a flow rate of 1.0 ml/min at 30°C column temperature in a linear run of 20 min with a retention time of 01:49.4 min. for the detection of cefixime.

**Keywords:** Antibiotic, HPLC; Ciprofloxacin; Tetracycline; Cefixime

EMT-10

### Exploration of Hydrocarbon degrading Bacterial diversity in Barmer Oil Basin, Rajasthan

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#### Abstract

Hydrocarbon is considered to be a primary source of energy in the current urbanized society. Considering the increasing demand, worldwide oil productions are declining due to maturity of oil field and also because of difficulty in discovering new oil fields to substitute the exploited ones. In order to meet current

and future energy demands further exploitation of oil resources is highly required. Microorganisms inhabiting in these areas exhibit highly diverse catabolic activity to degrade, transform or accumulate various hydrocarbons compounds. Enrichment of hydrocarbon utilizing bacteria in oil basin is caused by continuous long duration low molecular weight hydrocarbon microseepage which plays a very important role as an indicator for petroleum prospecting. Hydrocarbon resource in India is centred in several states including Rajasthan, Barmer basin. Soil and water samples were collected aseptically from surface and subsurface sites of Barmer oil basin. Bacterial communities were studied by culture-dependent methods. The hydrocarbon degrading bacteria was isolated by enrichment technique using Bushnell Haas broth with petrol and diesel oil as sole carbon source. Isolated bacteria were screened for hydrocarbon transformation and degradation potential via gravimetric and GC-MS analysis. Thus, this study on indigenous microbes in Barmer oil field site provides an improved understanding of potential bacteria for application in microbially enhanced oil recovery, bioprospecting and bioremediation of oil contaminated environments.

**Keywords:** Hydrocarbon; Degrading; Bacteria; Enrichment; Oil fields

EMT-11

### **Isolation and characterization of nitrogen fixing and nitrifying bacteria from mining impacted sites in Rajasthan**

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#### **Abstract**

Mining activities lead to tailing dumps, acid mine drainage and generation of huge amount of waste containing metallic contaminants. Considering the global impact of this activity most of the unreclaimed mining sites remain un-vegetated for several years. As N<sub>2</sub>-fixing microorganisms are considered to be the early and plentiful colonizers in most habitats deficient in nitrogen, then succession of other species occur. Therefore, it is highly significant to study the role these microbes in such devastated environments. Based on this hypothesis this study presents a survey of nitrogen fixing and nitrifying bacteria in Giral lignite mine sites, Rajasthan. The nitrogen fixing and nitrifying bacteria were isolated from soil and water samples aseptically collected from different sites in Giral mining region. Isolation of diazotrophs, was carried out by spread plate method on Norris Glucose Nitrogen free medium. In total 18 isolates were obtained from different samples. Isolation of nitrifying bacteria was done by suspending 0.5 g of soil samples in ammonium sulphate medium at pH 7.0-8.0, containing 0.5 mg/L of phenol red indicator. Colour change (from pink to yellow) was observed to check nitrite production. Confirmatory test was carried out on peptone-meat extract (PM) agar plates. The single colony of isolates was further tested for their capability to produce nitrite or nitrate by periodical spot tests for total oxidized-N (nitrite and nitrate) via Griess-Ilosvay method. The confirmatory test indicated the presence of 33 nitrifying bacteria. Indigenous bacteria are being evaluated for their nitrification potential. This study provides an understanding on the role of bacteria having role in nitrogen cycling and underlying mechanisms of ecosystem development in mining impacted systems.

**Keywords:** Nitrogen fixing; Nitrifying; Mining; Diazotrophs

## Microbial production of bioflocculant for the dye removal from waste water textile

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### Abstract

The effluent from the textile industries are continuously adding amount of hazardous dye and ions to water bodies, thus the non-biodegradable metal ions, cationic and anionic dyes create serious environmental issue. So there is an urgent demand of efficient and cost effective method for removal of dye. The scientists all around the globe is trying to cope up with this alarming issue. Many conventional and non-conventional methods are adopted to remove dyes and metal ions from the water bodies but the present investigation is carried out to explore the efficacy of bioflocculants produced by newly isolated bacteria. Bioflocculant producing bacteria strains are screened from different water sources and water treatment plant sediments. Screening is done in agar plate enrichment method using the Dextrose(15g/L), peptone(10g/L), yeast extract(5g/L) and NaCl (5g/L) as growth medium. Distinct bacterial colonies are picked and further cultured in flocculation screening media in Erlenmeyer flask of 250 ml at 30° C for 72 hours at 180 RPM. The flocculation screening media having Dextrose(10g/L), Casein Hydrolysate (5g/L), peptone(5g/L), KH<sub>2</sub>PO<sub>4</sub>(2g/L), K<sub>2</sub>HPO<sub>4</sub>(5g/L), MgSO<sub>4</sub>·7H<sub>2</sub>O(0.2g/L), NaCl (0.1g/L), pH 6.8(+)-1. After 72 hours, 5 ml of fermentation broth is centrifuged and supernatant is subjected to determination of flocculation activity by doing the assay with kaolin clay and optical density taken at 550 nm. In the flask level, using the bacterial strains we get high amount of flocculation activity. Optimization of absorbent dose, initial pH, temperature is done to check the decolourization rate and metal ion removal efficiency. Along with the above mentioned factors the carbon source of the media is also optimized to check decolourization rate and metal ion removal efficiency. Thus bioflocculant method is a green process of waste water treatment and is comparatively cheaper and easier method to use. Further investigation is going on to establish potential bioflocculant to treat waste water effluent.

**Keywords:** Bioflocculants; Flocculation screening media; Absorbent dose; Green process

## Biofilm formation and emulsification activity of marine bacteria for biodegradation of petroleum hydrocarbon

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### Abstract

Petroleum hydrocarbon (PHC) contaminations are the major sources of marine pollution due to port activities. PHCs belong to the family of carcinogens having highly toxic effects on humans as well as marine lives. These hydrophobic hydrocarbons have low water solubility and persist in the environment for longer time. Major concern arises for the removal of these toxic pollutants from the environment. In the recent years, microbial bioremediation has been a promising technology for degradation of contaminants due to its cost effective and eco-friendly nature. Biofilm forming marine bacteria are sheltered inside a protective layer of extracellular polymeric substances (EPS) which helps to degrade organic pollutants



and utilize as a source of carbon and energy. Moreover, EPS provides structural stability to the biofilms and maintain their metabolic activity. Additionally, EPS called as bioemulsifiers accumulates at the interface between two immiscible phases which results in increased water solubility of hydrophobic hydrocarbons. Thus, EPS plays role in emulsification of hydrophobic compounds which accounts for enhanced degradation abilities. Therefore, the present study was focused on the identification of hydrocarbon utilizing biofilm forming marine bacteria for degradation of petroleum hydrocarbon. Three isolates *Zobellella denitrificans* GPS-5, *Acinetobacter junii* PPS-16 and *Pseudomonas resinovorans* PPS-19 were isolated from petroleum hydrocarbon polluted sites of Gopalpur port and Paradeep port, Odisha, India, using Crude oil amended basal medium. These bacterial isolates showed good biofilm forming potential both in presence and absence of oil which was observed by microtitre plate assay. Biofilm components and morphology was characterized through various microscopic techniques. Further emulsification activity and stability was studied which presented their potential in effective oil cleanup.

**Keywords:** Petroleum hydrocarbon; Bioremediation; EPS; Bioemulsifier; Emulsification

EMT-15

### **Immobilized white rot fungal consortium: An innovative and green technology for textile effluent treatment**

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#### **Abstract**

White-rot fungi (WRF) have been investigated for the removal of a wide spectrum of contaminants from textile wastewater. The present study is to determine the efficiency of a white rot fungal consortium immobilized on coconut fibre balls to decolorize textile effluent. To maintain high biomass of fungal population and enhance the retention of isolated fungal strains in the contaminated sites, the fungi need to be immobilized. Hence, the WRF after being grown in free culture, were immobilized naturally onto sterilized coconut fibre balls by the means of "attachment", where they grew as active films on the fiber support. The optimized process parameters for immobilized fungal consortium were inoculum size of 5%, fibre quantity of 2.2g per litre of effluent at rotation speed of 100rpm. The textile effluent was found to be decolorized by 73.5% and 98.5% using free and immobilized consortium (WRF) respectively at optimized parameters of pH (4.5), temperature (30°C) and time (6h). Thus, it was found that the decolorization was enhanced by 25% with immobilized consortium. The reason may be due to the presence of lignin in coconut fibres, which induces ligninolytic enzyme secretion by WRF and during the course of WRF metabolism, lignin oxidation by ligninolytic enzymes catalyzes the degradation/transformation of dyes in the textile effluent and decolorize it.

**Keywords:** Textile effluent; White rot fungi; Immobilization; Coconut fibres; Decolorization

EMT-16

### **Biosorption of heavy metals by *Bacillus licheniformis* immobilized on hydroxyapatite nanoparticles**

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#### **Abstract**

Heavy metal contamination in drinking water has become one of the major health concerns now-a-days, which has been linked with various health issues. Since the mechanical methods for heavy metal removal

are neither cost effective nor environment friendly, therefore the removal of metals using biological approaches are gaining importance. The aim of this study was to increase the efficiency of heavy metal (Nickel, Arsenic, Chromium and Cadmium) adsorption by thermophilic bacteria *Bacillus licheniformis* immobilized on a matrix of hydroxyapatite (HA) nanoparticles so as to find an effective mean of metal removal. The functional groups present on HA was determined by FTIR while thermo stability was studied by Thermogravimetric analysis and morphological studies by Scanning Electron Microscopy. The various reaction parameters were optimized to achieve maximum biosorption of metals. The peaks in FTIR spectra indicated tetrahedral PO<sub>4</sub><sup>3-</sup> while the presence of -OH group was indicated by peaks at 3344.49 cm<sup>-1</sup> and 707.18 cm<sup>-1</sup>. The dead cell biomass immobilized on hydroxyapatite nanoparticles (HA) adsorb more metal concentration (84.98% of As<sup>3+</sup>, 98.82% of Cd<sup>2+</sup>, 99.39% of Cr<sup>3+</sup> and 93.89% of Ni<sup>2+</sup>) as compared to the living cell biomass. The maximum biosorption of heavy metals was observed at pH 5-7, temperature 55° C, 50mg dosage of adsorbent, at 24hr of incubation and agitation speed of 150rpm. It can be concluded from this study that *Bacillus licheniformis* is a potential candidate which can be used to remove heavy metals from contaminated water.

**Keywords:** Heavy metals; *Bacillus licheniformis*; Hydroxyapatite; Biosorption; Thermophilic Bacteria

EMT-17

### Isolation, screening and optimization of keratinolytic bacteria from feather dumping site of Taothong, Manipur

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#### Abstract

Keratinases are modern proteases that can valorize poultry and leather industry wastes and may find applications in prion degradation and treatment of neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's diseases. The present study deals with isolation, screening and optimization of feather degrading bacteria. Of 72 isolates obtained from feather dumping soil of Taothong, Manipur, 41 showed protease activity out of which 32 with relative halo zone sizes above 50% were screened for feather degrading ability (Keratinolytic screening) using different feather basal media (FBM-1, pH- 7; FBM-2, pH-7.5 and FBM-3, pH-7.5 at 30°C). Out of 32 isolates, 4 (TSIC-1, TSIC-3, TSIC-21 and TSIC-37) could digest chopped chicken feathers within 2-3 days. Optimum pH of TSIC-21 and TSIC-37 for enzyme (keratinase) production were found to be 9.5 and 8.5 respectively at 30°C.

**Keywords:** Keratinolytic bacteria; Feather degradation; Protease; Keratinase; Poultry; Prions; Neurodegenerative diseases; Optimum; Taothong

EMT- 18

### Isolation and Characterization of Arsenate Resistant bacteria form Khetri Copper Mines

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#### Abstract

Arsenic is a toxic metalloid compound that causes toxic effects on human as well as health of other living beings such as plants and animals. Microorganisms show natural resistance towards arsenic and different arsenic resistant bacteria such as *Bacillus* sp., *Aneurinibacillus aneurinilyticus*, *Enterobacter*

sp. and *Klebsiella* have been isolated which can be employed for arsenic bioremediation process as well as for biosensor development. The present work focuses on the isolation of arsenate and arsenite resistant bacteria from rhizospheric, waste water and slag dump site of Khetri copper mines, Jhunjhunu, Rajasthan, India, using enrichment culture technique. Amongst isolated bacteria, 7 isolates (SMSKVR-1, SMSKVR-3, SMSKVW-1, SMSKVW-2, SMSKVW-3, SMSKVW-4 and SMSKVW-5) were able to grow on 300 mM of arsenate containing media however, only SMSKVW-3 was able to grow with faster growth rate. Thus SMSKVW-3 was further studied for its morphological, biochemical and growth parameter studies. The SEM analysis of bacteria showed the average size to be  $1.620 \mu\text{m} \times 0.516 \mu\text{m}$ . This rod shaped, gram negative bacteria was able to grow in the temperature range of 25-40 °C with optimum pH as 7 and showed positive responses towards lysine, ornithine, citrate, malonate utilization, esculin hydrolysis, nitrate reduction and oxidase test. A comparison of growth curve in presence (300 mM) and absence of arsenate, suggests the presence of strong arsenate resistance mechanism. This was further confirmed by the SEM analysis of bacteria showing intact cells in the presence of arsenate. The molecular characterization of bacteria using 16S rDNA sequencing involving NCBI-BLAST, MSA and phylogenetic tree analysis, confirmed its homology with *Pseudomonas mendocina*.

**Keywords:** *Aneurinibacillus*; *Aneurinilyticus*; *Pseudomonas mendocina*; Malonate utilization

EMP-19

### Isolation, characterization and identification of eleven arsenic resistant bacteria from Purbasthali, Purba Bardhaman, West Bengal, India

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#### Abstract

Arsenic is very hazardous metalloid present in soil and water throughout the world and is very dangerous for every living organism. As physical and chemical arsenic remediation methods are not eco-friendly; bacterial bioremediation of arsenic draw much attention to the scientists. Here, in the present study; eleven potent bacterial isolates were isolated from water sample from ten sites of Purbasthali, Purba Bardhaman, West Bengal, India. For polyphasic characterization of isolates, their colony morphology, MTC of sodium arsenate and sodium chloride, antibiotic sensitivity test, physio-biochemical tests, growth curve, optimum pH and temperature along with microscopic studies as fluorescence, bright field microscopy, TEM etc. were performed. Bacterial isolates were identified by 16 S rDNA sequences. Preliminary trial for bioremediation of arsenic and PGPR activities of isolates were evaluated. Many bacteria produce extracellular enzymes like catalase, protease, amylase etc. Most bacteria are susceptible to maximum antibiotics and AKS4c were able to tolerate more than 12 g/L  $\text{Na}_2\text{HAsO}_4$  concentration. Ten isolates were gram positive and AKS7a was only gram negative bacterium. Nine bacteria showed similarity with different species of *Bacillus*. Optimum pHs for all isolates were more or less basic. The isolates have very little lag phase except AKS4a. These potent bacteria may be used in bioremediation of arsenic.

**Keywords:** Arsenic; Polyphasic characterization; Sodium arsenate; 16S rDNA sequence; Bioremediation

## Cytostatic drugs in aquatic environment and their detection by RP-HPLC methods

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### Abstract

The pharmaceutical occurrence in the aquatic environment is a public and scientific concern. Cytostatic drugs are also a group of pharmaceutical compound which are continuously increasing in the water bodies. These drugs are being used at large scale in the hospitals for the treatment of cancer patients and released in the environment through excretion, hospital effluent and household effluent. Cytostatic compounds are non-specific in nature, they inhibit topoisomerase activity when negatively bind with DNA and responsible for cause of mutation. So, the presence of these drugs in the aquatic environment is hazardous for environment and aquatic life and it is necessary to detect and remove these compounds from the aquatic environment. Cyclophosphamide, etoposide and paclitaxel are most widely used cytostatic drugs in the oncology ward for the treatment of patient. In present study, the simple, sensitive and robust RP-HPLC methods were developed for the detection of cytostatic compounds cyclophosphamide, etoposide and paclitaxel in the hospital wastewater.

**Keywords:** Antineoplastic; Hospital effluent; Non-specificity; Mutagenicity; Detection

EMT-21

## Optimization of effective dose of a newly isolated probiotic bacteria for the growth and disease resistance of *Clarias batrachus* (Linn.)

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### Abstract

The dose level determination of putative probiont *Bacillus cereus* PKA18 was carried out by supplementing the feed with different concentrations of the bacterial inoculum: control (without probiotic),  $2 \times 10^4$  (C1),  $2 \times 10^5$  (C2) and  $2 \times 10^6$  (C3) probiotic cells per 100 g feed for 60 days for the cultivation of *Clarias batrachus*. After the feeding trial, the fish were further challenged with pathogenic *Vibrio vulnificus* and the percentage mortalities were enumerated upto 15 days of post challenge. The morphometric measurements of the fingerlings were precisely calculated and the significant differences ( $\alpha = 0.05$ ) among various groups were analyzed by Duncan Multiple Range Test (DMRT) through SPSS 19. The application of probiotic-supplemented feeds has resulted enhanced growth performance compared to control. The fish fostered with C2 feed elucidates significantly ( $P \leq 0.05$ ) higher ADG (average daily growth) and SGR (specific growth rate) in contrast to the control. The C2-fed fish was observed with considerably higher live weight gain ( $29.77 \pm 1.24$  g), protein efficiency ratio ( $2.34 \pm 0.12$ ) and percentage weight gain ( $426.45 \pm 18.98$ ) than the

all other groups. The feed conversion ratio ( $1.72 \pm 0.11$ ) also has declined drastically in C2-fed fish followed by C3, C1 and control. The C2-fed fish has also displayed high rate of survivability (98.67%) followed by C3, C1 and control. Highest survival percentage was observed in fish fed with C2-feed compared to control while challenging with *V. vulnificus* which indicted the efficiency of the test organism in reducing vibriosis. The results also indicated that feed incorporated with the putative probiotic *B. cereus* PKA18 can safely be used for the sustainable development of *C. batrachus*. The findings of the study suggests that the dose level of probiotic *B. cereus* applied in feed C2 is the most effective dose to enhance growth performance and disease-resistance of *C. batrachus* juveniles.

**Keywords:** Aquaculture; *Clarias batrachus*; *Bacillus cereus*; Probiotic; Growth performance

EMT-22

### **Molecular characterization of thermo-alkaliphilic *Bacillus subtilis* strain producing lipase from a local water body near Vapi**

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#### **Abstract**

A thermo-alkaliphilic isolate C-1 producing lipase of extracellular origin, was isolated from a local water body at Kocharva village, near Vapi, Gujarat. Lipolytic bacterial strains were screened using Rhodamine B agar plates. It was observed that production of enzyme was markedly improved with consecutive optimization of carbon source, which included various carbohydrates and different oils; nitrogen source of inorganic and organic composition; inoculum size; incubation period; pH of the medium and temperature of incubation. Lipase activity of 6.83 U/ml was obtained in 96 h, at 55 °C and pH 10, with olive oil as carbon source and peptone as nitrogen source. Molecular characterization of the isolate C-1, identified it as *Bacillus subtilis*, based on 16S rDNA sequence. Phylogenetic tree construction revealed that the strain isolated was showing complete sequence identity for 16S rDNA with *Bacillus subtilis* strain IAM 12118.

**Keywords:** Thermo-alkaliphilic; *Bacillus subtilis*; Lipase activity; 16S rDNA; Peptone

EMT-23

### **Bioefficacies of microbes and micro-nanoparticles for mitigation of azo dyes and their heavy metal conjugates in the textile industry effluent**

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#### **Abstract**

Rapid industrialization and urbanization accompanied with the discharge of large amount of waste into the environment is a wide spread problem and creates great public health concern. India, over the last few decades, has emerged as a promising textile hub, where Rajasthan alone lends 7.5% of its produce of blended yarn, cotton and over 5% of the fabrics. This state enjoys prime position in the production of synthetic clothing material and polyester viscose yarn as well as in processing of light-weight and low-cost fabric especially at Bagru, Balotra (Barmer), Jaipur, Jodhpur and Pali. The effluent from these textile industries comprises of acids, bleaching agents, salts, heavy metals and dyes as contaminants. A

significant amount of mainly untreated textile dye effluent (10 - 15 kL day) is released into various water-bodies adjoining the textile dyeing and printing units thereby changing its physio-chemical properties. Subsequently the contaminated water takes the solvate (contaminants) to the fields in the vicinity and its consumers thereby adversely affecting quality of the agricultural produce, animal and human health. Indigenous microorganisms present in textile industrial area that contains a lot of synthetic compounds, metals and other toxicants, adapt themselves to the adverse environment. The present study aims to use those microbes for mitigation of azo-dyes respectively. The experimental data revealed that these microorganisms have great potential for bioremediation of such environments with huge quantities of industrial effluents. Recently, nanotechnology utilizing the nano-sized particles has become evident as a feasible alternative to the conventional methods, as robust, readily accessible, large surface area heterogeneous catalyst support. Keeping these facts in view the present study also deals with exploring micro based nano-particle synthesis to be used for the mitigation of dye and their heavy metal conjugates from textile waste water effluent. The study focuses on delivering microbe assisted and micro-nano assisted remediation methodologies to be used for *ex situ* processing of textile industrial effluents thereby serve the society and the environment.

**Keywords:** Bioremediation; Mitigation of dye; Heavy metal removal; Azo-dye degradation; Textile effluent

EMT-24

### **Natural and commercial poly-isoprene degradation by novel bacterial isolates: A potential approach for solid waste cleanup**

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#### **Abstract**

Polyisoprene is one of the most important indispensable polymeric materials used in a modern society because of its special properties for a wide range of applications. The cis-isomer of polyisoprene [poly(cis-1,4-isoprene)] is known to be the main component of natural rubber. On the other hand, the wide range of applications of polyisoprenes accordingly leads to problems in solid waste treatment due to the large volume of produced items and their durability. This is particularly the case for rubber. Two gram positive, non-motile, non-spore forming strains BSTG-01 and BSTN-01 which are able to considerably degrade natural rubber (NR) and synthetic polyisoprene rubber (SR) has been isolated from the bark, and from the water stored on latex collecting cup of *Hevea brasiliensis*, respectively, in a rubber plantation garden of Tripura, India. Adhesive growth of strains BSTG-01 and BSTN-01 directly on the surface of the NR and SR was observed. Surface investigation by scanning electron microscopy revealed the adhesive growth behaviour of strains BSTG-01 and BSTN-01 taking place by colonizing on the rubber surface, merging into the rubber and forming a biofilm. Schiff's reagent test indicated production of aldehyde groups during colonization. Weight loss of the rubber was 48.7% and 34.7% for NR and SR after 6 weeks of incubation; respectively for BSTG-01 while the weight loss of the rubber was 24% and 20% for NR and SR after 6 weeks of incubation, respectively for BSTN-01. Fourier transform infrared spectroscopy comprising the attenuated total reflectance technique was applied on NR and SR blocks overgrown by cells of the BSTG-01 and BSTN-01 isolates. Spectra demonstrated the changes of the overall chemical environment arising on the polyisoprene due to the degradation caused by the isolates. This is the first report of any microbe, exhibiting considerable rubber degradation properties isolated from the rubber plantation of Tripura, a microbiologically unexplored site.

**Keywords:** Polyisoprene; Biodegradation; Rubber Biodegradation; Solid Waste; Tripura

## Resistance of *E. coli*, *Salmonella* spp. and *S. aureus* to antimicrobials of human and veterinary importance in poultry-farms and poultry-retail shops of India

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### Abstract

Antimicrobial resistance (AMR) driven by inappropriate use of antimicrobial agents for growth promotion in food animals like poultry chickens is an expanding global health threat. In India, little attention has been paid on antimicrobial resistance in the context of developing “One Health” approach. Hence, antimicrobial resistance level and dynamics/pattern of multi-drug resistance (MDR) was tested, utilizing multi-disciplinary approach for drugs of great importance for both human and veterinary medicine, in chicken’s feces and ceca origin *E. coli*, *Salmonella* spp., and *S. aureus* circulating over the different environments or stages of poultry in India. A total of 342 bacterial isolates including *E. coli* (n=143), *Salmonella* spp. (n=104), and *S. aureus* (n=95) were recovered from fecal (n=80) and cecal (n=80) samples of chicken, collected across the different poultry-farms (n=16) and poultry-retail shops (n=16) located at urban and rural areas of Rajasthan, India, respectively. All bacterial isolates were analyzed *in vitro* antimicrobial susceptibility testing using the broth micro-dilution method for a number of antibiotics. High rates of AMR to antibiotics that are critically/highly important both in human and in veterinary medicine were observed among all the bacterial isolates. Interestingly, upward trends in AMR prevalence were observed in chicken’s cecal samples than in chicken’s fecal samples. Notably, >90% of all the bacterial isolates were MDR, of particular, pattern/prevalence of MDR was substantially varied across the chicken’s cecal samples than in chicken’s fecal samples. Our results indicate that AMR including MDR is highly prevalent in *E. coli*, *Salmonella* spp., and *S. aureus* distributed frequently in poultry-farms and retail-shops of India. Our findings persuades the prudent use of antimicrobial agents in poultry settings and may assist in drafting of the new policies for the judicious use of these lifesaving drugs, considering the “One Health” approach that can bring benefits to the human/animal, and the environment.

**Keywords:** Antimicrobial resistance; *E. coli*; *Salmonella* spp.; *S. aureus*; Poultry

## Potential of Microbial Autotrophy in Soil Carbon Fixation

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### Abstract

Ongoing global climate change caused by human induced increase in greenhouse gases represents one of the biggest scientific and political challenges of the 21<sup>st</sup> century. In the concern of increasing atmospheric CO<sub>2</sub> concentration, assimilation of atmospheric CO<sub>2</sub> into soil is of primary importance. Fate of terrestrial carbon (C) cycle depends upon the balance between the autotrophic C fixation and

soil respiration which ultimately controls the net effect of climate change on ecosystem carbon budgets. Although, our knowledge of photosynthesis and its response to climate change is well advanced, yet there are considerable gaps in our perception of the potential of microbial autotrophy in C cycle. The present study thus, focused on the role of RuBisCO activity in soil C fixation. For this, soils from natural forest (Bhopal, M.P) and grassland system (Jhansi, U.P) of semi-arid to sub-humid central India, were studied. Undisturbed soil core was collected in triplicate from two depths i.e. the 0-15 cm and 15-30 cm soil layers. Simple and most efficient method of sonication is employed for the soil RuBisCO extraction, followed by spectrophotometric determination of the enzyme activity. Result revealed that grassland system maintained for long term under different management had higher RuBisCO activity (1.33-2.28 nmol of CO<sub>2</sub> fixed g<sup>-1</sup> soil min<sup>-1</sup>) at both soil depths than natural grassland system (0.97-1.07 nmol of CO<sub>2</sub> fixed g<sup>-1</sup> soil min<sup>-1</sup>). Similarly, natural fig based and palash based forest soil had the RuBisCO activity in the range of 0.80-1.03 nmol of CO<sub>2</sub> fixed g<sup>-1</sup> soil min<sup>-1</sup>. Comparison among these two land use systems reflected that grassland system had more potential of autotrophic microbial assimilation of atmospheric CO<sub>2</sub> in soil carbon as compared to natural forest system in semi-arid to sub-humid central India. These preliminary data offer new insights into the importance of microbial autotrophy in terrestrial C cycling.

**Keywords:** Microbial autotrophy; RuBisCO activity; Soil carbon fixation

EMT-27

### Studying the effects of cold atmospheric plasma on bacterial cells towards enhancement of substrate utilization

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### Abstract

Production of value-added products through a fermentation-based manufacturing process that uses renewable feedstock such as lingo cellulosic biomass is a desirable alternative to petrochemicals. Ligno cellulosic raw materials hydrolysates commonly contain much higher amounts of D- glucose compared to other C6 and C5 sugars, and therefore, improving glucose uptake by the host-microbial strain and its subsequent fermentation has become a priority. Since inducible utilization pathways reflect widespread microbial strategies to uptake and consume sugars from the environment. As a unique approach, this research aimed at inducing the glucose uptake pathway and checking for enhances or reduced rate of glucose utilization by our thermophilic bacterial strain by the application of the technique cold plasma. The thermophile was subjected to the cold atmospheric plasma treatment at 9.1kV/cm for different time intervals 2, 4, and 8 min. Although, during our initial experimentation, there was no enhancement in the substrate utilization when treated for 2 and 4 min, and the cells died when the cells were treated for 8 min. Moreover, cold plasma treatment overall decreased the substrate utilization in the treated bacterium compared to the wild type cells. Hypothesizing, down-regulation of certain genes that is responsible



for substrate uptake in bacteria. Transcriptomics profiling of the expressed genes will help identify the potential transporter proteins in the bacterial cell membrane. The underlying research has the potential to design synthetic microbial strains with an improved lignocellulosic conversion.

**Keywords:** Cold Plasma; *Geobacillus*; Glucose Uptake

EMT-28

### Toxicity study of *Kocuria marina* DAG II treated Malachite green dye

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#### Abstract

In our study the gram positive bacteria *Kocuria marina* DAGII was isolated from the soil of NIT Durgapur. The bacterium was found to effectively decolorize 13ppm malachite green dye up to 99% at 8 hrs in batch study. 1.2% (v/v) of the culture having an absorbance of 0.4-0.6 was used as inoculum in the decolorization experiment. UV-Vis spectrophotometric analysis of the dye removal showed a single characteristic peak at wavelength 617 nm suggesting that no degradation metabolites was formed during the dye removal process. Toxicity study is very crucial to understand the toxic effect of treated dye. Phytotoxicity study was carried out by using ten different major cultivated seeds namely *Raphanus raphanistrum subsp. sativus*, *Vigna unguiculata subsp. unguiculata*, *Vigna radiata*, *Sinapis alba*, *Brassica nigra*, *Brassica juncea*, *Pisum sativum*, *Cicer arietinum*, *Macrotyloma uniflorum* and *Trigonella Foenum-graecum*. Phytotoxicity study was performed with distilled water as positive control, treated dye as sample and untreated dye as negative control. The study showed approximately 99% germination in distilled water and treated MG dye. Microbial toxicity was carried out in different microbes namely *Bradyrhizobium sp.*, *Saccharomyces cerevisiae*, *Dietzia maris NITD*, *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger*, *Pseudomonas aeruginosa* and *Penicillium chrysogenum*. Microbial toxicity showed no zone of inhibition for micro-organisms grown in presence of treated dye and distilled water. Thus the study showed that the bacterial treated MG dye is non-toxic and safe for microbial community and crops.

**Keywords:** Malachite green; Decolorization; Toxicity study; Phytotoxicity study; Microbial toxicity

EMT-29

### Fungal-mediated degradation of Polystyrene

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#### Abstract

Plastics are man-made long chain polymeric molecules of carbon and hydrogen atoms as the backbone, to which oxygen, sulfur or nitrogen atoms are attached. Polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and polyurethane (PUR) are the main types of plastics which correspond to over 80% of the total demand. Their properties such as chemical inertness, imperviousness to water, durability and stability have led to their use in versatile applications. A vast array of plastic products being used is daily discarded. However, the plastic waste recycling and management strategies still remain unrefined leading to its over accumulation in the land and aquatic ecosystems. Incineration is the globally used method for plastic degradation. But it leads to the release of toxic gases such as SO<sub>2</sub>, NO<sub>2</sub>, and persistent organic pollutants such as dioxins & furans, which are known to cause serious health issues. Biodegradation of plastics is an 'eco-friendly' method in which

the plastic waste is used as a carbon source by the microbes, and the products released are CO<sub>2</sub>, H<sub>2</sub>O and residual carbon, which further maintain the carbon cycle on the Earth. Despite of various reports published on the biodegradation of plastics, very little success has been achieved thus far. As fungi are the major degraders in the environment and are known to possess an efficient enzyme system, they can be explored to degrade plastics. In our study, the lab fungal isolates were screened for their ability to degrade polystyrene, which is a recalcitrant and non-biodegradable plastic. Out of 11 fungal isolates, *Aspergillus* sp. NJP02 and *Aspergillus flavus* NJP08, showed a potential to degrade polystyrene. For degradation studies, 5 g of fungal biomass (fresh weight) was separately inoculated in 50 mL of minimal salt media containing polystyrene as a sole carbon source and the experimental flasks were incubated at 28°C for 30 days on a rotary shaker (150 rpm) under dark conditions. After incubation period, an increase of 121 and 111.6 % was observed in the biomass fresh weight of *Aspergillus* sp. NJP02 and *A. flavus* NJP08, respectively. The obtained results suggest degradation and subsequent utilization of PS by these fungal isolates. Moreover, PS degradation was validated by the Raman spectroscopy analysis. Further research on biochemical and molecular basis is required to unveil the mechanism involved in the biodegradation pathways.

**Keywords:** Polyethylene; Polypropylene; Polystyrene; Polyvinyl chloride; Polyethylene terephthalate; Polyurethane; *Aspergillus flavus*

EMT-30

### Isolation and characterization of Bacteria from dairy industry effluent and sewage water for biological treatment of waste water

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#### Abstract

Water pollution is a major challenging issue in all over the World. Industrial effluent, sewage water and agriculturally waste water are major causes of water pollution. Waste water treatment involve three basic approaches e.g. Primary (physical treatment), secondary treatment (biological treatment) and tertiary (physico-chemical treatment). Biological treatment of waste water based on application of microorganisms. Microorganisms degrade the organic matter and accumulate or detoxify the heavy metals from the waste water. In our present study, sewage effluents and industrial effluents were collected from University sewage system, Hasanpur Sugar Mills and Sudha Dairy Plants of Samastipur and Muzaffarpur. Five different media (Nutrient agar media, King's 'B' media, Methylene Blue agar media, Eosin Methylene blue agar media, and Soil extract agar) were used for isolation of Bacteria from the samples. Samples were serially diluted and appropriate diluted were spread plated individually. The plates were incubated at 30°C temperature till the growth was observed. Morphologically distinct colonies were purified by sub-culturing on respective nutrient combination plates. Total 110 different bacterial morphotypes were isolated from dairy effluents, sugar mill effluents and University sewage water. Further these morphotypes were screened for starch, protein and fat degradation. Only 20 cultures showed the ability of starch, protein and fat degradation. These isolates further will be used to evaluate the BOD and COD removal efficiency.

**Keywords:** Water pollution; Sewage; Biological treatment; BOD; COD

## Role of *Saccharomyces cerevisiae* against arsenic induced toxicity in mice

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### Abstract

Arsenic found widely in the earth's crust. It is mostly found in organic, inorganic and gas form. In some areas of the world, high levels of arsenic are naturally present in drinking water which leads to high toxicological concern. Arsenic commonly causes arsenicosis, melanosis, renal dysfunction and prolonged exposure may cause cancer of skin, bladder and liver. Probiotics are reported to have antimutagenic, anticarcinogenic, hypocholesterolemic, antihypertensive, anti-osteoporosis and immunomodulatory effects. *Saccharomyces cerevisiae* is common yeast used in baking industry, it is used in many immunomodulating phenomenon. The present study is designed to evaluate hepatoprotective effect of *Saccharomyces cerevisiae* against arsenic induced toxicity in mice. Mice were administered arsenic for four weeks followed by four weeks administration of *Saccharomyces cerevisiae*. Blood were collected for biochemical assay. Liver tissues were fixed for Light microscopic study. Arsenic causes many fold increase in SGPT and bilirubin level. Hepatic cells and kupffers cells were degenerated to greater extent with fragmented nuclei in arsenic exposed group. Sinusoid and central vein were dilated in arsenic exposed group. While *Saccharomyces cerevisiae* causes marked restoration in biochemical and histological parameters of liver of mice.

**Keywords:** Yeast; Melanosis; Hepatic cells; Bilirubin

## Exploring the microorganisms for the production of biosurfactants

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### Abstract

Biosurfactants is an important product of biotechnology, reducing the surface and interfacial tension, possess potential applications to the area of detergency, emulsification, dispersion, paint, cosmetics, textile, agro-chemical, food, and pharmaceutical industries. Biosurfactants have various applications in green technology for the betterment of our environment. Isolates having capability of producing surfactant can be used for various different applications such as bioremediation, toxicity and other pharmaceutical etc. The present study was undertaken to evaluate the potential of bacteria isolated from soil sample to produce biosurfactants. Seven morphologically different bacterial strains were isolated from the agricultural soil sample collected from Brambey , Ranchi, Jharkhand,835205, India. All isolates were identified as gram negative and found potent biosurfactants producer. seven bacterial isolates were PS1,PS2,PS3,PS4,PS5,PS6,PS7. PS1 shows highest Emulsification activity (59%) whereas the strain PS5 (16%) shows the least. Highest positive result for oil displacement was found in PS1. The Result of the study provides the basis for the use of the bacterial isolate to produce biosurfactants or as a bioremediating agent.

**Keywords:** Biosurfactants; Bioremediation; Bacteria; Emulsification activity; Oil displacement

## Integration of bioelectrochemical system with constructed wetlands for municipal wastewater treatment

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### Abstract

Sewage and industrial waste are the two major sources of water contamination. Unconventional wastewater treatments are correlated with conventional wastewater treatments, are effortless to use, cost-effective and are complex in their operation and design. Constructed Wetland a green technology, is successful in wastewater treatment and provides a sustainable approach. In present study we investigated the role of CWs integrated with bio-electrochemical system (BES), can concurrently treat the wastewater effectively. This CWs integrated system utilizes nutrient species  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ ,  $\text{PO}_4^{3-}\text{-P}$  and  $\text{SO}_4^{2-}$  from municipal wastewater. Nowadays, bio-electrochemical system (BES) is being studied, consisting of integrating technology into natural ecosystems for environmental remediation technologies.

**Keywords:** Integration; Bio electrochemical; Constructed Wetland; Municipal Wastewater; Treatment.

## Abiotic stresses-induced alterations in metabolic processes of the bacterium *Cellulosimicrobium cellulans*

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### Abstract

Bacteria are among the first components of the biota in the ecosystems affected by diverse environmental stimuli. However, they are able to tolerate extreme environmental conditions such as ultraviolet (UV) radiations, higher temperature, heavy metal contamination and nutrient limitation. These attributes make them useful research models for understanding the stress response mechanisms and evaluating their bioremediation potential. Present study deals with the changes in the physiological status and expression of cellular proteins in the Gram positive bacterium *Cellulosimicrobium cellulans*. Employing standard microbiological techniques, four bacterial isolates growing in polluted sites were isolated and identified by 16S rRNA gene sequencing. Among these four bacteria, *C. cellulans* showed higher level of tolerance to UV-B radiation (280-315nm), temperature and mercuric chloride ( $\text{HgCl}_2$ ) treatment. *C. cellulans* exposed to UV-B radiation ( $2\text{W}/\text{m}^2$ ) for different time periods (0 to 6h) exhibited  $\text{LD}_{50}$  at 3h and complete killing occurred at 6h. Growth at varying temperature ( $37^\circ\text{C}$  to  $50^\circ\text{C}$ ) up to 6h showed  $\text{LD}_{50}$  at  $45^\circ\text{C}$  at 3h and complete killing occurred at  $50^\circ\text{C}$ . Exposure to  $\text{HgCl}_2$  (0 to 0.06mM) exhibited  $\text{LD}_{50}$  with 0.03 mM at 3h and complete killing occurred at 0.06 mM. Cultures exposed to above stresses induced generation of reactive oxygen species (ROS) and activity of antioxidative enzymes with increasing duration/concentration of exposure. The maximum increase in ROS production was noted at 3 h of UV-B exposure (2.83 fold),  $45^\circ\text{C}$  of temperature (3.46 fold), and 0.03 mM of  $\text{HgCl}_2$  exposure (5.63 fold). Similarly, activity of antioxidative enzymes such as superoxide dismutase (SOD) showed 2 to 4 fold increase, catalase (CAT) 2 to 2.5 fold, peroxidase (POD) 4 to 6 fold and ascorbate peroxidase (APX) 2 to 4 fold under the above stresses. Analysis of protein profile by SDS-PAGE showed significant alterations in the expression of proteins as compared to control cultures. Total proteome analysis by 2D

gel electrophoresis in cultures exposed to above stresses is being studied to identify and characterize differentially expressed proteins.

**Keywords:** Abiotic stresses; Bacteria; Reactive Oxygen Species; Antioxidative enzyme; SDS-PAGE

EMT-36

### **Detection and characterization of heavy metal resistant bacterial isolates from arable land of Uttar Dinajpur district of West Bengal**

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#### **Abstract**

The magnitude of toxic metals in soil is progressively increasing due to injudicious anthropogenic activities, emerging as a global concern. Agricultural soil gets contaminated with toxic metals through overuse of chemical fertilizers and pesticides, and use of untreated or poorly treated wastewater from industrial establishments. Consequently, soil dwelling bacteria has gained the resistance potentiality against toxic heavy metals for surviving in contaminated soil. Since development and propagation of resistance is mechanistically determined by selection pressure to a certain extent, the detection of resistant bacteria is an indicator of growing contamination. In this study we have focussed on some of the toxic metal contaminants of agricultural soil, and range of resistance against them by certain bacterial isolates obtained from the agricultural soil of Uttar Dinajpur district of West Bengal, which has a large area of arable land highlighted for its indigenous aromatic rice variety Tulaipanji and corn cultivation. We have studied the degree of resistance of bacterial isolates from agricultural soils to heavy metals like Cd, Fe, Co and Cu singly as well as in conjunction with each other. The morphology of the bacterial isolates both in normal as well as in metal stressed condition was worked out and found to display variations. We have also characterized the different biochemical properties of the isolates and detected the presence of lipolytic activity in most of the isolates which could be further exploited for production of lipases. Our results showed the presence of bacterial isolates with multiple metal resistance and resistance to certain antibiotics. Our study is the pioneering work in the region to address the concern of heavy metal toxicity in environment especially in arable soil, and manipulation of the soil bacterial population. Further understanding of the mechanisms behind the resistance phenomenon could help us to explore the isolates in potential bioremediation activities since the process of bioremediation will be effective if only microorganisms with established ability to remediate and tolerate heavy toxicity are employed.

**Keywords:** Heavy metal resistance; Soil contamination; Bioremediation; Resistant bacteria; Arable land

EMT-37

### **Genomics of denitrifying methanotrophic bacteria from municipal solid waste**

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### Abstract

Methanotrophs are capable of reducing nitrates and nitrites to nitrous oxide and nitrogen gas by virtue of the presence of methane metabolizing enzyme methane monooxygenase. Furthermore, methanotrophs can also utilize ammonia as an excellent nitrogen source. In this study, soil (Top soil: 0-10 cm depth from top; Bottom soil: 50-60 cm depth from top) and leachate samples were collected from Bhandewadi municipal solid waste (MSW) landfill dumpsite for isolation of denitrifying methanotrophs. Samples were added to Nitrate Mineral Salt (NMS) medium with methanol as sole carbon source for enrichment of methanotrophs in serum bottles closed with silicone rubber stopper and sealed with aluminium crimp seal. After three cycles of enrichment in NMS medium (each enrichment cycle of 10 days), samples were plated on NMS agar plates for isolation of pure cultures. 15 distinct isolates were picked from NMS agar plates on the basis of colony morphology. Individual isolates were then screened for methanotrophic and denitrifying activity in Syntrophic Nitrification Denitrification (SND) broth with methanol as carbon source. 5 isolates showed rapid utilization of ammoniacal nitrogen for growth as indicated by increase in turbidity. The isolates also demonstrated very high denitrification activity (100% NO<sub>3</sub>-N removal) at the expense of methanol as carbon source and electron donor. The denitrifying capacity of shortlisted methanotrophic isolates was also validated by PCR using primers specific for *pmoCAB* (particulate methane monooxygenase) and *mmoX* (soluble methane monooxygenase) genes. These isolates can be used for preparing consortia which can find potential application in methane mitigation by bioaugmentation at landfill sites.

**Keywords:** Methanotrophs; Denitrification; MSW; Enrichment; Bioaugmentation

EMT-38

### Mathematical modelling as a tool to optimize PHA production by *Massilia spp*

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### Abstract

Minimum Run Resolution IV screening design was employed to study important process factors to maximize DCW (dry cell weight) and PHA (polyhydroxyalkanoate) production by *Massilia*. Maltose, NH<sub>4</sub>HPO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub> and K<sub>2</sub>HPO<sub>4</sub> were found to be significantly affecting DCW and PHA production. A three-level-four-factor central composite design (CCD) was employed to optimize concentration of screened factors to maximize response. Statistical analysis showed that maltose and NH<sub>4</sub>HPO<sub>4</sub> had a positive effect whereas Na<sub>2</sub>HPO<sub>4</sub>, K<sub>2</sub>HPO<sub>4</sub> had a negative effect on DCW and PHA production. By using Response Surface methodology (RSM), optimum concentration of significant factors was found to be as follows: maltose: 30.0 g/l, NH<sub>4</sub>HPO<sub>4</sub>: 3.0g/l, Na<sub>2</sub>HPO<sub>4</sub>: 3.0g/l, K<sub>2</sub>HPO<sub>4</sub>: 3.0g/l. After optimization and verification of the model, 25.5 g/l of DCW and 6 g/l of PHA was produced.

**Keywords:** *Massilia*; DCW; PHA; Minimum Run Resolution IV; RSM

## Effect of Copper on the expression of Methanomonooxygenase Enzyme in *Methylosinus Trichosporium*

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### Abstract

Methanotrophs, methane oxidising proteobacteria that utilizes methane monooxygenase, an oxidative enzymatic machinery within the organism for the metabolization of methane as a source of energy. This enzyme machinery is comprised of two forms: the cytoplasmic form (soluble methane monooxygenase) and the trans membrane form (particulate methane monooxygenase). The disparity in expression of these forms is influenced by the copper-biomass ration within a cell. *Methylosinus trichosporium* OB3b, an obligatory aerobic methanotroph, is used as a model methanotroph to illuminate the expression of these enzymatic forms under altered copper biomass ratio in suitable minimal salt media. This study set one's sights on scrutinizing the effect of altered copper concentrations on the expression of methane monooxygenase and methane uptake rate. The experimental observations demonstrated that 10 $\mu$ M Cu concentration manifested the growth of the microbe to the maximum with an OD<sub>600</sub> equivalent to 0.2. It was also observed that at comparatively higher concentration of copper the organism experiences inhibition in growth. Ergo, the above-mentioned concentration of copper can be exploited to yield the membrane associated enzyme in a higher amount for industrial operation for production of value-added products such as purified enzymes, single cell protein and biopolymers. Forbye, study for quantitative analysis of expression level of both the enzymatic form is under progress.

**Keywords:** Copper; Growth; Methane; *Methylosinus*; Monooxygenase

## Bioremediation of Lignin in Paper and Pulp Mill Effluent

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### Abstract

Pulp and paper industry is usually related to pollution issues associated with high figure BOD, COD, toxicity, color, suspended solids, lignin and its derivatives and chlorinated compounds. Though many researchers have worked to get rid of COD, BOD, color etc. of pulp and paper effluents, the problem still persists. The biological colour removal process uses several classes of microorganism's bacteria, algae and fungi to degrade the polymeric lignin derived chromophoric material. Lignin is a most complex heteropolymer and does not contain identical, readily hydrolysable repeating linkages at regular intervals as in other natural polymers such as cellulose, protein or nucleic acids. Lignin-degrading enzymes have

been shown to be actively involved in these colour removal processes. Peroxidases and laccases are the two major classes of microbial lignin modifying enzymes. Laccases are taking centre stage of this attention, since these enzymes may help degrading lignin, using oxygen as the oxidant. Laccases is capable of eliminating the phenols through polymerization process. Till date, laccases have mostly been isolated from fungi and plants, whereas laccase from bacteria has not been well studied. Bacteria seem to be more effective than fungi for the bioremediation of environmental pollutants due to their immense environmental adaptability and biochemical versatility. Bacterial laccases have several unique properties that are not characteristics of fungal laccases such as stability at high temperature and high pH. Bacteria produce these enzymes either extracellularly or intracellularly and their activity is in a wide range of temperature and pH. It has application in pulp biobleaching, bioremediation, textile dye decolorization, pollutant degradation, biosensors, etc. it can be used effectively for treating the waste of paper and pulp industry.

**Keywords:** Lignin; Laccase; Bio-remediation

EMT-41

**Assessing the microbiological attributes of the Kulik River in Raiganj town of Uttar  
Dinajpur district of West Bengal**

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**Abstract**

Water, one of the most important element for existence of life is contaminated every moment, anthropogenic activities being the leading cause. An initiative was taken for assessing the microbial health and water quality of the Kulik River flowing past the town of Raiganj in Uttar Dinajpur district of West Bengal for determining its potability, faecal contamination, microbial diversity, resistant species of bacteria, and eutrophication level. Kulik is the lifeline of Raiganj and adjoining areas, but due to prolonged negligence and inaction is fast deteriorating. Raiganj Wildlife Sanctuary (aka Kulik bird sanctuary), home to a large population of Asian openbill storks and other migratory birds is situated at the bank of Kulik. The uncontrolled dumping of wastes has severely polluted the river, hastened by the reduction of flow due to siltation. Explosive growth of algal blooms and other plants is one of the major problem due to which the water quality is deteriorating and the aquatic ecosystem is getting destroyed. Some of the serious effects include death of fishes and aquatic plants making the water stinky with a high BOD, thus lowering the aesthetic value. Water samples from various locations along the bank of the Kulik was collected aseptically, and analysed using standard analytical procedures for estimating physico-chemical parameters like temperature, pH, alkalinity, TDS, dissolved oxygen and carbon dioxide, etc. MPN test was performed to detect coliforms acting as indicators of faecal contamination. Screening for detection of heavy metal and antibiotic resistant bacteria was also carried out followed by their molecular and biochemical characterization. Our initial study detected severe faecal contamination along with the presence of resistant bacterial isolates. All these will help to determine the sources, causes and types of pollutants, and also create awareness necessary to arrest pollution, carry clean-up activities and save the river.

**Keywords:** Kulik River; Resistant bacteria; Coliform; MPN; Microbial diversity



### Effect of microgravity on enzyme kinetics of purified enzymes

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#### Abstract

Microgravity has a significant effect on biological functions and cellular processes that is either beneficial or detrimental for an organism. Enzymes are responsible for catalysing biochemical reactions within cells essential for viability and producing key metabolic products. This study involves the study of enzyme kinetics in simulated microgravity environment which is achieved using a Rotatory Cell Culture System (RCCS) and High Aspect Ratio Vessels (HARV). The system developed by NASA mimics low shear environment as experienced under spaceflight condition. We used commercially purified enzymes to make a comparative study of enzyme kinetics by determining the  $K_m$  and  $V_{max}$  values under regular and microgravity condition. Enzyme activity was measured under different substrate and enzyme concentrations to determine  $K_m$  and  $V_{max}$  values through Lineweaver-Burk and Hanes-Woolf Plot. For industrially important hydrolytic enzymes like Amyloglucosidase, its  $K_m$  value was reduced by 40% indicating enhanced affinity towards the substrate. Similarly, another hydrolytic enzyme, Endo 1, 4- $\beta$  Xylanase is also being studied under microgravity conditions. The physical parameters of reaction medium, active site configuration and substrate enzyme complex structure would have cumulative effect on the enzymatic efficiency in low shear environment. This data will therefore help us interpret the structural or biochemical differences these enzymes experience under microgravity which leads to increase or decrease in their activities which can further be exploited for the improvement of several industrial processes.

**Keywords:** Microgravity; Enzyme Kinetics; RCCS; Amyloglucosidase; Endo 1, 4- $\beta$  Xylanase

### Isolation of novel methanotrophs for exploration of its biotechnological potential

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#### Abstract

Methane is the second most important greenhouse gas contributing substantially after carbon dioxide. The major global methane contributors are natural wetlands and rice fields. Methanotrophs or methane-oxidizing bacteria that use methane as their carbon and energy source. Methanotrophs are almost always obligate, growing only on methane or (sometimes) methanol which is the first intermediate in methane utilization. Almost 20% of methane emission is attenuated by methane-oxidizers in rice paddies, and wetlands. While in the surface of sediments or water-saturated soils, the attenuation rate is more than 90% of the emitted methane. Methanotrophs have been reported to be useful for production of bulk chemicals, such as biodiesel, methanol, single-cell protein (SCP), Polyhydroxybutyrate (PHB), liquids, and for bioremediation purposes. For the cultivation of methanotrophs, samples were collected from

different habitats such as rice paddy and marshy water canal followed by enrichment and isolation. As a result, diverse methanotrophs were isolated. A total of 21 isolates of the genus *Methylomonas*, five of the genus *Methylocystis*, four of the genus *Methylosinus*, and one genus of *Methylomagnum*. In addition to this, three putative novel methanotrophic taxa were isolated including one putative novel genus, *Methylococcaceae bacterium* FWC3 (NCBI accession no. for 16S rRNA is MN080433.1) and two novel species, *Methylobacter* sp. KRF1 (NCBI accession no. for 16S rRNA is MK511847.1) and *Methylomonas* sp. WWC4 (NCBI accession no. for 16S rRNA is MH764454.1). These all isolates were set for screening of their biotechnological potential such as PHB and carotenoid production. We could detect measurable carotenoids from the *Methylomonas* strains, whereas PHB could be extracted from *Methylocystis* strains. These methanotrophic strains are being explored for the further use for enhanced production of these metabolites.

**Keywords:** Methane; Methanotrophs; Carotenoids; Polyhydroxybutyrate (PHB); Biotechnological potential

EMT-44

### Characterization and antiproliferative activity of *Pseudomonas* mediated rhamnolipids

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#### Abstract

Microbial surfactants are structurally diverse group of surface active molecules synthesized by microorganisms. The majority of surfactants used in industry is synthetically derived from petro-chemical feedstock, and are thus not sustainable and often environmentally unfriendly. Hence, there is a need to identify natural counterparts to synthetic surfactants, produced via green production routes and that are less toxic in the environment. Biosurfactants produced through microbial fermentation are an alternative that have been shown to have these characteristics. Microbial surfactants are widely used for industrial, agricultural, food, cosmetics, pharmaceutical, and medical applications. Rhamnolipid is the common commercially existing microbial surfactant from *Pseudomonas* spp. In this study, *Pseudomonas aeruginosa* DNM50, isolated from oil contaminated soil, and capable of producing biosurfactant, was used. Bacterial cell-free culture broth showed positive results toward seven screening tests (hemolysis in blood agar, CTAB and lipase assay, drop collapse, oil displacement, emulsification activity, and surface tension reduction). They reduced the surface tension of growth medium from  $62.50 \pm 0.01$  to  $23.23 \pm 0.02$  mN/m. Based on TLC pattern, FT-IR, MALDI TOF and NMR analysis, the solvent extracted biosurfactant was designated as Rhamnolipids. The analysis of the extracted Rhamnolipids by thin-layer chromatography revealed the presence of two compounds corresponding to those of authentic Mono- and Di-rhamnolipid. In the extract obtained from *P. aeruginosa* DNM50, MALDI TOF confirmed the presence of Mono and Di-rhamnolipid congeners, specifically Rha-C8-C8:1, Rha-C10-C8:1, Rha-C10-C10, Rha-C10-C12:1, Rha-C16:1, Rha-C16, Rha-C17:1, Rha-Rha-C10:1-C10:1, Rha-Rha-C10-C12, Rha-Rha-C10-C8, Rha-Rha-C10-C8:1, Rha-Rha-C8-C8. Screening of anticancer studies also reveals that, the extracted Rhamnolipids effectively suppress the proliferation of breast cancer cell lines MDA - MB - 231 showing an IC<sub>50</sub> value of 5  $\mu$ g/ml. The present study concludes that the ecofriendly biosurfactant produced by *Pseudomonas aeruginosa* DNM50 shows significant antiproliferative activity. These findings suggest that the produced Rhamnolipids would be of potential for application in biomedicine as a new and promising therapeutic agent.

**Keywords:** Biosurfactant; Rhamnolipids; *Pseudomonas aeruginosa*; Anti-proliferative activity

## Leachate: A potent source of surface and subsurface groundwater contamination

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### Abstract

Solid waste generation is increasing rapidly due to fastest rate of urbanization and industrialization across the world including India. Landfill is an area of land where large amount of waste are dumped. Moisture trapped in the landfill seeps in the form of black brown liquid known as leachate. Leachate contains heavy loads of organic carbon, nitrogen other chemical contaminants and pathogens which works as a potent source of surface and subsurface groundwater contamination. After mixing with stream of fresh water or ground water it causes eutrophication, depletes oxygen level, and makes drastic changes in microbiota and chemical composition of water. Data on landfill leachate microbiology is lacking from India. Considering the role of leachate in spread of pathogen and antimicrobial resistance and their ill effects on human health and environment, in current study we have isolated and identified nearly 200 anaerobic and aerobic Bacteria and Archaea by 16S rRNA gene sequencing. Strain (AnLec124B) showed low sequence similarity (97.4%) with *Ochrobactrum anthropi* indicates it is supposed to be a novel species. Nitrate reductase and biofilm formation abilities of the strains were tested to assess their environmental sustainability. Metagenomic analysis shows that *Proteobacteria*, *Bacterioides* and *Fermicutes* are the dominant phyla and *Pusilimonas*, *Pseudomonas*, *Aquamicrobium*, *Membranicola*, *Soehngenia*, *Denitrimonas*, *Fermentimonas*, *Methanosarcina*, are the dominant genus in leachate. X-ray Fluorescence(XRF) data of leachate shows that the concentration of heavy metals such as sulfur, vanadium, chromium, cobalt, nickel, copper, tin, cesium, molybdenum, barium, lanthanum, tungsten are several fold higher than freshwater system and play crucial role in spread of heavy metal resistance in the environment

**Keywords:** Leachate; Anaerobe; Methanogens

## Potential lignolytic fungi for rapid decomposition of rice stubbles

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### Abstract

Open burning is a predominant practice to manage rice stubble in northern regions of India. Stubble is the most recalcitrant part in rice residue due to higher lignin content. In the present study, fungal strains with lignolytic potential were isolated, characterized and identified from decomposing rice stubbles. The isolates were subjected to both qualitative and quantitative analysis for lignolytic potential. After enrichment with various lignin related substrates like tannic acid, guaiacol, 19 morphologically different fungal strains were isolated and designated as LN-1, LN-2 to LN-19. In qualitative analysis, 11 out of 19 isolates showed positive result for laccase activity whereas, 14 isolates showed positive lignin peroxidase activity. Only three isolates were capable of tannic acid degradation and 12 fungal isolates were able to

decolorize bromophenol blue indicating lignolytic potential. Further quantitative analysis was carried out for eight selected isolates from the qualitative test. The results indicated that isolate LN-1 and LN-14 produced higher amount of lignin and manganese peroxidase as compared to rest of the six isolates. On the basis of morphological and microscopic studies at ITCC, Division of Plant Pathology, IARI the lignolytic fungal isolates LN-1 and LN-14 were identified as *Aspergillus terreus* and *Aspergillus fumigatus* respectively while molecular identification is under progress.

**Keywords:** Recalcitrant; Rice Stubbles; lignolytic Fungi; Lignin; Manganese peroxidase activity

EMT-47

### Effect of anticipated climate change on soil physico chemical characteristics and enzyme activities in flooded soil planted with rice

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#### Abstract

The global atmospheric carbon dioxide concentration has sharply increased to the current level exceeding 400 ppm. In future, their level in the atmosphere is expected to project further and would serve as feature of climate change. Elevated carbon dioxide ( $e(\text{CO}_2)$ ), has profound effect on plants, which is centre of concern, especially on food crops. It is noteworthy to analyse their impact on world's most important staple food, paddy. A field study was carried out to observe the impact of  $(\text{CO}_2)$ -550 ppm in open top chambers (OTC) on soil characteristics. Rhizosphere and bulk soil samples from three critical stages namely, active tillering, flowering and maturity were analysed for various physico chemical and biological properties. The evaluated physico chemical parameters *viz.*, soil pH, EC, available N, P and K displayed no contrasting differences irrespective of different concentration of  $\text{CO}_2$  and stages of crop. Analysed biological parameters exhibited distinct changes during flowering stage particularly under  $(\text{CO}_2)$ . Further studies were done with varied concentrations of  $\text{CO}_2$  and different soil nutrient amendments. Under  $e(\text{CO}_2)$ , biochar application (1 tonne/ hectare) significantly enhanced total soil organic carbon by (23 %) and soil microbial biomass carbon (SMBC) by (37 %). The result elucidates biochar and carbon content in them acting as matrix for soil microbes which in turn positively influence SMBC. Acid phosphatase and alkaline phosphatase activity followed AM fungi + Rock phosphate > AM fungi > biochar application. While the activity of  $\beta$  glucosidase, soil dehydrogenase were found in the order of  $e(\text{CO}_2)$  biochar > AM fungi + RP > AM fungi and the same trend was observed at lower levels under ambient conditions. A similar pattern was also observed for total viable bacterial count, heterotrophic fungi and actinobacteria. In contrast, population of methanotrophs were found sharply increasing steadily (active tillering < flowering < maturity). The result suggests the impact on physico-chemical properties, soil population dynamics and enzyme activity. Principal component analysis depicted three different factors *viz.*,  $\text{CO}_2$ , nutrients and crop growth stages influences the overall soil properties and microbial community.

**Keywords:** Elevated Carbon dioxide; Paddy; Active tillering; Flowering; Maturity

## Screening, identification and characterization studies of Azo dye decolourization by Mangroves rhizospheric isolates

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### Abstract

Removal of synthetic textile dye possesses a challenge and a threat to environment flora and fauna. Various bioremediation studies involving whole plants, plant roots and microbes in single or consortium have been used as environment friendly methods for the removal of textile dye. In the present study potent bacterial dye decolourizers were isolated from mangroves rhizospheric soil collected from Kamothe, Navi Mumbai, India. Of the 20 isolates obtained after enrichment, 6 isolates were used for further screening employing MBM medium containing 10 % glucose, trace element and 0.1% of dye concentration. All these 6 isolate's CFE were analysed for effect of physiological parameters like pH, temperature and incubation time to optimize the dye decolourization. Based on decolourization pattern, the effects of two formulated consortiums were also studied on methyl orange and on mixture of azo dyes. Biodecolorization of these dyes were confirmed through UV-Vis spectral analysis and biodegradation of Methyl Orange dye was confirmed by FTIR spectral analysis. The 6 isolates were characterized morphologically, biochemically, and molecular identification of these strains were performed by 16S rRNA sequence analysis. The isolates were identified as *Pseudomonas taiwanensis*, *Acinetobacter pittii*, *Exiguobacterium acetylicum*, *Psychrobacter celer*, *Citrobacter murlinae* and *Aeromonas taiwanensis*.

**Keywords:** Azo dye decolourizers; Bioremediation; Rhizospheric soil; Decolourization; Bacterial consortium; Molecular studies

## Designing of microbial consortia for the efficient wastewater treatment: A lab-scale study

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### Abstract

Water is the most essential entity for both the evolution and survival of life. Extensive rise in global population and subsequent rise in pollution generation accompanied by global climate change is posing serious threat to the fate of existing water bodies. Therefore, to limit the stress of water demand on natural freshwater reserves, recycling of greywater is required using the combination of microbial and engineered approaches. Microbes play essential role in the bioremediation of toxic and hazardous waste. This study presents a designed microbial consortia for treatment of wastewater. The consortia member were selected after testing lipolytic, amolytic, proteolytic and cellulolytic activity as markers. Optimization studies were carried out to test performance on removal of COD, ammonia, nitrate, nitrite after immobilization on various matrix. The results indicate that 90% removal of COD and 80% removal of ammonia, nitrite, nitrate when coir was used as matrix. It is envisaged that application will be used to treat flowing drain.

**Keywords:** Wastewater; Microbial consortia; Immobilization; Bioremediation; COD

## Developing novel associations to mitigate methane emissions in flooded rice fields

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### Abstract

Methane is a powerful greenhouse gas, second only to carbon dioxide in its importance to climate change. Flooded conditions in paddy fields facilitate anaerobic conditions, promoting methanogenesis. The net balance in methane emission from paddy fields is the result of the activity of methanogens and methylotrophs, by way of production and consumption. In our earlier investigation, efficient plant growth promoting methane oxidizing bacteria (*Methylobacterium oryzae*, *Hyphomicrobium facile* and *Burkholderia sp*) were found to reduce cumulative methane emission from flooded paddies by 20-25%; they also improved the yield parameters and growth of rice. However, this requires maintaining the population of inoculated MOB above threshold level in both the rhizosphere and phyllosphere. A need is felt to develop an efficient delivery mechanism for field scale application of efficient methane oxidizers and plant growth promoting bacteria. In this context, cyanobacteria which are oxygen liberating photosynthetic prokaryotes, can be explored as a physical support for methane oxidizing bacteria and such cyanobacterial mats, through commensal interactions, may help in reducing methane emissions effectively. A set of four cyanobacteria were screened for their ability to grow in the presence of different concentrations of methane (2, 3 and 4%). Among them, *Anabaena torulosa* and *Anabaena laxa*- both rice rhizospheric isolates from Aduthurai were promising, with 3-6fold enhancement in chlorophyll at all concentrations of methane, upto a period of 3 weeks. Growth media capable of supporting growth of MOB and cyanobacteria were developed and synergistic effect on the methane reduction, plant growth promotion and population dynamics was evaluated. Such an association of MOB and cyanobacteria can help to reduce methane emissions, both by directly stimulating methane oxidation at the soil-water interface and indirectly by promoting methane oxidation in flooded paddy soils by increasing soil. Future research may provide better insights into the nutrient dynamics of such associations.

**Keywords:** Methane; Phyllosphere; Rhizospheric; Cyanobacteria

EMT-51

## Evaluation of soil isolates obtained from polychlorinated biphenyl contaminated soil for PCB degradation potential

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### Abstract

Polychlorinated Biphenyls (PCBs) are ranked fifth in priority list of hazardous substances and consist of a biphenyl skeleton where 1-10 hydrogen atoms are substituted by chlorine giving rise to 209 congeners. The relative volatility of PCBs contributes to their spread throughout the globe as far as Antarctica. PCBs bio-accumulate and bio-magnify in the food web due to its lipophilic nature. PCB congeners can be co-metabolized aerobically and anaerobically by bacteria through the biphenyl catabolic pathway. These bacteria are capable to use biphenyl as carbon and energy source.

Therefore, the aim and objective of the present study was to investigate the biodegradation of PCBs in contaminated soil from Bhilai steel plant. Soil Samples were collected and identified for the presence of PCB through GC-MS/MS. In addition to the presence of PCB like 1,1'-Biphenyl, 4,4'-dichloro, 1,1' biphenyl 2,2',3',5,6 pentachloro and 1,1 biphenyl 2,2',3,3',6,6' hexachloro, the soil was also found to be contaminated with Polyaromatic hydrocarbon (PAHs) viz. phenanthrene, pyrene, benz(a) anthracene, perylene. After repetitive enrichment of the PCB degrading bacteria in the contaminated soil, 9 isolates were obtained. The 16S rRNA sequence analysis resulted in identification of five strains of *Pseudomonas*, two strains of *Ochrobactrum*, one strain of *Brevibacterium*, and one strain of *Pantoea*. Further GC-MS/MS studies revealed that among these isolates, *Ochrobactrum* OA-3, *Pseudomonas* sp PA-2, *Pseudomonas* sp. PP-6 and *Brevibacterium* sp. BI-7 exhibited 100%, 67.5%, 97.1% and 53.3% biphenyl degradation respectively after 48h with 200 ppm concentration. In addition, *Ochrobactrum* OA-3 isolate was also selected for biphenyl dioxygenase and toluene dioxygenase gene amplification by PCR and the amplicon bands were observed at 726 bp and 520 bp respectively. Overall results demonstrated the potential of strain OA-3 to degrade and survive in PCB contaminated soil and therefore it can be explored further for its role in bioremediation and environmental management.

**Keywords:** Polychlorinated Biphenyl; Bioremediation; Biodegradation; Persistent organic pollutant; Biphenyl dioxygenase

EMT-52

### Decolorization of the textile dye blue herd by bacterial isolates from industrial contaminated soil

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#### Abstract

Industrial effluents and sewage mixing is a major cause of environmental and water pollution. Synthetic dyes and colorants are being increasingly used in paper, textile, food, cosmetics, and pharmaceutical industries. Among these, textile industries are the largest consumer, accounting for 80 % of total production. The inefficient dyeing process results in discharge of 10–15 % of unused dye in waste water. Blue HERD dye is one of the azo dyes, widely used in textile industries and are recalcitrant, highly toxic compounds, carcinogenic and genotoxic, this impose need of their removal. In present study screening of dye decolorizing bacteria from industrial contaminated soil was done using nutrient broth medium with 5 ppm Blue HERD dye. Total 20 isolates were obtained, amongst these three potent decolorizing bacterial isolates were selected with ability of more than 90% dye decolorization. The dye decolorization by selected bacterial isolates was observed in various concentrations (10 to 200 ppm) of dye. A bacterial consortium using three potent dye decolorizing different bacterial isolates showed 95% Blue HERD dye decolorization in nutrient broth medium pH 7.0 at 37 °C in 24 hrs. These were identified using morphological and 16S rDNA as *Bacillus amyloliquefaciens*, *Proteus vulgaris* and *Brevibacillus reuszeri*. The maximum extent of dye decolorization was at concentration 20 ppm in nutrient broth medium pH 7.0 whereas media designing showed significant influence of yeast extract, 95% decolorization and static condition. The present study highlights the Blue HERD dye decolorization potential by three bacterial isolates and their possible use for dye remedial process.

**Keywords:** Dye decolorization; Blue Blue HERD dye; *Bacillus amyloliquefaciens*; *Proteus vulgaris*; *Brevibacillus reuszeri*

## Biodeterioration process of heritage buildings: Tracking through biofilm forming genes *pel A* and *alg A*

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### Abstract

Conservation and restoration of historical monuments is currently one of the major worldwide issue. It has been envisaged that, monuments deteriorates due to the interaction of microorganisms with building surfaces exposed to environmental factors. Biofilm bacteria are reported to mainly contribute towards deterioration process of potential surfaces by virtue of their capability to initiate adherence, secrete extracellular polysaccharides and create microenvironment to degrade surface structures. Biofilms comprises of group of microorganisms capable of producing extracellular polysaccharides. In the present study, such biofilm forming bacteria were isolated and biofilm associated genes (*alg A*, *pel A*) were tracked from four heritage structural sites of Maharashtra viz.; Ajanta Caves, Chhatrapati Shivaji Maharaj Terminal Mumbai (CSMT), Elephanta Caves and Sion Hillock fort. Key biofilm forming bacteria from each site were isolated and characterized by 16S rDNA sequencing as *Pseudomonas* sp. (MN253546, MN253099, MN253101, MN253097), *Bacillus* sp. (MN254971, MN254972, MN254973, MN253102, MN253100), *Staphylococcus* sp. (MN2535547), *Ochrobactrum* sp. (MN253098), *Acinetobacter* sp. (MN253099). PCR amplification of biofilm associated genes i.e. *alg A* and *pel A* from isolates and from total DNA (metagenome) extracted from heritage surface samples showed the prevalence of biofilms on heritage structures. The results indicated that biofilm associated genes could serve for tracking biodeterioration occurrences of heritage and historical surfaces.

**Keywords:** Biodeterioration; Heritage; Biofilms; Gene; Tracking; *alg A* & *pel A*

## Biofilm characterization of *Enterobacter cloacae* SBP-8

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### Abstract

Biofilm is predominant lifestyle for most of the micro-organisms rendering them a set of emanating properties such as antimicrobial resistance, enhanced survival, social cooperation and resource assemblage. Bacterial biofilms are produced by communities that are engrained in a uniformly self-produced extrapolymeric substance (EPS). Biofilm-associated infections are mostly culpable in causing majority of nosocomial infection. *Enterobacter cloacae* is one of the commonly implicated nosocomial pathogens and causes pneumonia, urinary tract, infections, ophthalmic infections and specifically catheter-related infections, where it has been reported to form biofilms in dwelling catheters of hospital patients. It is therefore important to characterize the biofilm forming ability of *E. cloacae* under different physiological conditions in order to understand the biofilm formation and its role in pathogenicity. In this study, we have evaluated the biofilm formation and progression under various physiological conditions which includes the effects of growth medium, temperature, by *Enterobacter cloacae* SBP-8. Biofilm formation was also confirmed for catheter and feeding tubes under conditions. The architecture of the biofilms was



examined using scanning electron microscopy technique. The heterogeneous composition of the complex biofilm was studied using Surface Enhanced Raman spectroscopy (SERS). Our results indicate that the biofilm formation of *Enterobacter cloacae* SBP-8 at 48 hrs and 96hrs old cultures exhibit the optimum biofilm formation. Addition of nutrients, such as glucose also vitally enhances the biofilm formation. SEM analysis revealed a uniform layer of extracellular matrix. The SEM analysis also revealed presence of a tubular membranous bridge which is termed as *nanotube* which serves as conduit for intercellular connection. The Surface Enhanced Raman spectroscopy gave us an insight of the heterogenous nature of biofilm which showed variation in biofilm formation over a time period of 24-120 (hrs). It was found that the carbohydrates production was consistent throughout the duration of 24-120 hrs. The lipid and protein production were significantly higher at 72hrs which contributed maximum to the biofilm formation. Our study concludes that crystal violet assay, SEM and SERS helped us to understand the biofilm formation and the composition of the matrix.

**Keywords:** Infections; Biofilms; *Enterobacter cloacae*; Surface Enhanced Raman Spectroscopy

EMT-55

### Enhancing the lipid content in oleaginous fungi for biodiesel production

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#### Abstract

Many countries in the world facing the problem of depletion in the percentage of the fossil fuels due to increase in the demand of fuels like petrol, diesel etc. Biodiesel is the good alternative as a replacement of fossil fuel. The present work emphasizes on enhancement of lipid content in oleaginous fungi for biodiesel production. In this study, 12 different fungal isolates were purified from different soil sample. Primary and secondary screening were performed for the maximum lipid accumulation. In primary screening, fungal isolates growing on potato dextrose agar were stained with the Sudan Black B for detection of blue or greyish lipid globules within the cell. Out of 12 isolates, 11 were found to be oleaginous fungi. Secondary screening was performed by inoculating fungal isolates in submerged medium and lipid was extracted by Folch method. On the basis of secondary screening, fungal isolate RD6 was found to be best lipid producer and identified as *Aspergillus niger*. The environmental parameters such as incubation time, medium pH and incubation temperature were optimized for maximum biomass production and lipid accumulation and found to be 120 h, pH 5 and 30°C, respectively. The maximum biomass and lipid content were found to be 0.79 g/l and 0.0039 g/l, respectively.

**Keyword;** Biodiesel; Oleaginous; Accumulation; *Aspergillus niger*

EMT-57

### Assessment of factors operative for biosurfactant production using yeast *Meyerozyma guilliermondii* YK21

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#### Abstract

Biosurfactants are surface-active compounds produced by microorganisms such as bacteria, yeast and fungi. These offer lower toxicity, inherent good biodegradability and ecological acceptability over chemical surfactants. Commercial production of biosurfactants is, restricted due to increased cost of

production which could be lowered down by optimization of production process using high yielding strains. Present investigation was designed to optimize biosurfactant production by yeast *Meyerozyma guilliermondii* YK21 evaluated in terms of oil displacement technique and emulsification index. Among carbon sources, olive oil at 8% (v/v) concentration was optimized to achieve maximum oil displacement of 6.4cm and emulsification index of 42.20% after five days of incubation. Diesel being complex hydrocarbon source was not utilized easily and showed poor biosurfactant production. Incorporation of yeast extract at 1.5% (w/v) concentration improved the biosurfactant yield as evident from increased oil displacement of 7.20cm and emulsification index of 46.60%. Addition of sodium chloride [3% (w/v)] further improved the oil displacement to 7.7cm. However, a further increased level of sodium chloride beyond this concentration negatively influenced the biosurfactant production. An incubation temperature equivalent to 30°C supported maximum oil displacement of 7.7cm which reduced significantly with any alteration in temperature to 25°C (4.80cm), 35°C (7.0cm) and 40°C (1.5cm). Biosurfactant production was found to be highly sensitive to pH as indicated by significant reduction in emulsification index to 48.80% at pH 7 as compared to 58.80% at optimum pH 6. A concomitant reduction in biomass production was also observed at pH 7 (6.3g/L) as opposed to pH 6 (8.6g/L). Stationary conditions were not found to be supporting biosurfactant production as observed by reduction in emulsification index from 58.80% to 34.10% after 5 days of incubation. The ability of yeast *Meyerozyma guilliermondii* YK21 to produce biosurfactant with efficient emulsification properties suggests its potential applications in bioremediation. Moreover, the application of final characterized product could be investigated towards bioremediation of petroleum hydrocarbons and degradation of agrochemicals.

**Keywords:** Biosurfactant; Yeast; *Meyerozyma guilliermondii*; Oil displacement; Emulsification Index

EMT-58

### Chemo-enzymatic hybrid system for CO<sub>2</sub> sequestration and conversion into industrial products

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#### Abstract

Industrialisation, advancement in technologies and anthropogenic activities are leading to the increasing concentration of carbon dioxide in the atmosphere. It has led to imbalance in natural carbon cycle and is the major reason of increasing global warming. More technologies need to come up to sequester carbon dioxide at reasonable rates and to upscale current technologies to meet the carbon capturing requirements. Approximately 60% energy is lost which affects the economy and the environment, the leakage rates of carbon dioxide should be monitored. Nowadays, various amine solvents have been used for CO<sub>2</sub> absorption among these monoethanolamine and diethanolamine are mostly used. These amines react with CO<sub>2</sub> and result in formation of stable carbamates. However, these amine solvents suffer from multiple inherent shortcomings such as higher energy demand, low absorption efficiency and solvent loss. Recently it has been proved that green biocatalyst carbonic anhydrase increases the CO<sub>2</sub> absorption rate of above mentioned amines. CA is a zinc containing metalloenzyme which catalyse the hydration of CO<sub>2</sub> into bicarbonate. CA catalyse the reaction very fast and can make large scale conversion economically viable. At present there is an urgent need of hybrid system in which chemicals and enzyme can be used together to control the tremendous rise in CO<sub>2</sub> level. We attempted to design a hybrid system for CO<sub>2</sub> conversion where CA can convert the atmospheric CO<sub>2</sub> in the presence of amine solvents. Furthermore the CA will be immobilized onto a nanotextured material which has ability to absorb the CO<sub>2</sub> efficiently. Overall these hybrid technologies will help to mitigate the climate changes by efficient CO<sub>2</sub> conversion.

**Keywords:** Carbondioxide; Climate change; Amines; Carbonic anhydrase; Hybrid system

## Recent advances in CO<sub>2</sub> sequestration and conversion using microbial enzymes

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### Abstract

We all know that, emission of carbon dioxide has resulted in serious climatic problems due to increase in earth's temperature and global warming. Therefore, effective methods to capture CO<sub>2</sub> and reduce CO<sub>2</sub> emissions are needed. A number of techniques have been attempted so far to reduce carbon dioxide including pre-combustion, oxyfuel and post combustion. However, these techniques have several limitations such as high cost, less effective, energy intensive *etc.* To overcome all these problems an enzyme-based approach may prove efficient and cost-effective method for carbon dioxide sequestration. CA can efficiently catalyze the hydration of CO<sub>2</sub> to form bicarbonate ion and a proton; the bicarbonate ion can further react with a calcium ion to form calcium carbonate. In the present study, twenty bacterial isolates isolated from soil sample were screened based on production of a yellow color colony on nutrient agar plate containing *p*-NPA as a substrate. The effect of different media and various fermentation conditions for production of carbonic anhydrase was studied. T4 isolate shows the higher CA production when grown in nutrient agar medium at 40 °C with pH 7.0. Maximum carbonic anhydrase production was resulted when 4% (v/v) inoculum used in production medium and incubated in shaking condition at 150 rpm. The results obtained are very motivating as the crude enzyme extracted from bacterial cell lysate able to convert the CO<sub>2</sub> into different metal carbonates.

**Keywords:** Carbonic anhydrase; Isolate; *p*-NPA

## A novel cellulase from indigenous fungal strain NSF-2 using agro industrial waste and its use in waste paper recycling

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### Abstract

The study, present the production of cellulase using agro-industrial waste, its purification and characterization. A fungal strain NSF-2 isolated from the plant litter produce high amount of cellulase. 15.59 fold purified enzyme achieved specific activity 71.421 U/mg. Under the optimum condition,  $V_{max}$  and  $K_m$  for the enzyme were 1.5 mg/ml/min and 3.2 mg/ml respectively. Purified cellulase stable in non-ionic surfactant like TWEEN -20, Triton X-100 on the other hand salt like urea, EDTA and SDS inhibited the enzyme activity up to 65%, 50% and 55% respectively. The study also reveals that Hg<sup>2+</sup> is a non-competitive inhibitor of cellulase. The  $V_{max}$  was decreasing with increasing concentration of metal inhibitor. The purified and crude cellulase produce by NSF-2 strain is well suitable in industrial application such as pulp and paper for waste paper recycling which also reduces the use of chemical thus help in waste water management also.

**Keyword:** Cellulase; Agro-industrial waste; Waste paper recycling

## Isolation and screening of potential cellulolytic thermophilic fungi from compost

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### Abstract

Cellulose is a polysaccharide consists of glucose subunits. It is present in abundance on planet earth. Cellulosic biomass has a wide spread application in industries producing variety of products. To make it in usable form, degradation is necessary. Cellulase enzyme is required to hydrolyse the crystalline structure of cellulose. Cellulase enzyme is expensive and makes the process costly. So to make it cost extensive, researches has been made to obtain the enzyme from the microorganism. Fungi is one of the microorganism that has great potential of producing cellulose degrading enzyme. It is commonly categorized into two types- mesophilic and thermophilic. Thermophilic enzyme has an advantage over mesophilic fungi i.e. it can produce extracellular thermostable enzyme. It can catalyse the reaction at elevated temperature and also has a less chance of contamination as in mesophilic fungi. So, the aim of present study is to isolate the thermophilic fungi from samples of compost for cellulase enzyme production. Isolated fungi were screened for cellulase enzyme production by using Congo red plate assay method. A total number of 3 thermophilic fungi have been screened as potential cellulolytic fungi depending upon zone of hydrolysis.

**Keywords:** Thermophilic fungi; Cellulose; Cellulase enzyme

EMP-62

## Effect of arsenic resistant bacteria on seedling germination and vegetative growth of rice (*Oryza sativa* L.)

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### Abstract

Rice (*Oryza sativa* L.) belongs to family Poaceae. It is one of the most ancient food crops and a primary food source for over one third of the world's population being cultivated in 117 countries across the world and hence called as "Global Cereal" Rice (*Oryza sativa* L.) Heavy metals are commonly found elements in the universe. The density should be larger than 5 g/cm<sup>3</sup> in order to be considered as heavy metals. In the present study, the arsenic resistant bacteria were isolated from contaminated water samples. These isolates were screened for their ability to resist higher concentration of arsenic salt (250 ppm, 300 ppm, 400 ppm and 500 ppm). ASR-7 isolate was found to be efficient and identified as *Stenotrophomonas pavanii* based on molecular characterization. In order to confirm the arsenic resistance of these isolates on seedling germination of rice a pot experiment was conducted at UAS, Raichur. The data obtained from study revealed that, these isolates would be used as potential bioremediating agent for the lowering As concentration and also results proved that these isolates play an important role in enhancing the vegetative growth parameters of paddy.

**Keywords:** *Oryza sativa*; *Stenotrophomonas pavanii*; Arsenic resistant bacteria

## Evaluation of *Exiguobacterium profundum* PHM11 for alleviation of salinity stress in wheat and maize

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### Abstract

Salinity is one of the major constraints for agriculture all over the world. High salinity in soil can inhibit water uptake leading to reduction in plant growth and development. Microorganisms offer economically feasible eco-friendly solution to address the salinity stress in crop plants. In the present study, *Exiguobacterium profundum* PHM11 which is a gram positive salinity tolerant bacteria was evaluated for its ability to alleviate salinity stress in wheat and maize. Inoculation of PHM11 resulted in significant increase in root length, root volume, projected root area, surface area, number of links and forks in both wheat and maize with or without salinity stress (100mM NaCl). Bacterial inoculation resulted in reduction in root diameter which actually helped the plants to take up more water under salinity stress. Similarly, increased root length, projected area, root volume and root branching resulted in better water mining by the plants. However, the effect of inoculation was more prominent on maize under salt stress. The results indicate that PHM11 has potential to be developed as a inoculants for maize crop under saline conditions.

**Keywords:** Exiguobacterium; Salinity; Wheat; Maize

EMT-64

## Microalgae based waste-water treatment coupled with CO<sub>2</sub> fixation

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### Abstract

In recent times, surface water pollution leading to water crisis, been recognized at the global level has become one of the grave environmental problems. Enrichment of wastewater with organic nutrients stimulates the growth of algal species producing massive surface growths, known as algal blooms, frequently being toxic to the water dwelling organisms. Cost-effective wastewater treatment along with biological CO<sub>2</sub> fixation by microalgae is becoming essential nowadays. The water demand for algal cultivations can be met with low-quality sources such as wastewater or sea water. Many microalgae can be used to transform CO<sub>2</sub> to the organic matters through biological mitigation. The current study emphasizes on the implementation of a partially identified *Chlorella sp.* in waste-water purification. The microalgae, *Chlorella sp.* was grown in waste water for 15-16 days with continuous monitoring of various water parameters viz., pH, temperature, etc at every 24 h interval. Various parameters viz., COD have revealed a decrease in the treated waste-water (106 mg/L) as compared to the untreated one (395 mg/L) in bench scale trials. The degradation performances by algae fixation trials are underway. The detailed outcomes will be exhibited in the conference.

**Keywords:** Microalgae; Waste-water; *Chlorella sp.*; CO<sub>2</sub> fixation; COD; Biofertilizer

**A detailed investigation on *Planococcus maritimus* derived biosurfactant and its potential application in enhanced oil recovery process**

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**Abstract**

Genus *Planococcus* represents a marine halotolerant bacterial group where *P. alkanoclasticus* known for hydrocarbon degradation, *P. maitriensis* reported for Biosurfactant (BS)/ bioemulsifier production. The practical potential of genus *Planococcus* has been reported as oil cleansing activity. This published literature proposes enormous application of genus *Planococcus* in diverse industries. In spite of knowing this genus *Planococcus* since 2001; it has remained largely unexplored. The halotolerant *Planococcus* genus provides huge opportunities for researchers to investigate it thoroughly for basic and applied research. BS producing marine bacteria possesses oil scavenging activity in the oil polluted environments. This assists them to survive in extreme and adverse environmental conditions. With this literature background we aimed to extract BS from *Planococcus maritimus* isolated from sea water of Indian Arabian coastline. The integrated approach was used for genomic insight of the genes involved in BS biosynthesis. This detailed genome analysis of *P. maritimus* revealed involvement of terpenoid encoding genes in BS synthesis. Initial screening procedures namely, surface tension, oil displacement and drop collapse identified *P. maritimus* as a potent BS producer. BS extracted using solvent system of ethyl acetate: methanol (4:1, v/v) and reduced the surface tension of phosphate buffer saline from 72 to 30 mN/m with a lower CMC value of 1.3 mg/mL. Further investigation on chemical characterization using TLC, FT-IR, <sup>1</sup>H NMR and <sup>13</sup>C NMR and LC-MS disclosed the glycolipid nature of BS. The *Planococcus* derived BS demonstrated antimicrobial activity against few selected pathogens. Evidences in decrease in surface tension proposed potential applications of BS in crude oil degradation and also in microbial enhanced oil recovery. The potential application of the BS in oil recovery evaluated using the 'Pumice stone assay', 'Oil saturated gravel' and 'sand pack column' techniques. These results are promising for the remediation of oil-contaminated soil and water.

**Keywords:** *Planococcus maritimus*; Biosurfactant; Genome sequencing; Glycolipid; Oil recovery

**Solving the mystery of dark green waters of Amalnala, a lake in Chandrapur district, Maharashtra**

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**Abstract**

Amalnala dam is located at the foothills of Manikgarh hillock in Gadchandur town of Chandrapur district in Maharashtra state. The medium-scale irrigation dam is a main source of water for surrounding villages and local industries. It is also a well-known tourist spot. From last couple of years, water of

the dam is turning dark green, especially, in the month of August resulting in the increased viscosity and stinking of water. This increased our curiosity to test the water sample for any biological origins. Preliminary microscopic examination showed the presence of *Microcystis*, a neurotoxic cyanobacteria which is green in color and probably imparts green color to water. The excessive growth of single cell cyanobacteria can be due to rise in nitrates and phosphate levels in water. Thus it becomes necessary to study the source of nitrates and phosphates and its possible bioremediation with the help of microbes and effect of neurotoxin produced by cyanobacteria on animal and human health.

**Keywords:** Amalnala; Neurotoxic cyanobacteria ; *Microcystis*; Bioremediation

EMT-67

### **Biosurfactant yield enhancement via process optimization using bacterial isolate BK34**

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#### **Abstract**

Microbially produced biosurfactants are biodegradable and have high ecological acceptability making these an ideal alternate to chemical surfactants in various applications. Large scale production of biosurfactants is restricted because of the high cost. The production cost however, could be effectively lowered down by improving yield of the final product *via* optimization of fermentation process. In this work, different physical, chemical and biological parameters were optimized for biosurfactant production by isolate BK34. Five independent variables were varied; carbon source and its concentration, nitrogen sources and its concentration, pH, temperature and pitching rate. Emulsification index, oil displacement, biomass production and parafilm M test were used to measure biosurfactant production. Maximum oil displacement of 6.8cm, emulsification activity of 20.0% and 5.55fold increase in biomass production was observed when olive oil at the rate of 2% was used as carbon source. The influence of three nitrogen sources, sodium nitrate, urea and ammonium sulphate was tested. BK34 showed maximum oil displacement of 9.2cm, emulsification index of 75% and drop diameter of 0.80cm with urea as a nitrogen source. Highest biosurfactant production was obtained at 30°C as maximum oil displacement (9.3cm), emulsification index (75%), drop diameter (0.80cm) and biomass production (2.16fold) was obtained after 5 days of incubation. Biosurfactant production was found to be optimum at pH 7 as revealed by maximum oil displacement (9.2cm) and emulsification index (75%) after 5 days of incubation. Oil displacement, emulsification index and parafilm M was found to be increasing up to 0.05% (w/v) biomass reaching a level equivalent to 8.9cm, 70.0% and 0.80cm respectively. There was no significant increase observed beyond this level of pitching. The ability of isolate BK34 to produce biosurfactant with efficient emulsification properties suggests its potential application in bioremediation procedures.

**Keywords:** Biosurfactant; Emulsification index; Oil displacement; Parafilm M test; Biomass production

EMT-68

### **Polyhydroxyalkanoate production from banana peel blended with potato peel**

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### Abstract

The plastic wastes after consumption has not been completely collected for recycling and its safe disposal is a matter of concern. The biodegradable plastic is a feasible alternative to combat with environmental impacts of petroplastics. The potato and banana are two crops of importance in human diet all over the world. These are good sources of protein, carbohydrates, calcium and fats. But the consumption and processing of these crops generates peels as wastes. The peels are rich source and used by microbes in polyhydroxyalkanoate production. The potato peels had high starch content and banana peel had high glucose content. The sugar content in peels has high potential for value added products. The aim of the present study was to evaluate the capability of *Pseudomonas fluorescens* for polyhydroxyalkanoates (PHA) production from banana peel blended with potato peel at low solid loading rate of 1 percent w/v. The PHA production by *Pseudomonas fluorescens* from blended peels by hot water pretreatment and filtrate mix from potato and banana peels were more or less similar. The maximum PHA yield from the sugar recovered from blend of potato and banana peel was 12.2 percent at 48h. But the biomass production was higher in the mix of filtrate of banana and potato peel. *Pseudomonas fluorescens* was able to utilize reducing sugar from potato and banana peels as carbon and other nutrient sources.

**Keywords:** Biodegradable plastics; Polyhydroxyalkanoate; Blend; Potato peel; Banana peel

EMT-69

### Effect of different pretreatment method and supplements on biowaste for PHA production

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### Abstract

Plastic waste is contaminating our surrounding rapidly. Bioplastic is alternative for petroplastic. Economic and sustainable bioplastic production to a large extent is dependent on use of biodegradable waste. PHA (polyhydroxyalkonate) is a type of biopolymer produced by microbes as food reserve material. Its sustainable production is the aim of our experiment. To produce high content of PHA we treat our desire waste like sweetlime (peel, pomace, peel+pomace) 4%, 6% for 4 days from different different method e.g high temperature and pressure, acid+alkali and microbial hydrolysis treatment. We add different supplements for *Bacillus* species e.g Glucose, peptone, NH<sub>4</sub>Cl. *Bacillus cereus*, *Bacillus thuringiensis* these two strains used in our experiments to produce PHA. Maximum yield occur on 4<sup>th</sup> day in 1% glucose added as a supplement in culture for PHA production.

**Keyword:** Biowaste; Petroplastic; PHA production; Pre-treatment method; *Bacillus thuringiensis*

EMT-72

### Genome-wide screening for identification of Polystyrene degrading genes in *Exiguobacterium* strain DR11 and DR14

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### Abstract

Polystyrene (PS) is a chemically inert synthetic aromatic polymer. This widely used form of plastic is recalcitrant to biodegradation due to the hydrophobic nature of the PS surface. The exponential production and consumption of polystyrene in various sectors has presented a great environment risk and raised the problem of waste management. Biodegradation by bacteria has previously shown great potential against various xenobiotics but there are only a few reports concerning polyolefins. In a previous study, it was reported that two bacterial species – *Exiguobacterium sibiricum* strain DR11 and *Exiguobacterium undae* strain DR14 showed promising biodegradation potential against polystyrene. Members of *Exiguobacterium* are considered extremophiles, as they possess extreme tolerance to temperature (12°C to 55°C), halo tolerance (up to 13%) and can grow in a wide range of pH (5–11). *Exiguobacterium* strains DR11 and DR14 have the ability to degrade non-irradiated solid polystyrene material after incubation with these isolates. Growth studies suggested that these strains utilize polystyrene as a carbon source and initiates polymer degradation by biofilm formation over the PS surface. This leads to alteration in the physical properties of the material. Genomic DNA was extracted by a conventional isolation method and the whole genome of *Exiguobacterium* strain DR11 and DR14 was sequenced using the HiSeq 2500 sequencing platform (Illumina), with a paired-end module. We then performed bioinformatics analysis of the whole genome sequence data and mined the genome for PS degrading genes. Traditionally, there are two pathways of styrene degradation, yielding either 3-vinylcatechol or phenylacetate as end-products. Our analysis revealed the presence of genes involved in both the pathways. Biochemical and functional characterization of these genes would reveal deeper insight into the mechanism of PS degradation. Studies on these bacteria have potential to develop novel PS bioremediation pathways in future.

**Keywords:** Polystyrene; *Exiguobacterium*; Bioremediation

EMT-73

### Assessment of dumpsite soil biocover suitability for reducing methane emission from landfills under interactive influence of nutrients

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### Abstract

Biocovers are known for their role as key facilitator to reduce landfill methane (CH<sub>4</sub>) emission on improving microbial methane oxidation. Methanotrophs existed in the aerobic zone of dumped waste are the only known biological sink for CH<sub>4</sub> being emitted from the lower anaerobic section of landfill sites and even from the atmosphere. However, their efficacy remains under the influence of landfill environment and biocover characteristics. Therefore, the present study was executed to explore the suitability and efficacy of dumpsite soil as biocover to achieve enhanced methane bio-oxidation under the interactive influence of nutrients, carbon source and environmental factors using statistical-mathematical models. The Plackett Burman Design (PBD) was employed to identify the significant factors out of 07 tested factors having considerable impact on CH<sub>4</sub> bio-oxidation. The normal plot and student t-test of PBD indicated that ammonical nitrogen (NH<sub>4</sub><sup>+</sup>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N), CH<sub>4</sub> dose and copper (Cu) were found significant. A 3-level Box-Behnken Design (BBD) was applied to optimise the significant factors identified from PBD. The BBD results revealed that interactive interaction of CH<sub>4</sub> with NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>

-N affected the CH<sub>4</sub> oxidation significantly. The sequential statistical approach predicted that maximum CH<sub>4</sub> bio-oxidation of 27.32 μg CH<sub>4</sub> h<sup>-1</sup> could be achieved with CH<sub>4</sub> (35%), NO<sub>3</sub><sup>-</sup>-N (250 μg/g), NH<sub>4</sub><sup>+</sup>-N (25 μg/g) and Cu (50 mg/g) concentration. Conclusively, waste dumpsite soil could be a good alternative over conventional soil cover to improve CH<sub>4</sub> oxidation and moderate the emission of greenhouse gas from waste sector.

**Keywords:**Waste soil biocover; Methane oxidation; PBD; BBD; Nitrogenous nutrient

# **FOOD AND DAIRY MICROBIAL TECHNOLOGY**



# ORAL PRESENTATION



**Selection and Characterization of Bacteriophage against the Foodborne Pathogen *E. coli* O157:H7****B. H. Joshi<sup>a</sup>, D. S. Chavda<sup>b\*</sup>**<sup>a</sup>*College of Food Processing Technology & Bio-Energy, Anand Agriculture University, Anand-388110, Gujarat.*<sup>b</sup>*Shree Ramkrishna Institute of Computer Education & Applied Sciences, Surat-395001, Gujarat.*

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**Abstract**

*Escherichia coli* O157:H7 is an endemic food borne pathogen causing a variety of human diseases including mild diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura. This study concerns the exploitation of bacteriophages as biocontrol agents to eliminate the food borne pathogen *E. coli* O157:H7. Three distinct lytic phages (EHD1, EHD2 and EHH1) isolated against a human pathogenic strain of *E. coli* O157:H7 from domestic sewage from different area of Anand district and screened more potent phages by plaque morphology & lytic assay. Most potent bacteriophages were characterized based on Temperature & pH assay, Growth curve and TEM microscopy. One-step growth curve analysis showed logarithmic growth of phages and relatively short eclipse latent periods. TEM analysis revealed that *E. coli* phage (EHD2) belongs to the family pathogens in food, suggesting its potential as a novel biocontrol agent.

**Keywords:** Bacteriophage; Foodborne Pathogen; *E. coli* O157 H7; Characterization; Food safety**Antimicrobial and technological activities of lactic acid bacteria isolated from locally fermented foods in Ogun state, Nigeria****Bamidele Odumosu<sup>a\*</sup>, Tajudeen, Bamidele<sup>b</sup>, Daniella, Ofem<sup>a</sup>, Friday, Agbozo<sup>a</sup>, Ram Chandra<sup>c</sup>**<sup>\*</sup>*bodumosu@unilag.edu.ng (corresponding Author)*<sup>a</sup>*Department of Microbiology, University of Lagos, Akoka, Lagos State, 100001, Nigeria*<sup>b</sup>*Department Molecular Biology and Biotechnology, Nigeria Institute of Medical Research, Yaba-Lagos, 100002, Nigeria*<sup>c</sup>*Department of Microbiology, School of Environmental Sciences, BabasahebBhimraoAmbedkar University, Lucknow, Uttar Pradesh, 226025, India***Abstract:**

Several Lactic Acid Bacteria (LAB) have been applied in food preservation as a result of their antimicrobial properties and versatilities. Application of LAB in the control of human pathogens that causes gastroenteritis holds promise provided appropriate strains are scientifically chosen and a suitable mode of delivery is utilized. The aim of this study was to determine the antibacterial and essential vitamins producing properties of LAB isolated from *Parkia biglobosa* (fermented locust beans) from selected markets in Lagos and Ogun states in Nigeria against clinical isolates of *Escherichia coli* and *Salmonella typhimurium*. Thirty-two LAB were isolated from 50 samples of *Parkia biglobosa*. and were investigated for their antibacterial activities against *E. coli* and *S. typhimurium* by agar spot test and well diffusion methods while the vitamins producing abilities was determining by High Performance Liquid Chromatography (HPLC). Of the 32 isolated LABs, 5 isolates showed antibacterial activities with inhibitory zones of 1.4mm, 1.3mm, 1.3mm and 1.4mm on *E. coli* and zones of 1.3mm, 1.2mm, 1.0mm and 1.0mm on *S. typhimurium* while the other isolates showed no inhibitory properties. The HPLC results indicated abilities of the investigated strains to produced vitamins B1, B2, B3, B12 and folic acid. The result of this study suggest *P. biglobosa* may harbor LAB which could help to reduce the health risk of bacterial gastroenteritis and improve nutritive diets respectively

**Keywords:** Lactic Acid Bacteria; Vitamins; Antimicrobials; fermented foods; *Parkia biglobosa*

## Biogenic synthesis of bacteriocin-coated silver nanoparticles and their efficacy against food-spoiling bacteria

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### Abstract

Food spoilage has become a major concern throughout the world, as consumption of unsafe food is leading to an increase in the mortality rate. The use of chemical preservatives in marketed food products is affecting the global population at an alarming rate. Therefore, the need to replace the chemical preservatives with natural preservatives has become a topic of interest for the entire research fraternity. In this direction, "bacteriocins", the biological preservatives have been investigated to be competent enough to replace the chemical additives from food products. However, although bacteriocins are efficient in inhibiting the growth of food pathogens, but they hold a few limitations such as: a narrow antibacterial spectrum and a high dosage requirement, which limit their use as efficient food preservatives. To encounter these major limitations, in the present research, attempts were made to involve the upcoming field of nanotechnology by undertaking biogenic synthesis of bacteriocin-coated silver nanoparticles. The conditions for synthesis were optimized and the resultant nano-conjugates were characterized using various physio-chemical techniques such as UV-spectroscopy, transmission electron microscopy, fourier transform infrared spectroscopy, X-ray diffraction and zeta potential. The size range of the nanoparticles synthesized was found to lie within 21-34 nm. *In-vitro* antibacterial efficacy of the synthesized nano-conjugate was also evaluated against six food pathogens, the activity of synthesized nanoparticles was found to be higher than the bacteriocin alone. Moreover, the minimum inhibitory concentration value as low as 8µg/mL was recorded against *Staphylococcus aureus*. The present study thus reports that bacteriocin-coated silver nanoparticles can be used to fight against food spoilage pathogens and aid in food preservation.

**Keywords:** Antimicrobial efficacy; Bacteriocin; Biogenic synthesis; Food pathogens; Nanoconjugates

## Metataxonomics and functionality of *kinema*, an ethnic fermented soybean food of the India, Nepal and Bhutan

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### Abstract

*Kinema* is an ethnic fermented soybean food which is sticky and flavorsome consumed by people of the Eastern Himalayan regions of India, Nepal and Bhutan. Presence of diverse microorganisms in *kinema* plays important role in the hydrolysis and conversion of protein and starch for development of flavor of the product. The present study is aimed to profile the bacterial community of *kinema* samples collected from India, Nepal and Bhutan by 16S rRNA amplicon gene using high-throughput sequencing method and also to determine the predicative functionality using gene sequences. DNA was extracted and carried out for high-throughput amplicon sequencing (Illumina Miseq platform) targeting the V1-V3 region of the 16S rRNA gene and analyzed using QIIME2 version-2019.7 and analysis of predictive functionality using PICRUSt2-2019. *Firmicutes* was dominant phylum in all samples of *kinema*. More



than 1% relative abundance of genera were *Bacillus*, *Acinetobacter*, *Myroides*, *Corynebacterium*, *Proteus*, *Brevibacillus*, *Lactobacillus* and *Lysinibacillus*. At species level, *Bacillus subtilis* was found to be the most dominant species among the species of *Bacillus* detected after taxonomy assigned. In the functional profiles, metabolism has a higher rate, specifically with the amino acid metabolism (Alanine, aspartate and glutamate metabolism) and followed by carbohydrate metabolism. This study provides the unknown information regarding profiling of bacterial communities in *kinema* from three different countries-Nepal, India and Bhutan, which lays a foundation for further investigation into the food ecology. Additionally, the insight metabolic activity shows the potential of *kinema* as functional food.

**Keywords:** *Kinema*; 16S rRNA gene; QIIME2; *Bacillus subtilis*

RF/FDMT-18

## An indicator-independent colorimetric assay for the detection of membrane-acting bacteriocins

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### Abstract

The bacteriocins are ribosomally synthesized secretory peptides generally targets cell membrane of related and pathogenic bacteria through membrane permeabilization and extensive pore formation. An indicator-independent vesicle based colorimetric assay has been used for the determination of membrane-acting bacteriocins. The polydiacetylene (PDA) vesicles were exhibited the strike colour changes upon interactions with AMPs/bacteriocins due to structural perturbation of the lipid vesicles. The change of the percentage of blue into pink colour is calculated in the form of colorimetric response (CR%). In this study, PDA vesicles were prepared from dimyristoylphosphatidylcholine (DMPC) and 10,12-tricosadiynoic acid (TRCDA) using sonicator. The plantaricin LD1 was purified from *Lactobacillus plantarum* LD1 using tangential flow filtration (TFF). The PDA vesicles were treated with plantaricin LD1 at pH 8.0 with different strengths (2, 10, 25, 50 and 100mM) of tris-Cl buffer and found CR% 16.12±0.09, 20.74±0.2, 28.51±0.08, 51.49±0.3 and 51.50±0.5, respectively with colour change blue to red. The PDA vesicles treated with different concentrations of plantaricin LD1 0, 5, 20, 80, 100, 150 and 200µg/ml and found the CR% 4.17±0.2, 7.85±0.5, 16.45±0.09, 25.73±0.4 and 30.26±0.1, respectively with colour change blue to red. The untreated PDA vesicles and treated with 5 and 20µg/ml of plantaricin LD1 did not change the colour and showed the nil CR%. The untreated PDA vesicles showed round shape with clear boundary whereas PDA vesicles treated with plantaricin LD1 showed large in size and ruptured cell boundary under scanning electron microscopy. The membrane-acting nature of plantaricin LD1 was further confirmed by Fourier Transform Infrared (FTIR) analysis suggesting interaction with phospholipid in vesicles which confirm the membrane-acting nature of the plantaricin LD1.

**Keywords:** PDA vesicles; Antimicrobial peptides; Bacteriocins; Colorimetric



# POSTER PRESENTATION



## Application of MALDI-TOF MS approach for detection of bacteria in fruit juices

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### Abstract

Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) is one of the emerging tools for high-throughput and rapid microbial identification. The technology has started replacing existing phenotypic identification systems prevalent in clinical diagnosis and in some cases DNA sequence-based identification practices due to its relatively higher accuracy and low-cost. Accurate profiling of whole cell peptides and short proteins of microorganisms using MALDI-TOF MS approach gives a fair idea about their taxonomic affiliations and assist in their identification. The pomegranate and sweet lime juice are the main preferable juice among the consumer because of medical properties and nutritional value. These juices frequently get spoiled because of yeast and bacteria. In order to isolate these bacteria from juice sample use of rapid method is highly essential. In present study raw juice sample was inoculated on Malt extract Peptone Yeast extract Dextrose agar medium (MPYD) and plates were incubated at 35°C for 48 hrs. The colonies obtained on plates were subjected to colony morphology study total six colonies were decided to characterize, out of which four colonies from citrus juice and two from pomegranate juice were subjected for MALDI-TOF MS identification comparison with the Bruker taxonomy database using Biotyper 3.1 software the data shows the best match with *Klebsiella pneumoniae* ssp. *pneumoniae* 9295\_1, *Klebsiella variicola* 37924 PFM, *Pantoea dispersa* DSM 30073T DSM, *Enterobacter cloacae* MB11506\_1 in sweetlime juice while *Lactobacillus fermentum* DSM 20391 in pomegranate juice, while one colony isolated from pomegranate juice cannot be identified. From obtained results it can be anticipated that MALDI TOF identification system is also efficient tool for identification of juice spoiler bacterial species.

**Keywords:** MALDI-TOF MS; Citrus juice; Pomegranate juice; Yeast spoilage

## Camel Milk: A revolutionary probiotic for sustainable development of humans

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### Abstract

Demand of probiotics is evidently on the rise globally due to its various benefits on human health. The search of novel bacteria with potentials of probiotics is the necessity of time. Several probiotics available in the market at high cost claims to show large number of health benefits but are usually found ineffec-

tive. Therefore, the main objective of our study is to evaluate the probiotic potentials of Lactic Acid Bacteria (LAB) isolated from camel milk. As camel milk is considered to be highly nutritious and is a natural source of probiotics. Camels can easily survive in both the extreme temperatures of summer as well as winter. They can also survive with little availability of water for long period. Hence, we felt it desirable to study LAB of camel milk. Our study found numerous Lactic Acid Bacteria in camel milk. On biochemical characterization, the isolates were found to be *Lactococcus*, *Lactobacillus*, *Pediococcus* and *Bifidobacterium*. Majority of the isolates fulfilled all the standard requirements of probiotics such as growth at low pH, bile salt tolerance, growth at different temperatures, resistance against antibiotics and antimicrobial activity. Many of the isolates showed great antibacterial activity against common human pathogens. These strains can be highly effective against several common infections and diseases caused through common pathogens. The entire work will briefly describe the role of Lactic Acid Bacteria in sustainable development of humans.

**Keywords:** Lactic Acid Bacteria; Camel Milk; Probiotics; Gut Microflora; Human Health

FDMT-3

### **Antifungal attributes of *Lactobacillus casei* against mycotoxigenic *Fusarium graminearum* associated with cereal-based foods**

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#### **Abstract**

Cereals are important source of nutrients which are rich in carbohydrates, proteins and fiber. However, they can be contaminated by mycotoxigenic fungi; because of which efficient management strategies for these fungal contaminations is being studied. Many lactic acid bacteria (LAB) have proven their capability of inhibiting fungal growth and mycotoxin production in food/ feed products and transform it into nontoxic derivatives. In this work, 5 LAB were tested for their ability to inhibit the growth and mycotoxin production of *Fusarium graminearum*. Antifungal activity was assessed in agar medium as well as in maize seeds. Results showed that LAB significantly inhibited *Fusarium graminearum* but none of the strains completely inhibited fungal growth. The highest inhibition was obtained using *Lactobacillus casei* (87.61% growth of *Fusarium graminearum*). The cell free supernatant (CFS) from LAB reduced fungal growth with average growth ranging from 75% to 90% for *Fusarium graminearum*. It was also noted that CFS at pH 7 after neutralization from the entire tested LAB failed to inhibit fungal growth proving the activity of organic acids against *Fusarium graminearum*. *Lactobacillus casei* was most effective strain in mycotoxin suppression for trichothecene (T-2). Moreover, the inoculation of *Lactobacillus casei* in maize seeds artificially contaminated *Fusarium graminearum* decreased the infection incidence in seeds. This study revealed the potential of *Lactobacillus casei* to be used in the control of fungi growth and mycotoxin production in cereals.

**Keywords:** Lactic acid bacteria; Antifungal; Mycotoxigenic; Trichothecene (T-2); Cereals

## Phytic acid estimation by colorimetric method in different flours

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### Abstract

The phytic acid is the free-acid form of *myo*-inositol hexaphosphate whereas the term phytate refers to the salt when phytic acid binds with cations Zn, K, Ca, Mg and forms complexes, thus affecting their digestion in monogastrics. In the present study, the quantitative colorimetric wade reagent method was used to estimate phytic acid in different flours. Phytic acid was estimated by two different methods, Hydrochloric Acid (HCl) and Trichloro Acetic Acid (TCA). Each flour (rice flour, wheat flour, gram flour and pearl flour) was suspended in 2.4% HCl and in 5% of TCA respectively. The supernatant was diluted suitably and Wade reagent was added for color appearance. Absorbance will be measured at 500nm for phytic acid determination. In HCl method, wheat flour reported highest phytic acid (3881.6 µg/ g of flour) followed by gram flour (2701.8 µg/ g of flour), pearl millet flour (2639.7 µg/ g of flour) and, rice flour (962.40 µg/ g of flour). The phytic acid of rice flour (1149.06 µg/ g of flour) was very low as compared to wheat flour (4875.7 µg/ g of flour), pearl millet flour (5900.6 µg/ g of flour) and, gram flour (1430.4 µg/ g of flour), in TCA method. The partially purified phytase of *A.oryzae* SBS50 was used for biodegradation of food phytates. Maximum inorganic phosphate was liberated in pearl millet flour (13065 µg/ g of flour) followed by wheat flour (10415 µg/ g of flour), gram flour (8715 µg/ g of flour) and rice flour (890 µg/ g of flour).

**Keywords:** Phytic acid; Colorimetric method; Wade reagent; Biodegradation

## Shelf life optimization for Tofu prepared using Himalayan black soybean

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### Abstract

Tofu is one of the most popular protein rich soybean-based food. Himalayan Black soybean, locally known as Bhatt (*Glycine max* L.) is a variety of soybean grown in Uttarakhand hilly areas as well as bordering states of Indian Himalayan region. Black soybean is popular because of its high nutritional value and medicinal properties and this can be used as source of tofu. This study was conducted to examine the effect of storage time and temperature (5-35°C) on physico-chemical and microbiological properties (shelf life) of tofu prepared using Himalayan black soybean. Citric acid was used as coagulating agent for preparation of tofu. Microbiological parameters (Total aerobic bacterial count, fungus, yeast and mold) was analysed using standard plate count method and for achieving this sample was analysed for microbial load at every 2h for higher temperatures (25 and 35°C) and at every 6h for lower temperature (5 and 15°C). Physical properties (pH and moisture) and chemical properties (proximate nutrient content and phytochemicals) were also analysed at different storage time until the spoilage occurs. Results showed that the storage

time of 6 h at higher temperature, (i.e. 35°C) has shown negative effect on microbiological properties of tofu along with change in nutritional content. Total aerobic bacterial count at 6 h was  $4.29 \times 10^8$  CFU mL<sup>-1</sup> but no fungal, yeast and mold contamination was found, whereas at controlled temperature i.e. 5°C, there is no microbial growth was found upto 6 days of storage. Protein and fat content of tofu was declined with extended storage time. Further, in view of commercialization of the Himalayan black soybean based tofu, it is important to maintain the storage temperature at 5°C for not more than 5 days as the microbiological quality is directly depends on the storage time and temperature.

**Keywords:** Himalayan black soybean; Soymilk; Shelf life; Nutritional value

**FDMT-6**

### **Exploring fructophilic probiotic bacteria from fructose rich niches of North Maharashtra region**

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#### **Abstract**

The theme of employing effective probiotic strain in the human gut microbiota to improve metabolism has gained significant interest nowadays. Currently, several scientific investigators have predicted and confirmed the fructose as an inducing factor for obesity in human. The potential fructophilic probiotic strain could be utilized effectively for prevention of the high-fructose diet-induced metabolic syndrome. The fructophilic lactic acid bacteria commonly present in fructose rich niches. In the present study various fructophilic lactic acid bacteria were isolated from highly diversified fructose rich habitats, particularly in North Maharashtra Region. Among total 113 different fructose rich sources, a fructophilic bacterial strain - MPP-76 was isolated and characterized for various probiotic parameters. The strain is capable to grow in the range of 1-35% fructose with optimum 5% w/v, this designates its fructophilic property. The studied fructophilic strain MPP-76 utilizes fructose quicker than glucose and grow at optimum concentration of fructose - 5% w/v. with the capability to withstand 35% fructose. Also, in vitro assessments of the MPP-76 strain have examined the survival in acidic situation of gastric juice. The superior auto aggregation capacity in the presence of hydrolytic enzymes, co-aggregation with pathogenic bacteria, hydrophobicity properties, and no hemolytic activity; denotes that the strain is novel probiotic strain. The significant antagonistic activity against several harmful bacteria. This probiotic strain could be potentially used as starter culture, constituent of food-formulation contributing in improvement food aroma and taste, prolonged storage, and nutritional aids of foods especially rich in fructose.

**Keywords:** Fructophilic lactic acid bacteria; Probiotics; Fructose rich niche

**FDMT-7**

### **Probiotic viability, nutritional, functional and sensory attributes of beetroot juice fermented with lactic acid bacteria**

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### Abstract

Consumers are demanding for the diet with balanced nutrient profile along with metabolic, physiological, health and functional benefits rather than energy providing diet. Probiotic microorganisms have various health promoting functions like prevents intestinal tract infections, improves lactose metabolism, reduces serum cholesterol level, enhance immunity, stimulates calcium absorption, improves protein digestibility, synthesis of vitamins (vitamin B, nicotinic acid and folic acid), and counteracts the effects of food-borne pathogens. Probiotic products available in the market today are usually dairy based and cannot be consumed by individuals who suffer lactose intolerance and allergies due to milk proteins. This has led to development of rapidly emerging fruits and vegetables based non-dairy probiotics. Fruits and vegetables offer healthy alternatives for the production of probiotic foods due to their large distribution and nutritive value. While searching for alternative food matrices, the suitability of beetroot juice as a raw material taken for the production of probiotic drinks by lactic acid bacteria (*Lactobacillus rhamnosus*, *Lactobacillus plantarum* and *Lactobacillus delbrueckii*) was investigated. Growth profile and strain compatibility of the three microorganisms were examined and discovered to be compatible with each other. The vegetable juice was fermented at 37°C for up to 24 h and changes in the microbial count, total phenols, antioxidants, pH, sugars, titrable acidity and sensory evaluation were observed during the fermentation period.

**Keywords:** Beetroot; Probiotic; Polyphenols; Compatibility; Sensory

FDMT-8

### Screening and molecular identification of fluoride-tolerant bacteria from

Nasipur village, Birbhum district, West Bengal, India

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### Abstract

Fluorine is mostly electronegative, toxic and highly reactive element in earth's crust. Due to its high reactivity it combines easily with many other metals and decreases the amount of many trace elements which are beneficial for environment. When fluoride is present in excessive amount in drinking water it shows many adverse effects on human health. Fluoride-tolerant microbes play a dominant role by accumulating the anions so that they become less toxic. The objective of this study is to isolate and characterize fluoride-tolerant bacteria from fluoride contaminated soil and water samples of Nasipur village, Birbhum district; West Bengal. From water sample analysis through SPADNS photometric procedure, significantly higher fluoride concentration of 1.95 mg/L was found in tube well samples of Nasipur village. For primary screening, different concentrations of sodium fluoride (NaF) are used and 7 isolates are selected. Among of them MNF9 were able to tolerate maximum fluoride concentration i.e. 0.80g/l. For further screening, colony morphology, microscopic analysis, antibiotic susceptibility test, extracellular enzyme production and carbohydrate fermentation assay, were performed of those 7 isolates. Most of the isolates produce extracellular enzymes such as catalase, amylase, protease etc. Isolates were susceptible to many important antibiotics, shows eco-friendly nature of the isolates. They utilize galactose, glucose, sucrose and lactose as a carbon source. Through 16S rRNA gene sequencing, bacterial isolates show maximum similarity with genera *Acinetobacter*, *Aeromonas*, *Bacillus* and *Pseudomonas*. Further we use these fluoride-resistant bacterial stains for application in water defluoridation in contaminated sites.

**Keywords:** Fluoride; SPADNS Photometric; Bioremediation; 16S rRNA; Defluoridation

## Extraintestinal Pathogenic *Escherichia coli* (ExPEC) in backyard and broiler chickens of Rajasthan, India

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### Abstract

In recent years, some studies have found that poultry may contain intestinal pathogenic *Escherichia coli* (*E.coli*) as well as extra intestinal pathogenic *Escherichia coli* (ExPEC). More importantly, extra intestinal pathogenic *Escherichia coli* (ExPEC) causes significant infections in both poultry and in humans such as colibacillosis in chicken and urinary tract infections, meningitis, and septicemia in human. The zoonotic risk to humans from chicken source *E.coli* is not fully elucidated. Hence, the objective of this study was to characterize *Escherichia coli* isolates from healthy backyard and broiler chickens from different regions of Rajasthan, India. In this present study we have isolated, 156 *E.coli* from poultry sampled over a wild geographical area of Rajasthan. The prevalence of ExPEC among, these isolates 21/156 (13.4%) were screened by PCR for virulence genes associated with human-sourced ExPEC. Genetically selected isolates were assessed *in vitro* virulence-associated phenotypic assay. In addition, 19 (90.4%) ExPEC were show strong biofilm producers and 6(28.5%) were showing hemolysis activity among isolated ExPEC. In summary, our study demonstrated that poultry could be a source of the potential reservoir of virulent ExPEC strains that could be transmitted to humans via food that could pose serious zoonotic risks to human public health.

**Keywords:** *Escherichia coli*; Extraintestinal pathogenic *Escherichia coli* (ExPEC; Zoonotic); Biofilm; Hemolysis

FDMT-11

## *Listeria monocytogenes*: threat to fresh vegetable industry

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### Abstract

The fresh-cut horticultural produce market has huge potential and fastest growing marketplace in past ten years, providing consumers convenient, suitable and wholesome food. *Listeria monocytogenes*, a member of the genus *Listeria*, is widely dispersed in agricultural environments, such as soil, manure and water. This bacterium is a recognized food borne pathogenic causing numerous diseases, from mild gastroenteritis to severe blood and central nervous system infections. Usually, ready-to-eat cold-stored meat and dairy products are considered high-risk foods for causing listeriosis. Moreover, numerous studies have identified *L. monocytogenes* in fresh and slightly processed vegetables as these microorganisms are present in the growing environment (soil and water). The microorganisms also get adhered to biotic and abiotic surface resulting in biofilm formation accounting for contamination of food. Prevention of biofilm formation, maintenance of proper hygiene plan and implementation of food safety management systems from farm to fork are important control measures to reduce the prevalence and survival of *L. monocytogenes* on fresh produce.

**Keywords:** *Listeria monocytogenes*; Biofilm; Horticultural; Gastroenteritis

## Microalgae as a source of bio-products application for food supplementation

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### Abstract

Microalgae is a sustainable and potent source of various bioactive natural compounds, including  $\beta$ -carotene, lutein, astaxanthin, fatty acids. Nowadays, the large-scale cultivation of microalgae for production of various bioactive compounds has gained significant interest with respect to commercial and industrial use for food supplementation. For the enhancement of their exploitation, a suitable extraction method is fundamental to reach high yields, optimal extraction condition, without degrading process caused for high temperature, air and light exposure, and to eliminate trace of hazardous solvents. A well-known innovative method is CO<sub>2</sub> supercritical fluid extraction (CO<sub>2</sub>-SFE) that uses CO<sub>2</sub> as safe solvent and operate in mild conditions avoiding degrading process. This method is advantageous for the reduction of solvent use and extraction time, and the yield increase. However, the CO<sub>2</sub>-SFE of carotenoids from microalgae have still needed to be enhanced to improve the yield and extract quality. For the optimization of this technique, key parameters should be tested on microalgae biomass, as sample matrix pre-treatment, quantity of loaded material, process parameters (temperature and pressure) and entrainer co-solvent. This study review effect of different operative conditions for extraction of high value-added chemicals from algal biomass, which can be used for as food supplementation compounds.

**Keywords:** Microalgae; Astaxanthin; CO<sub>2</sub> mitigation; Nutraceuticals; Circular economy.

## Screening and selection of L-asparaginase producing bacteria

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### Abstract

L-asparaginase catalyzes the deamination of asparagine to aspartic acid and ammonia. It is used to treat childhood acute lymphocytic leukemia. asparagine is an essential amino acid for the growth of certain malignant cells whereas the normal cells are independent of its requirement. Among the antitumour drugs, L-asparaginase has been employed as the most effective chemotherapeutic agent in pediatric oncotherapy, especially for acute lymphoblastic leukemia. L-asparaginase is also intended for use as a processing aid during food manufacture to reduce the level of asparagine by its hydrolysis to L-aspartic acid and ammonia. Free asparagine present in food is the main precursor of acrylamide, which is considered to be a probable human carcinogen. Acrylamide is formed from asparagine and reducing sugars primarily in starchy foods that are baked or fried at temperatures above 120°C. So, the main agenda of the study was to screen L-asparaginase producing bacteria from the environmental source. In the present

studies, extensive screening of the L-asparaginase producing bacteria was carried out. Total of 72 bacterial isolates were obtained from 34 different plant rhizospheric samples. Primary screening of these isolates revealed 23 potential L-asparaginase producers. Further quantitative evaluation was carried out for the selection of most efficient L-asparaginase producers. Result showed, BB2I4 isolate found to produce maximum 1386 IU of L-asparaginase. Strain improvement of the selected isolate was attempted by using random UV mutagenesis. The result showed about 2 fold increases in the L-asparaginase productivity.

**Keywords:** L-asparaginase; Lymphoblastic leukemia; Acrylamide; UV mutagenesis

FDMT-15

### **A Pilot Study on the Application of Functionalized Magnetic Nano Particles for Detection and Extraction of Pathogens in Beverages**

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#### **Abstract**

Food borne diseases are an important cause of morbidity and mortality, and a significant impediment to socioeconomic development worldwide. Advances in agronomic, processing, preservation, packaging, shipping, and marketing technologies have not only enabled the food industry, but has also introduced an increased risk for human illness associated with pathogenic bacteria. Some of the common pathogens associated with the food, beverage and dairy industry are *Escherichia coli* O157:H7, *Salmonella Sps*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Campylobacter Sps*. etc. Elimination of pathogens from consumables has been the object that almost all food processors strive to achieve. Targeted affinity for isolation, extraction and removal of pathogens from food matrices, without heat treatment could prove to be a boon to the food industry on the whole. Our study is twofold. In this pilot study we have attempted to use functionalized Magnetic Nano-particles (fMNP) as a non-thermal, target specific elimination of pathogens and have used fMNP for concentrating pathogens from beverages, which can be an alternate for enrichment step that is common to all microbiological testing (culture, ELISA or PCR methods) protocols that are in use for pathogen detection. Our experimental results corroborate earlier reports on affinity of fMNPs to pathogens and thus prove to be an effective tool in both extraction, isolation and concentration of pathogens from food matrices.

**Keywords:** Food borne diseases; Food industry; Pathogenic bacteria; Human illness

FDMT-17

### **Competitive Inhibition of *Listeria monocytogenes* by Lactic Acid Bacteria**

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#### **Abstract**

Three species of Lactic Acid Bacteria as pure cultures were screened for their ability to inhibit/suppress growth of *Listeria monocytogenes*. The test was carried out for 4 days and the growth of *Listeria monocytogenes* was compared regularly. The strains used were *Lactobacillus delbrueckei*, *Lactobacillus plantarum* and *Lactobacillus casei*. The cultures were inoculated in SCDM Broths with *Listeria monocytogenes* and incubated for 24, 48 and 72 hours at 37°C. The CFU and log values were determined by serial dilution and pour plating of the broths in Hichrome *Listeria* Differential Agar. *L. casei* and *L. plantarum* were observed to effectively suppress growth of *Listeria monocytogenes*.

**Keywords:** Hichrome *Listeria* Differential Agar; *Listeria monocytogenes*; SCDM Broths; CFU

## Survivability of antibiotic resistant bacteria in fish and processing conditions

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### Abstract

Antibiotic-resistant bacteria can enter into seafood through secondary contamination of the harvesting waters or during various stages of handling, transportation, storage and sale. This study was conducted to determine the survivability of artificially inoculated multidrug resistant (MDR) *bla*<sub>NDM</sub>-harbouring *Escherichia coli* EC121 in fish stored at -20°C and in ice, in comparison with an antibiotic-sensitive *E. coli* K12. Bacterial counts were taken bi-weekly and on alternate days for frozen and chill stored fish, respectively. At -20°C frozen storage, EC121 and K12 showed 2.01 log and 1.83 log reductions in 161 days when inoculated into fish at 8 log CFU/g level. At 4 log CFU/g inoculation, 1.97 log and 1.23 log reductions in numbers occurred for EC121 and K12, respectively. In the case of chilled storage, 8 log CFU/g-inoculated fish showed 2.71 log and 3.2 log reductions in counts for EC121 and K12, respectively over 14 days storage period, while at lower (4log CFU/g) inoculation levels, the corresponding decreases in counts were 1.43 log and 2.49 log units. Both the strains exhibited better survival in frozen storage than in chilled storage. Irrespective of storage medium and the temperature, EC121 did not get cured of its plasmids throughout the storage period suggesting that antibiotic resistance markers are stable under temperature and starvation stresses. Conjugation experiments showed that the *bla*<sub>NDM</sub>-harbouring plasmid could be readily transferred to a recipient strain of *E. coli* in ice storage temperature but not at -20°C in fish matrix. The results suggest that the multidrug resistant bacteria such as the *bla*<sub>NDM</sub>-harbouring *E. coli* in seafood are stable at low temperature storage, being able to survive and transfer resistance markers by conjugation. Survival of MDR bacteria for extended periods of time in seafood provides ample opportunities for wider dissemination of the bacteria in food chain and the resistance genotypes among related bacteria as well.

**Keywords:** Survivability; Conjugation; Storage

FDMT-20

## Impact of stress adaptation on antimicrobial susceptibility profile of *Cronobacter sakazakii*

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### Abstract

*Cronobacter sakazakii* is an opportunistic food borne pathogen and primarily associated with the contamination of powdered infant formula (PIF). Although occur at low level in PIF, the pathogen can survive severe environmental stresses (heat, cold and desiccation) and known to grow in reconstituted PIF to infectious level that could ultimately lead to severe diseases in infants and neonates. Currently, *Cronobacter* infections are treated with antibiotics, however; the level of antibiotic considered adequate today may be inadequate in the future. Moreover, there have been evidences regarding changes in antimicrobial susceptibility profile due to prior exposure to stress conditions. Present study evaluated the antimicrobial efficacy of five pure polyphenols *viz.*, quercetin, rutin, caffeic acid, *p*-coumaric acid and ferulic acid against *C. sakazakii* and compares their efficacy against unstressed and stress adapted *C.*

*sakazakii*. Only two polyphenols, ferulic acid and *p*-coumaric acid showed antimicrobial activity against *C. sakazakii*. The MIC of unstressed and stressed *C. sakazakii* was compared by broth micro-dilution method. Overnight cultures (18 h) were prepared from five cultures of *C. sakazakii* that were either unstressed, desiccated (4 d at room temperature in desiccators), starvation stressed (48 h in 0.89% saline solution at 37 °C), or cold stressed (24 h in potassium phosphate buffer at 4 °C). Among all stressed cultures, different degrees of inactivation were obtained for ferulic acid and *p*-coumaric acid treated samples compared with the control and were found to be more resistant to polyphenols after stress adaptation. Results obtained will help in understanding the effect of environmental stresses during processing on *C. sakazakii* susceptibility to antimicrobials.

**Keywords:** *Cronobacter sakazakii*; Powdered infant formula; Polyphenols; Stress adaptation

FDMT-21

### **Analysis of biochemical changes in pearl millet based functional food during fermentation with *Lactobacillus sporogenes***

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#### **Abstract**

The continuous increase in the population of nutrient deficiency diseases is a serious public health concern worldwide. Traditional fermented foods have been used widely for various health promoting effects; however, large scale production through traditional methods are not suitable because of poor shelf life and nutritive value protection. Therefore, unlimited prospects exist in Indian traditional fermented foods for value addition by incorporating the probiotic microorganisms as well as by improving fermentation conditions and storage practices. The probiotic fermentation can bring specific added advantages apart from the nutritional improvements. The aim of the present study was focused on the determination of biochemical changes of pearl millet based product through fermentation using probiotic culture of *Lactobacillus sporogenes*. Pearl millet is a rich source of protein, minerals and fats as well as contains different phenolic compounds. During the fermentation process, pH, fat, hemicelluloses, and ADF was found to decrease; while NDF, acidity, dry matter, ash, moisture and organic matter was reported to be in an increasing order.

**Keywords:** Pearl millet; probiotic; *Lactobacillus sporogenes*; Fermentation; Health promoting effects

FDMT-22

### **Analysis of bacteriocins activity from lactic acid bacteria in food and dairy products by different molecular techniques**

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#### **Abstract**

Bacteriocins is found in the food that is transposed during food souring (in situ), bacteriocins by LAB can be considered in excessive proportion during in vitro fermentations under finest physical & chemical atmosphere. Many functions have been intended to the detection, purification and characterisation of bacteriocins, besides we can apply food preservation scheme. Bacteriocins are a contrasting type of ribosomally integrated, extracellularly released, bioactive peptides or proteins showing antimicrobial

action versus further bacteria. Its reactivity enhances amylolytic enzymes & lipase proposed like a lipid & carbohydrate element could be essential for the activity. The amino acid content of the filtered bacteriocin was evaluated to 29 amino acids. The bacteriocin was appeared by distinct technique like SDS-PAGE & amino acid contents. The report of analysis is conducting with the utilization of bacteriocins to the preservation of food products obtained from animal source is too convenient in compositions, but its drought for an abstract on the exploitation of bacteriocins in vegetable meals. The promoter strain was recognizing by molecular typing to be held by the species *Lactobacillus delbrueckii*, which is an isolated maker of bacteriocins. The preventor factor was boiling coherent & vital against LAB (lactic acid bacteria) classification & various food borne disease *Escherichia coli* and *Yersinia enterocolitica*.

**Keywords:** Bacteriocins; Soured food; *In situ* production; Lactic acid bacteria; Dairy industry

FDMT-23

### **Bacterial cellulose as an excipient for probiotics**

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#### **Abstract**

Cellulose, synthesized by plants or microbes is an inexhaustible carbohydrate polymer source. In recent times, bacterial cellulose (BC), special cellulose has wide industrial applications, due to its chemical purity, biocompatibility and ultrafine architecture. BC fibres impart varied beneficial effects to the host system by improving the gut transit rate and has been accepted as an insoluble dietary fibre by Food and Agricultural Organization (FAO) and American Association for Clinical Chemistry (AACC). Therefore the usage of BC as a carrier material for the targeted delivery of probiotic organisms is gaining interest. Apart from the delivery of probiotics, these fibres also help in modulating the gut microbiota and improves health besides useful in prevention or treatment of disease. In these present studies, BC produced from *Acetobacter senegalensis* MA 1 was evaluated for its application as an excipient for the probiotic delivery. BC in wet form was utilized for the development of probiotic coconut water and probiotic jelly desserts. Of the two developed products, probiotic jellies received maximum overall acceptance value (4.1). The probiotics immobilized in BC maintained a viable population of 5.95 log CFU g<sup>-1</sup> in the jelly dessert by the end of 28 d. In dry form, the BC mats were used for the production of probiotic spray dried and freeze dried formulations with maltodextrin as a co-polymer. The spray dried BC formulation was more efficient compared to the freeze-dried formulation by way of maintaining viable cell count of probiotics population. Furthermore, spray-dried BC maintained 7.11 log CFU g<sup>-1</sup> over a period of 28 d, while the freeze-dried formulations maintained approximately only 2.00 log CFU g<sup>-1</sup>. Such spray-dried formulations also showed improved survival of probiotics in the GI conditions, compared to the freeze dried cells.

**Keywords:** Bacterial cellulose; Probiotics; *Acetobactersenegalensis*; Excipient; Formulations

FDMT-24

### **Enhancement of bioavailable micronutrients and reduction of antinutrients in Pearl Millet through selective fermentation**

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#### **Abstract**

Pearl millet (*Pennisetum glaucum*) is generally grown in northern region of India and consumed thoroughly during winter season by almost all rural population. It considered as one of the most edible

crop which contains ample amount of Iron (Fe), Zinc (Zn), etc. Regardless of containing ample amount of micronutrients, millets contains antinutritional factors i.e. saponins, tannins, phenol, flavonoids, phytic acid, etc. These antinutrients factors make complex with essential nutrients or by other way decrease the bioavailability of nutrients. A selective fermentation is one of the best methods which reduce these antinutrients effects and make various micronutrients available for absorption. In this study, attempts are going on to enhance bioavailability of micronutrients (i.e. Fe, Zn) by reducing the concentration of antinutrients using selective microbial consortium under specific fermentation conditions.

**Keywords:** Pearl millet (*Pennisetum glaucum*); Bioavailability; Antinutritional factors; Selective fermentation

FDMT-25

### **Isolation, identification and antagonistic effect of *Lactobacillus* isolate from raw milk of cows and goats for bacteriocin production**

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#### **Abstract**

Lactic acid bacteria occur as natural micro flora in fermented dairy and food products. Its exert a strong antagonistic activity against many food contaminating microorganisms as a result of the production of organic acid, H<sub>2</sub>O<sub>2</sub>, diacetyl, inhibitory enzyme and bacteriocin. The present study was an attempt to isolate some *Lactobacillus* spp. from raw milk of cow and goat for determining their antagonist effect of the isolates against some food borne pathogens i.e. *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus*. Raw milk samples of cow and goat were collected from various localities of Uttarakhand from which fifty six of bacterial colonies were isolated. Out of fifty six, twenty seven bacterial isolates from cow milk and twenty nine bacterial isolates of goat milk, out of fifty six, eighteen were showing the characteristic of lactobacillus isolates on the basis of morphological and biochemical characterization These were further evaluated for antagonistic activity against food borne pathogens viz. *E. coli* (ATCC-25922), *S.aureus* (ATCC-25923) and *B.cerus* (ATCC-14579) in which eighteen were exhibited good antimicrobial activity but three isolates Cm5, Gm6 and Cm12 were resulted maximum zone of inhibition against various pathogens. Finally, these selected isolates would be characterized for bacteriocin production.

**Keywords:** Probiotics; Lactic acid bacteria; Raw milk; Bacteriocin; Dairy Products

FDMT-26

### **Development and quality evaluation of prepared functional food products from wild pomegranate (*Punica granatum*)**

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#### **Abstract**

Underutilized fruits are the key component of balanced human diet and also the main drivers in achieving global nutritional security by providing nutrients, vitamins and minerals. This fruit is



adapted to low-input agriculture. Focusing attention on this neglected and underutilized fruit is an effective way to help maintain a diverse and healthy diet. The study was conducted to develop and evaluate functional food products i.e. RTS beverage and tablets with respect to nutritional composition, sensory acceptability and microbiological parameters at different storage intervals i.e. fresh, 3, 6 and 9 months. From the study, it was found that the fruit contain high amounts of fibre (5.58 %), anthocyanin (21.38 mg/100g), vitamin C (17.00 mg/100g), pectin (1.86 % as calcium pectate), potassium (155.00 mg/100g) and phosphorous content (86.66 mg/100g). The results for wild pomegranate beverage blended with cultivated pomegranate in different ratios (0:100, 25:75, 50:50,75:25 and (100:00) and *tablets* showed that the TSS, acidity, ascorbic acid, total and non-reducing sugars decreased significantly while reducing sugars increased significantly with the increase of storage interval. The TSS, acidity and reducing sugars increased from 17.49-17.86, 0.37-0.51 and 4.72-5.49 while pH, ascorbic acid, total and non-reducing sugars decreased from 3.94-3.89, 2.17-1.75, 15.27-12.61 and 9.99-6.70, respectively. The prepared functional food products viz., RTS beverage and tablets were subjected for sensory evaluation to a panel of members at different storage intervals and the products were found to be acceptable in terms of colour, taste and consistency/texture. Even up to storage interval of 9 months at ambient conditions, no microbial load was observed in tablets during storage of 9 months. The pure/wild pomegranate based RTS beverage was free from microbial load up to 6 months of storage. As the concentration of cultivated pulp increased, the bacterial count increased with corresponding total bacterial count for 6 and 9 months of storage for different proportions 75:25, 50:50, 25:75 and 00:100 was recorded as 1.24, 1.34, 1.39 and 1.42 and 1.36, 1.41, 1.46, 1.51 (log CFU/ml), respectively.

**Keywords:** Pomegranate; Evaluation; Anthocyanin; TSS; Ascorbic acid; Food product

FDMT-27

### Functional analysis and whole genome sequencing of a prospective probiotic strain

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#### Abstract

Probiotics are live microorganisms intended to provide health benefits when consumed in adequate quantity. Most of the probiotic strains sold in India are imported from China or the western countries. In the present study, we have developed an indigenous strain that has requisite properties of a probiotic as per the WHO guidelines. *Lactobacillus plantarum* MSD-1 is a rod-shaped, gram-positive lactic acid bacterium, isolated from soil. Lp MSD-1 is acid and bile salt tolerant, displays BSH activity and has high bactericidal and fungicidal activity. The culture filtrate of the strain was very effective in inhibiting the growth of Uropathogenic *E. coli*, *Klebsiella pneumoniae*, *Vibrio cholera*, *Staphylococcus aureus* and *Salmonella typhimurium* along with *Candida albicans* and *Schizosaccharomyces japonicus* var *japonicus*, *Schizosaccharomyces pombe* and *Zygosaccharomyces bailii*. The whole genome of Lp MSD-1 was sequenced revealing 2972 genes, 2876 CDS, 2972 exons, 76 tRNAs, 16 rRNAs, 1 mRNAs, 3 ncRNA, 11180 assembly gaps, 83876 reference junctions, 84 SNPs and 621 repeat regions. So far, we have identified 46 genes that are known to influence human health. These include Riboflavin synthase (Vit. B12); Bactericin; Diacyl Glycerol Kinase; Lead, Copper, Mercury Transporting ATPase; EntA, etc. Many genes involved in cholesterol uptake and metabolism have also been detected. Apart from these, the strain also has antibiotic resistance gene, namely Chloramphenicol Acetyltransferase (*cat*), Beta Lactamase (*blaA*) and Tetracyclin resistance gene (*tetM*) that can be very helpful in antibiotic-associated diarrhoea (AAD). The genome sequence also revealed many adhesins genes that are helpful in adherence of the probiotic strain

to the gut mucosa where they impart their health promoting effects. This preliminary data makes for a very strong case for the strain to be a prospective probiotic.

**Keywords:** *Lactobacillus platarum*; Probiotic; Whole genome sequencing; Cholesterol sequestering; Commercial product

FDMT-28

### **Mycotoxigenic fungi in food chain and their novel biocontrol agents**

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#### **Abstract**

Mycotoxins are toxic metabolites of fungi, which are chemically stable and survive in all food processing. *Aspergillus*, *Penicillium* and *Fusarium* species produce toxins leading to contaminations emerging in the food chain. More than fifty genera of fungi are known to produce toxins and unfortunately, identification of closely related toxigenic species is overwhelming task even for taxonomic experts. Further, polyphasic approach is necessary in any taxonomic study and important facets of fungal biology in addition to morphology, nucleotide sequences that creates molecular markers for the straightforward identification of fungal species to solve taxonomic difficulties within species. Furthermore, molecular detection of mycotoxins, can serve as good alternatives to conventional methods targeting their biosynthetic pathway gene regarded as an efficient method for spotting of toxigenic *fungal species in fields, food and feed*, to ensure the food safety and quality. Aflatoxins (AFs), ochratoxins (OTs), Trichothecenes, Zearalenone (ZEA), Fumonisin (FUM), and citrinin (CIT) are significant toxins in food safety concern in the grain supply chain. At present, the use of synthetic chemicals/antifungal agents for control of fungi is in practice and is abundant hazard to the humans and animals due to increasing in numerous drug resistant microbes, and its residual toxicity to animals and humans. Therefore, there is a tendency to use bio-agents to control the fungal growth and the elimination of mycotoxins in food commodities by several agricultural and food industries. Toxigenic fungi and toxin detoxifying agents produced by diverse source of fungi used for the management of mycotoxins are important for the food safety. Furthermore, detailed studies need to be undertaken in mycotoxin-contaminated food in developing countries including India.

**Keywords:** Fungi; Mycotoxins; Trichothecenes; Biocontrol agents; Zearalenone (ZEA); Fumonisin (FUM)

FDMT-29

### **Valorisation of Dairy by-product for cost effective production of a potent antibacterial food preservative: Gutter to Gold**

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#### **Abstract**

'Paneer' or 'soft cottage cheese' is increasingly becoming popular in India due to its high nutritional attributes and ease of availability. India produces approximately 12 million tonnes of paneer annually.

Paneer production is associated with around 5-7 times of liquid 'paneer whey' due to processing steps. Historically, paneer whey has been considered a waste stream and nuisance by panner-makers in both organized and unorganized sector of India. India's annual production of whey is estimated around 2 million tons. Protein market is projected to grow at a CAGR of around 13% during 2017-2022. The very extensive polluting power of whey, with a BOD about 175-fold higher than typical sewage effluent promoted us to explore ways to utilize it for the production of potent antimicrobial peptides. Bacteriocins, which are antimicrobial peptides produced by certain lactic acid bacteria, warrant serious consideration as alternatives to traditional antibiotics. Pediocin is a cationic peptide produced by *Pediococcus pentosaceus*, having antimicrobial properties against several Gram-positive food spoilage bacteria, especially *Listeria monocytogenes*. They exhibit thermo-stability, non-immunogenicity, non-toxicity and retaining of antimicrobial activity at a wide pH range, making them an important class of bio-preservatives. Production of the pediocin by *P. pentosaceus* was investigated in batch cultures using a paneer whey medium containing formulated mixture of components. We optimized the production of pediocin using Response Surface Methodology (RSM) which were based on the results obtained from three level factorial design. The optimized parameters were statistically validated using logistic model of cell growth. The rate of pediocin production was measured in both the synthetic and our formulated whey-based media. Surprisingly, pediocin titre was approximately four times higher than MRS medium (a conventional commercial medium used for growth of lactic acid bacteria). The produced food-grade pediocin from paneer whey can, therefore, be exploited further to confer a rudimentary form of innate immunity to various foodstuffs, helping processors and food industries to extend their control over the food flora. We believe we have successfully converted a so-called nuisance into a valuable and prized raw material for the production of an antibacterial food preservative.

**Keywords:** Bacteriocin; Paneer; Whey; RSM; *Pediococcus pentosaceus*

FDMT-30

### Screening of indigenous *Lactobacillus* spp. strains for glucosidase and DPP IV inhibitory activities under *in vitro* conditions

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#### Abstract

Glucosidase(s) and di-peptidyl peptidase IV (DPP IV) are amongst the key enzymes that are involved in glucose metabolism. Glucosidase(s), primarily alpha glucosidase metabolizes complex carbohydrates leading to rapid increase in postprandial glucose. DPP IV, on the other hand, cleaves the incretin hormones for e.g. GLP-1 and GIP that are vital for pancreatic insulin secretion. Several alpha glucosidase and DPP IV inhibitors are clinically prescribed to patients with type 2 diabetes to check postprandial hyperglycaemia. In this study, attempts were made to screen *Lactobacillus* spp. strains from different origin (human milk, goat milk, infant faecal samples) for their alpha glucosidase and DPP IV enzyme inhibitory potential. Different *Lactobacillus* preparations in HEPES buffer *viz.* untreated cell free supernatant (CFS), heat treated CFS, sonicated cell free extract (S-CFE) and heat treated CFE were screened *in vitro* for enzyme inhibitory potential. Acarbose and berberine were included as positive control for alpha glucosidase and DPP IV enzyme inhibition, respectively. The enzyme inhibitory potential varied between different strains and their preparations. Overall, high enzyme inhibitory activity was recorded with sonicated preparations.

The study indicates that *Lactobacillus* spp. strains following safety validation can be explored as a safe and cost effective dietary intervention for management of type 2 diabetes.

**Keywords:** Alpha glucosidase; DPP IV; Human milk; Goat milk; Infant fecal samples; *Lactobacillus*; Type 2 diabetes

FDMT-31

### Pilot scale production of natural food biopigment from *Monascus purpureus* utilizing agro-industrial waste

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#### Abstract

Food colour is one amongst the most straight forward feature that fascinates consumers. For this purpose, synthetic colours are currently popular in the food industry. Synthetic colours are reported to have harmful effects on human health. Due to increasing awareness, need for a natural and safe alternate is being witnessed. *Monascus purpureus* is known to produce several bioactive secondary metabolites such as, pigments viz. orange, red, and yellow;  $\gamma$ -aminobutyric acid and monacolins (Lovastatin). However, production of these bioactive compounds is limited under natural conditions. The study aimed at scaling up production of natural red biopigment from *Monascus purpureus* at pilot scale. Solid state fermentation was carried out with broken rice as a substrate, followed by biopigment extraction. The biopigment yield was quantified spectrophotometrically at 500nm. With tray type fermenter, colour value of 41.2 OD Units/g dry matter substrate (dms) with 25.8% recovery using static extraction with ethanol was obtained. Following optimization of extraction parameters, maximum colour value of 44.8 OD Units/g dms with 27.5% recovery was obtained. Further, the physicochemical nutrient characteristics, solubility and the stability of biopigment were evaluated. The biopigment was non-hygroscopic and water and oil soluble. Following fermentation, the carbohydrate content and pH of broken rice decreased, while moisture, protein, crude fat and fiber, and ash content increased. The extracted red biopigment was stable against freeze-drying, vacuum oven drying, heating up to 100°C and 0.6 % (w/v) of various preservatives. However, the biopigment was sensitive to UV and sunlight. The biopigment was free from citrinin and had aflatoxin within permissible limits. The study provides an effective method for production of a stable and nutrient-enriched natural food biopigment that can serve as safe and cost effective alternate to current day synthetic colours in food industry.

**Keywords:** Natural food Biocolour; *Monascuspurpureus*; Pilot scale production; Solid-state fermentation; Broken rice

FDMT-32

### Post-harvest loss management through value addition for health benefits

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### Abstract

A large amount of by-products are generated from cereals, pulses after harvesting in milling process. They are mostly being used for livestock feed in spite of being a nutrient rich source for nutritional and therapeutic purposes. Based upon ongoing studies, valorization of these post-harvest losses, also known as agricultural by-products from highly consumed cereals or pulses, such as Bengal gram, wheat bran, and rice bran can be exploited for better health management. This study is all about the determination of physiochemical properties and nutritional composition of the by-product of Bengal gram for further development of value added products, incorporating by-products of cereals and pulses in combination. For the nutritional evaluation of raw ingredients, physiochemical property and nutrient composition were generally evaluated according to AOAC methods. According to the prepared study, most of these by-products showed promising levels of nutrients. Bengal gram husk showed highest amount of crude fibre, ash content, total and insoluble dietary fibre, and total calcium. Anti-nutritional factors were also found in significant level. Lowest content of trypsin inhibitor activity and higher amount of starch was found in broken rice. Soluble dietary fiber was present in wheat bran. Combining these by-products various products can be prepared using various processing techniques. These products seem to contain higher amount of protein, dietary fibre, available minerals, In vitro protein digestibility, higher anti-oxidant activity as compared to control products. Novel formulated food products developed from these agro wastes can be utilized as an unconventional source of nutrients and therapeutic weapon for the diseases such as diabetes, hypertension etc along with the normal human consumption. Exploitation of such agro wastes for food product development will also be helpful to manage malnourished state besides rural development and maintenance of food security in cheap prices for a healthy seeks.

**Keywords:** By-products; Post-harvest loss; Value added food products; Nutrition security; Therapeutic purpose

FDMT-33

### Immunomodulatory activity of colostrum derived bioactive proteins

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### Abstract

Buffalo colostrum, the first mammary secretion after calving, is a complex fluid rich in nutrients particularly high level of bioactive proteins such as immunoglobulins, lactoferrin, lactoperoxidase, lysozyme, growth factors and growth hormones. Purpose of the present study is to separate colostrum derived bioactive proteins fractions with immunomodulatory and antimicrobial properties that may improve immune system in immune-compromised persons. In the present work, colostrum whey was separated by rennet treatment and the bioactive proteins were separated from colostrum whey using ultrafiltration membrane of 100kDa and 50kDa molecular weight cut-off membrane (MWCO). The immunomodulatory activity of these bioactive protein fractions was checked by phagocytic assay. Macrophage from mice peritoneal fluid was extracted and incubated with yeast stain *Kluyveromyces marxianus* MTCC 1389 and cell proliferation was checked by MTT assay ranging from 10 ng/ml to 1mg/ml of protein concentration. Antimicrobial activity of the proteins was determined by well assay method against diarrheagenic *E.coli* (MTCC strains). 100 kDa retentate showed higher cell proliferation as compared to 100 kDa permeate and 50 kDa permeate and retentate. Further, phagocytic activity of macrophages showed increase in size and phagocytic activity against yeast as the concentration of protein increased from 10 ng/ml to 1mg/ml as compared to control (PBS). 50 kDa (Permeate and Retentate) showed more antimicrobial activity as compared to 100 kDa (Permeate and Retentate). *E. coli* MTCC 723 and *E. coli* ATCC 25922 were more sensitive than *E. coli*

MTCC 724 and *E. coli* MTCC 725. Therefore, it may be concluded that colostrum derived bioactive proteins showing immunomodulatory as well as antimicrobial activity against diarrheagenic *E. coli* strains may be used for strengthening the immune system of immune compromised people.

**Keywords:** Colostrum; Immunomodulatory activity; Bioactive proteins; Diarrhea; Antimicrobial

FDMT-34

### Effect of inulin on growth and antimicrobial activity of Probiotic bacteria against oral pathogens

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#### Abstract

In the recent years, combination of probiotics and prebiotics attracted attention because of the synergistic effect on health benefits to the host. The effect of prebiotics on proliferation of probiotics can be evaluated in terms of their growth response. The objective of this study was to determine the effect of inulin (a standard prebiotic) on growth of different lactic acid bacteria and the antibacterial activity of this synergistic combination. This experiment was arranged in completely randomized block design with three replication. There were six probiotic strains belonging to different genera of *L. sporogenes*, *L. rhamnosus* GG, *Lactobacillus* sp. and *Streptococcus faecalis*. For the growth response, three different media formulations were used (glucose free-MRS, 2% glucose-MRS, and 2% inulin-MRS). The antimicrobial activity of the synergistic combination was evaluated against three oral pathogenic strains (*Pseudomonas aeruginosa*, *Bacillus* sp, and *Staphylococcus aureus*). The result of this study showed that glucose supplementation led to highest population of all the probiotic strains in terms of log CFU/ml. However, substitution of glucose with inulin on MRS significantly decreased growth for all the strains ( $p < 0.05$ ). Furthermore, significantly lower antimicrobial activity was observed against all the pathogenic bacteria with supplementation of inulin as prebiotic substrate in MRS. The cell-free supernatant of *L. rhamnosus* GG showed the highest antibacterial activities against *P. aeruginosa* and *Bacillus* sp. The study indicated that in comparison to inulin, glucose is still a preferred substrate for the metabolic activities of probiotic strains.

**Keywords:** Inulin; Probiotic; Antimicrobial activity

# **GENOMICS, PROTEOMICS AND METABOLOMICS**





# ORAL PRESENTATION



## Metabolomic footprint of stress resilient *Rhizobium esperanzae* CRB6 from Finger millet (*Eleusinecoracana* L. Gaertn) in conferring abiotic stress tolerance

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### Abstract

Finger millet (*Eleusinecoracana* L. Gaertn) is an annual herbaceous nutriceal cultivated predominantly as a rainfed crop. In the current scenario of unprecedented climate changes, drought is the major limiting factor affecting finger millet production. Plant microbiome is an unsung entity in mediating drought tolerance *via* induced systemic tolerance (IST), which includes bacterial production of cytokinins, production of antioxidants, blends of microbial volatiles (mVOCs) and degradation of the ethylene precursor 1-aminocyclopropane-1 carboxylate (ACC) by bacterial ACC deaminase. In the present investigation, *R. esperanzae*CRB6, a root bacterial endophyte of Fingermillet genotype CO15, tolerated upto -6.25 MPa in PEG infused agar plates. Moreover, the strain possessed plant growth traits such as P and Zn solubilization, IAA production and exhibited ACC deaminase activity. While exploring the volatile blends (mVOCs), nonanol, 1-pentanol, benzothiazole and glucopyranoside benzo sulphonate were unraveled as signature volatiles of *R. esperanzae*CRB6. These mVOCs are involved in trehalose and sorbitol synthesis and are identified as a potential mechanism for abiotic stress tolerance by *R. esperanzae* CRB6. Moreover, untargeted metabolic fingerprinting of *R. esperanzae* CRB6 under induced stress showed upregulation of osmoprotectants such as proline, phenol, ethanol, ascorbic acid and geranyl isovalerate, the precursor of Abscisic acid (ABA). However under uninduced condition (NS), amino acids and other organic acids were produced during the ideophase. Also it was inferred that, with increase in stress intensity, the area intensity of the metabolites also increased suggesting upregulation. Hence the isolate *R. esperanzae* CRB6 it can be considered as a potent isolate for abiotic stress mitigation. Further scope on applying the metabolites produced by *R. esperanzae* CRB6 in conferring drought stress open new vistas in developing microbe mediated stress alleviation technology.

**Keywords:** Finger millet; Microbiome; Microbial volatiles; Metabolites; Abiotic stress

## Plant and microbial interaction studies in *Withania somnifera* with special reference to Secondary Metabolites

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### Abstract

*Withania somnifera* (L.) Dunal commonly known as Ashwagandha is a traditional Indian medicinal plant used by medicinal practitioners to cure wide range of ailments such as inflammation, tumor, stress, microbial infections etc using different plant parts or even whole plant. The medicinal properties of the plant are attributed to the availability of unique steroidal lactones of terpenoid ancestry called withanolides; synthesized via two major pathways mevalonate and non-mevalonate in the plant. With the increasing inclination of people towards herbal source of medicines owing to the high cost and side effects associated with allopathic drugs. The gap between the supply and demand of plant based drug is also increasing due to various environmental constraints in plant growth in natural conditions such as seasonal dependence, effect of biotic and abiotic stress factors etc among which one of the major factor is microbial infection which effects the crop yield. Secondary metabolites are potent anti-microbial agents, this knowledge prompted us to investigate that what role these secondary metabolites plays on the microbes which are detrimental for the growth of plant itself. Thus to understand the experiments were designed wherein endophytic fungus were isolated from the plants and its growth pattern in response to crude and purified extracts was studied. The responsive fungus was then used to validate the function of a key gene i.e. cycloartenol synthase (CAS), responsible for the steroidal biosynthesis and production. The study found that there is an interesting observation between endophytes and fungal infection. Further infection of over-expressed and silenced *W. somnifera* lines with candidate fungus showed that rate of fungal growth and CAS gene expression are inter-dependent which in turn regulated secondary metabolites biosynthesis. The relationship between microbes, secondary metabolites and associated pathways involved in the biosynthesis using knock-out and over-expressed lines will be discussed.

**Keywords:** *Withania somnifera*; Withanolides; Endophytes; Cycloartenol synthase

RF/GPM-01

### Epigenetic modulation alters the secondary metabolite profile in the endophyte *Nigrospora sphaerica* by HDAC and DNMT inhibitors

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### Abstract

Endophyte interactions with plant have various beneficial aspects to its inhabiting host, along with contribution to diverse structural attributes with biological potential. Phomalactone producing *Nigrospora sphaerica* from *Adiantum philippense* L. was subjected to epigenetic modification by chemical treatment under standard laboratory conditions. Most microbial biosynthetic gene clusters are either silent or expressed at very low levels. The culture manipulation of fungi in the presence of DNA methyl transferase and/or HDAC inhibitors led to the enhanced expression of biosynthetic pathways and further production of new secondary metabolites. In this present work we selected histone deacetylase inhibitors: Sodium butyrate, Suberoylanilide Hydroxamic Acid and valproic acid and DNA methyl transferase inhibitors: 5-azacytidine and hydralazine hydrochloride to carry out modulation epigenetic studies with endophytic fungus *N. sphaerica*. The liquid cultures of the *N. sphaerica* were supplemented with 500 $\mu$ M of each epigenetic modulator and cultured for 14 days. The ethyl acetate extracts were analyzed by TLC and HPLC. The chromatography profiles showed different responses to the small-molecule epigenetic modifiers, which induced the production of additional compounds. Results showed that this approach may trigger cryptic biosynthetic pathways for the production of silent secondary metabolites.

**Keywords:** Endophytic fungi; Phomalactone; Epigenetic modification; *Nigrosporasphaerica*; HDAC; DNMT

## ***Helicoverpa armigera* larvae as an *in vivo* model for evaluation of *Yersinia enterocolitica* strain 8081 virulence**

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### **Abstract**

*Yersinia enterocolitica*, belonging to the family Enterobacteriaceae, is a human pathogen with extensive serotype diversity, differentiated into six biovars (1A, 1B and 2-5) and above 70 serotypes. The highly virulent strains of *Y. enterocolitica* subsp. *Enterocolitica* have *Yersinia* virulence plasmid (pYV), *Yersinia* heat-stable toxin (YstA), high-pathogenicity islands (HPI), resistance-gene loci, prophages, metal-uptake operons, genomic islands, putative hemolysins, adhesions, type II (T2SS) and type III (T3SS) secretion systems. Mouse models are used routinely to study the pathology of *Y. enterocolitica* but due to costly animals and expensive maintenance facilities, there exists a need for rapid-inexpensive *in-vivo* model. Interestingly, *Y. enterocolitica*, also confers toxicity to insects. Therefore, the present work was undertaken to develop the larvae of *H. armigera* (Hübner) (Lepidoptera: Noctuidae), as a rapid-inexpensive *in-vivo* model system to study the effect of *Y. enterocolitica* strain 8081 (serotype O:8, biovar 1B) on its midgut. Significant decrease in body weight and damaged midgut of third instar larvae of *H. armigera* was observed when virulent *Y. enterocolitica* was supplemented to its artificial diet. Higher malondialdehyde and reduced antioxidants marked the enhanced lipid peroxidation in the midgut of *Y. enterocolitica*-fed *H. armigera*. The impact of pathogen on host was evaluated using the gel-free proteomics technique i.e. Orbitrapnano-LC-MS/MS and the bioinformatics tools. Conclusively, the cotton bollworm can be used to study the effect of pathogen on the physiology, anatomy and survival of the host and to assess the host-pathogen interactions.

**Keywords:** *Helicoverpa armigera*; *Yersinia enterocolitica*; Proteomics; Orbitrapnano-LC-MS/MS

## **Genomic insights into CRISPR-Cas System in the *Mycobacterium* genus and its evolution**

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### **Abstract**

CRISPR-Cas (clustered regularly interspaced short palindromic repeats–CRISPR-associated proteins) systems are now known to regulate processes like gene regulation, group behavior, and virulence in prokaryotes apart from providing adaptive immunity against invading foreign genetic elements including viruses. CRISPR-Cas systems are divided into two classes based on effector module composition. *Mycobacterium* genus constitutes a number of pathogens including *Mycobacterium tuberculosis* and many environmental bacteria, suggesting diverse genome evolution and adaptability. Recently *Mycobacterium* genus has been reclassified into five genera, based on a large set of core proteins of the genus into five

distinct monophyletic clades. Although CRISPR-Cas system has been identified in *M. tuberculosis* complex (MTBC), we are at a nascent stage to understand the implication of its presence in them. We performed a comparative genomic analysis of CRISPR-Cas system in 141 *Mycobacterium* species obtained from NCBI repository. Our analysis revealed two major types of CRISPR-Cas system in *Mycobacterium* genus, Type-I and Type-III, both belonging to class I system and characterized by presence of multisubunit effector-complex. The presence of type III-A system, denoted by the signature *cas10/csm1* gene, was found unique to MTBC. Though *Mycobacterium marinum* and *Mycobacterium leprae* are closely related to MTBC, we did not find presence of CRISPR-Cas system in them. We speculate that acquisition of CRISPR-Cas system has occurred independently in MTBC after the divergence of these three species from their last common ancestor. This hypothesis is supported by the results of the 16S rRNA and *cas10* gene phylogeny as well as CRISPR repeat structural and sequence similarity-based analysis. Bayesian analysis was performed to estimate divergence time-points deducing the events of horizontal gene transfer and IS6110-element derived deletion of some *cas* genes in MTBC. More detailed work can provide us valuable insights about the evolutionary advantage conferred to MTBC by retention of CRISPR-Cas system in their genomes.

**Keywords:** CRISPR-Cas System; Evolution; Horizontal gene transfer

# POSTER PRESENTATION





## Immunoinformatics studies for prediction of potential vaccine candidates against *Nipah Virus*

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### Abstract

*Nipah virus* belongs to the family paramyxovirida and contains a non-segmented, single stranded negative sense RNA. In addition to direct contact with infected bats, peoples also acquire the virus through contact with pigs or people. A recent outbreak in India of *Nipah virus* has infected at least 15 peoples and killed at least 13, according to WHO reports. The current *Nipah virus* outbreak in Kerala was first alerted with the death of three members of a family in May-2018. The etiological cause of their death due to *Nipah virus* encephalitis was confirmed by the National Institute of Virology in Pune. *Nipah virus* has 75% of the fatality rate. Outbreaks of *Nipah virus* in South East Asia have a strong seasonal pattern and have a limited geographical range. All the outbreaks occurred in winter and spring. There is no specific drug to treat the infections caused by this virus. Being the most recent in the list of emergent viruses more efforts are required to hunt for effective vaccine candidates against it. The Reverse Vaccinology approach definitely has the potential to pace up this entire task by exploiting the advancements in DNA sequencing techniques. Reverse Vaccinology is based on a pure bioinformatics approach to identify potential vaccine candidates that are intractable using other conventional approaches. Our current study is the initial effort to use this strategy for analysis of proteins of *Nipah virus* to reveal 11 epitopes of Nucleocapsid protein, 18 epitopes in Fusion protein and 60 epitopes of Attachment glycoprotein with potential to be treated as vaccine candidates. Though the docking studies has revealed their ability to efficiently bind with HLA molecules but to further validate the efficiency or usefulness of the predicted epitopes as vaccine candidates there in vivo immunogenicity must be confirmed. We believe that the proposed workflow is straightforward, user friendly and can be extended to target other pathogens/viruses for elimination of persistence infections.

**Keywords:** Paramyxovirida, *Nipah virus*, Reverse Vaccinology, Nucleocapsid protein

## Docking studies to predict potential targets of antifungal compounds identified from bacterial extracts

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### Abstract

There is no effective drug available to fight with the resistant strains of *Aspergillus fumigates*. Though numbers of antifungal compounds were already identified but their potential targets or the proteins with which these compounds interact to show the antifungal activity still remained unknown. So, in the present study we have tried to unveil the potential targets of 4 identified antifungal compounds using docking studies (Ergotamine, Hexadecanoic Acid, 2-butoxyethyl Acetate). To identify their targets, either we could have gone for wet lab validations but we have opted for *in-silico* studies that are more efficient in terms of time, money and labor. After performing literature searches we were able to list the 17 pathogenic proteins from *A.fumigatus* as potential receptor for the selected antifungal compounds. All

the considered antifungal compounds except Cepaciamide B have shown best interactions with most of the pathogenic proteins. Out of 17 selected pathogenic proteins, 7 proteins have shown most favorable interactions with Ergotamine, 9 proteins have shown the favorable interactions with Hexadecanoic Acid and 10 proteins have shown the favorable interactions with 2- butoxyethyl Acetate. Only two proteins were observed to interact with Cepaciamide B. Also the unknown mechanism of action of compounds like Cepaciamide B and 2-butoxyethyl Acetate can be unveiled through these interaction studies. These studies suggested that Cepaciamide B compound, must be interacting with 2 pathogenic proteins like Aspergillo pepsin F and cyp51A to exhibit its activity, whereas 2-butoxyethyl Acetate compound might be interacting with proteins like Aspergillo pepsin F, Mittogillin, cyp51 A, pkaR, Ribosomal L3 protein, Pep2, GpaA, pptA, Asf13and PMP20.

**Keywords:** *Aspergillus fumigates*; Cepaciamide B; Aspergillopepsin F; Mittogillin; cyp51 A; pkaR; Ribosomal L3 protein; Pep2; GpaA; pptA; Asf13and PMP20

GPM-05

### Reverse vaccinology studies to predict potential vaccine candidates for *Influenza virus*

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#### Abstract

*Influenza viruses* are members of the family orthomyxoviridae. Special attention was drawn to the public by the deaths caused in humans by the highly pathogenic avian influenza A/H5N1 subtype. These cases have shown that influenza can cause deadly infections in humans. The approach of Reverse Vaccinology (RV) is an *in-silico* technique to predict the potential vaccine candidates by the analysis of the genome of pathogen. RV has represented a breakthrough in the field of vaccinology and is able to considerably narrow down the search space for discovery of novel vaccine candidates. Our current study is the initial effort to use this strategy for analysis of proteins of Influenza virus to reveal 5 epitopes of Hemagglutinin protein and 4 epitopes in Neuraminidase with potential to be treated as vaccine candidates. The information generated using the mentioned workflow will be of great interest to biochemical scientists for development of vaccines. The attempted workflow has the potential to reduce the number of experiments and cost as compared to conventional vaccinology approaches. Also, there is no requirement to cultivate the pathogen/virus, so no risk of accidental exposure to virus exists. Another advantage of the approach is that even the antigens which are not able to express in vitro conditions can be accessed using this approach.

**Keywords:** *Influenza viruses*; Orthomyxoviridae; Reverse Vaccinology; Hemagglutinin protein

GPM-06

### *sarA* mediated antibiofilm and antivirulence efficacy of myrtenol against Methicillin-resistant *Staphylococcus aureus* (MRSA)

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#### Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a harmful human pathogen and attained high priority level in World Health Organization (WHO)-Multidrug-resistant (MDR) pathogens list.

Antibiotic treatment failure is occurring in *S. aureus* strains due to developing of MDR. Biofilm formation is one of the major reasons for the antibiotic resistance of *S. aureus*. Therefore, targeting the biofilm formation of *S. aureus* is an alternative strategy to treat its infections. In the present study, antibiofilm and antivirulence efficacy of myrtenol was analysed against MRSA and its clinical isolates. Myrtenol showed a concentration-dependent biofilm inhibition activity without affecting the cell growth and viability of MRSA strains. In addition, microscopic analysis validated the antibiofilm potential of myrtenol against MRSA. Furthermore, myrtenol decreased the production of major virulence factors such as slime, staphyloxanthin and autolysin. Staphyloxanthin inhibition is enhancing the sensitivity of MRSA cells to healthy human blood and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Myrtenol treatment inhibited the extracellular DNA (eDNA) mediated autoaggregation since eDNA releasing autolysis was reduced by myrtenol. Further, trypan blue and Alamar blue assays were validated the non-cytotoxic effect of myrtenol on human peripheral blood mononuclear cell (PBMC). Transcriptional analysis revealed the down-regulation of global regulator *sarA* and its mediated virulence genes expression in myrtenol treated cells which is well associated with results of phenotypic assays. Therefore, the present study revealed the *sarA* mediated antibiofilm and antivirulence efficacy of myrtenol against MRSA.

**Keywords:** Alamar Blue; Biofilm; Myrtenol; Methicillin-resistant; *Staphylococcus aureus*; Transcriptional analysis

GPM-08

### Reactive Oxygen Species (ROS) mediated regulation of *Candida tropicalis* virulence factors by a plant-derived bioactive compound

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#### Abstract

Clinically, Non-albicans *Candida* (NAC) species were increasingly reported as both colonizers and pathogenic in bloodstream infections. The occurrence of *C. albicans* infection is high in European nations but in case of US, countries in Latin America and India, the occurrence of NAC infections are higher than *C. albicans* infections. Among NAC species infection, *C. tropicalis* is the most prevailed when compared to other NAC species such as *C. glabrata*, *C. parapsilosis*, *C. krusei* and *C. kefyr*. A study at Indian rural tertiary hospital reported that the recurrence of *C. tropicalis* infection is phenomenal in urine, blood and oral scrapings from candidemia patients compared to *C. albicans*. Also, *C. tropicalis* infection is found in surgical related infections such as osteomyelitis and endophthalmitis. Conventional antifungals such as Amphotericin B, Flucytosine, Echinocandins and Azoles drugs are available for treatment of *C. tropicalis* infections. Most of the antifungals induce intracellular Reactive Oxygen Species (ROS) in *Candida* species. Reactive oxygen species (ROS) are the aerobic by-product in both prokaryotes and eukaryotes. The production of ROS is an unavoidable consequence of aerobic metabolism and the building up of ROS causes damage to DNA, RNA and protein. Also, generation of ROS plays a vital role in altering virulence processes of the cell. Similarly, a plant based bioactive which is a saturated fatty acid by nature, induced the intracellular ROS in *C. tropicalis* with increasing concentrations at different time points. Furthermore, the present study unveiled that the ability of bioactive at various concentrations induced apoptosis mediated regulation of prominent virulence factors such as biofilm formation, cell surface hydrophobicity, sterol synthesis and phospholipase in *C. tropicalis*.

**Keywords:** *Candida tropicalis*; Bioactive; Reactive Oxygen Species; Apoptosis; Virulence

## Inhibition of aquaculture pathogen *Vibrio* sp. by signal peptides of probiotic bacteria *Lysinibacillus phaericus*: A bioinformatics insight

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### Abstract

Vibriosis, one of the major diseases caused by *Vibrio* species, becomes a major threat in aquaculture. *Lysinibacillus phaericus* is one of the established probiotic bacteria which shows pattern of inhibition towards different species of *Vibrio*. A molecular docking approach has been made to understand the mode of action of *L. sphaericus* signal peptides to inhibit the growth of *Vibrio* species. 31 signal peptides were studied among which 20 were found to interact with 103 common transmembrane proteins of three *Vibrio* species- *V. harveyi*, *V. vulnificus* and *V. parahaemolyticus*. Most of the signal peptides of *L. sphaericus* showed higher affinity to two protein diacylglycerol kinase (DAGK) and phosphoenol pyruvate dependent sugar phosphotransferase system (Sugar PTS). Inhibition of DAGK leads to accumulation of membrane disrupting component diacylglycerol which sequentially destabilize the lipid bilayer. On the other hand, inhibition of PTS system leads to cessation of fructose uptake within the cell followed by energy depletion and death of the cell.

**Keywords:** Vibriosis; *Lysinibacillus phaericus*; Molecular docking

## Study of gut metabolites during anti-tubercular therapy

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### Abstract

The metabolic products produced by the gut microbes are known to regulate host metabolism. Most of the microbes that are present in the human gut reside in a symbiotic association with the host. It is well established that many diseases and long-term usage of antibiotics are associated with a state of microbial dysbiosis in host. The change in microbial community exerts its influence on milieu by changing the delicate balance of metabolite pool. Microbe-derived metabolites are produced by degradation of various plant-derived phytochemicals. These gut metabolites exert their influence on various physiological processes such as energy metabolism and host immunity and thus are likely to influence microbial

pathogenesis. Tuberculosis (TB), one of the most deadly diseases caused by *Mycobacterium tuberculosis*, is also associated with microbial dysbiosis and the treatment of TB requires a long anti-tubercular treatment (ATT) regimen. Therefore, the present study aims to understand the modulation of gut microenvironment by the disease and treatment process by studying the metabolite pool associated with the patients. We studied a cohort of TB patients (n=20) and healthy unrelated individuals (n=20) through untargeted metabolomics approach to reveal metabolic signatures before and during ATT. Fecal samples collected from patients in nodal TB centers, Delhi region were processed for metabolite identification using <sup>1</sup>H-NMR. The findings would elucidate the difference in abundance of different short-chain fatty acids, amino acids and corresponding catabolites in TB patients and healthy individuals. Such findings can potentially go a long way in development of nutritional and personalized therapies for restoration of alteration and subsequent relief.

**Keywords:** Gut microbes; Metabolites; Tuberculosis (TB); Anti-tubercular treatment (ATT); <sup>1</sup>H-NMR

GPM-10

### Investigation of *Caenorhabditiselegans* phosphoproteome unveils the involvement of a molecular chaperon, HSP-90 during bacterial infection

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#### Abstract

Insights into the protein players and their corresponding Post Translational Modifications (PTMs) provide the basic knowledge to understand host-pathogen interactions. By being a key PTM, protein phosphorylation mediates various biological processes and the defense mechanisms that control host defense mechanism against bacterial pathogen attack. In this context, the present study was intended to determine the role of immune regulatory proteins and their corresponding PTMs with special reference to phosphorylation during a pathogen exposure, using *C. elegans* as a model host and *S. Typhi* as an interacting pathogen. The analysis of regulation patterns of the candidate immune regulatory kinases such as PMK-1, SGK-1, and JNK-1 through immunoblotting analysis revealed an up-regulation of kinases kinetically at 48 h of *S. Typhi* exposure. Phosphoproteome profiling of *S. Typhi* exposed *C. elegans* with the utility of TiO<sub>2</sub> Column Chromatography followed by MALDI-ToF-ToF-MS, gel-free and gel-based analyses resulted in the identification of 166 and 54 proteins, respectively. In particular, HSP-90 was found to be a central player from the interactome analysis among the common proteins and its role during pathogenic exposure was subsequently validated using immunoblotting. Furthermore, the common proteins found between both analyses were extensively studied for their intrinsic protein disordered regions *in silico*. From the comparative analysis, the present study indicated that *S. Typhi* interferes with the protein homeostasis of chaperone molecules by kinetically interfering with the phosphorylation event of downstream pathway players of MAPK and JNK pathways. Our data on the regulatory phosphoproteome during host-pathogen interaction will pave the way for understanding the dynamics of PTMs on regulatory proteins for developing future therapeutic molecules against bacterial infections.

**Keywords:** Post Translational Modifications (PTMs); Immunoblotting; Phosphoproteome

## Analysis of post translational modifications during bacterial exposures in the eukaryotic model system, *Caenorhabditis elegans*

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### Abstract

Studying the Host pathogen interaction in a definite model organism is being employed by the researchers for the past few decades. By using the OMICS technologies, the underlying mechanisms of the pathogenesis can be studied very well during host pathogen interactions. Most importantly, functional proteins of a living system play a crucial role in all types of cellular mechanisms (such as defense mechanism, cell-signalling, homeostasis, etc.). Undeniably, the translated proteins will be activated or inactivated soon after undergone for a biological phenomenon called post translational modification (PTM). Hence, studying the importance of PTMs will give a clear view on pathogen mediated immune response in the host system. Previously, host pathogen interaction studies have been reported that *C. elegans* can be used as a better model system to study the bacterial pathogenesis. Our research group is currently focusing on the impact of various PTMs such as phosphorylation, glycosylation, acetylation, etc., against various bacterial exposures. By studying the impact on *C. elegans* phosphoproteome using TiO<sub>2</sub> column chromatography followed by MALDI-ToF-ToF analyses, we identified the involvement of molecular chaperone, HSP-90 and ubiquitin mediated proteolysis pathway during *S. typhi* exposure. Meanwhile, the analysis of O-GlcNAcylation revealed that it confers protection against *S. aureus* through ubiquitination. Subsequently, the analysis of acetylated proteins and Sumoylated proteins are also currently on progress to understand their involvement during bacterial pathogenesis. The identification and characterization of the molecular mechanism and the responsible PTMs during host pathogen interactions is a challenging task which will pave the way for future therapeutic applications.

**Keywords:** 2DGE; Glycoproteins; Phosphoproteins; MALDI-ToF-MS; STRING analysis; Bioinformatics

GPM-13

## To explore the expression analysis of nectin family genes in human glioblastoma

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### Abstract

Gliomas are the most prevalent primary tumors of the brain and spinal cord. Histologically, they share characteristics of normal glial cells. Despite advances in therapeutics, the molecular and cellular mechanisms of nectins that initiate and establish human brain cancer have not been completely elucidated. The Nectin family belongs to Ca<sup>2+</sup>-independent immunoglobulin-like molecules consisting of four members (Nectin-1, Nectin-2, Nectin-3, and Nectin-4) having an important role in cell-cell adhesion. Nectins 1, 2, and 3 are widely expressed in adult tissues, but Nectin-4 expressed specifically in the embryo and placenta. Accumulating evidences indicated that Nectins have been originally investigated in cell adhesion, and play critical roles in various physiological conditions including tissue development. A few reports have shown that Nectin-4 expression contributes to the postoperative prognosis of patients with malignant tumors. Multiple studies suggested that Nectin-4 may play functionally important roles in tumor progression and metastasis independently of the TNM classification but not in human glioma. The aim of the present study was to analyze the expression of Nectin-4 in Human Glioblastoma cell

Lines (U373MG, LN 18, LN 229 U87MG) using RT-PCR. In particular, till date there are no published reports which showed association of nectin4 expression in human glioma. The present study revealed the presence of Nectin-4 in U373MG Human Glioblastoma cell line which showed connecting linkage between Nectin-4 and glioma. The mRNA expression analysis showed absence of nectin4 mRNA expression in all human glioma cell lines U87MG, LN18, and LN229 except U373MG. Furthermore, normal human brain also showed no mRNA expression of nectin4 gene in comparison to these cell lines. This is the first report which showed nectin4 mRNA expression in U373MG human glioblastoma cell line. It could be concluded in line with the present findings that Nectin-4 might be playing an important role in the tumorigenesis in human glioblastoma.

**Keywords:** Glioma; Brain tumor; Nestin; RT-PCR; Cell line

GPM-14

### Immunohistochemical profiling of cancer stem cells in human glioma

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#### Abstract

Glioma are the most common primary tumors of CNS and represent an important cause of cancer related morbidity and mortality worldwide. Gliomas are composed of a heterogeneous mixture of cells, which includes cancer stem cells (CSC) that contribute to tumor initiation and therapeutic resistance. The present study was designed to study the clinical significance of various cancer stem cell markers in different grades of astrocytoma such as Pilocytic astrocytoma (PA; WHO Grade I; N=10), Diffuse astrocytoma (DA; WHO grade II; N=13), Anaplastic astrocytoma (AA; WHO grade III; N=9), Mixed grade astrocytoma (MGA; WHO III/IV; N=3) and Glioblastoma (GBM; WHO IV; N=21). A total of fifty six cases of different grades of astrocytoma were analyzed to study the expression of CD133, CD44, BMI-1, SOX2, Nestin, Musashi-1 and CD90 by immunohistochemistry. Their expression was correlated with histological subtypes and their survival status. The cytoplasmic immunoreactivity of CD133 in PA, DA, AA, MGA and GBM cases were 20%, 23%, 33% 67% and 33%, respectively. All cases of GBM showed strong cytoplasmic positivity for Nestin protein. All cases of PA, DA, AA, MGA and GBM showed strong nuclear positivity for Sox-2 antigen. The frequency and intensity of strong nuclear immunopositivity of BMI-I were observed in AA. All cases of DA and GBM showed similar nuclear medium immunoreactivity for BMI-I protein. MGA showed no nuclear positivity for BMI-I. All cases of PA and MGA showed no cytoplasmic positivity for CD90 antigen. Mortality was highest among the MGA (67%) followed by GBM (48%), DA (15%), AA (11%) and PA (10%), respectively. All cases of PA and MGA showed no cytoplasmic immunopositivity for CD90. A weak cytoplasmic positivity was observed with cases of GBM (19%), AA (22%) and DA (54%), respectively. There was a significant positive correlation between different histological grades and cytoplasmic immunopositivity of Nestin, Musashi-I and BMI-1, respectively ( $P < 0.05$ ). Among 56 cases, 16 cases (28%) showed mortality which was highest among GBM cases followed by AA, MGA, DA and PA, respectively. This study gave an insight into role of cancer stem cells as potential diagnostic and prognostic tool in differentiating different grades of astrocytomas.

**Keywords:** Cancer stem cells; Glioma; Astrocytoma; Immunohistochemistry

## Defining denitrifying capabilities of *Citrobacter* sp. AAK\_S5 using draft genome analysis

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### Abstract

In this study, a high-quality draft genome sequence of *Citrobacter* sp. AAK\_S5 was explored to predict the nitrogen removal potential of the isolate. Whole-genome sequencing showed a genome size of 5.93 Mb, and annotation suggested a total of 6,826 protein-coded genes. Phylogenetic analysis of the 16S rRNA gene showed a close relationship to *C. freundii* complex sp. CFNIH9, and *C. portucalensis* strain P10159 (homology of 99.87%). The genome sequence was mined for the presence of different genes involved in the removal of nitrogen. Ammonia removal was attributed to assimilation catalyzed by the action of enzymes glutamate ammonia ligase adenylyl transferase and [protein II] uridylyl transferase which encoded by genes *glnE*, and *glnD* which were annotated on contigs2, and 14 respectively. On the other hand, four gene clusters associated with denitrification such as for respiratory nitrate reductase (*narG*, *narH*, *narI*, *narJ*), nitrite reductase (*nirK*), nitric oxide reductase (*norQ*, *norB*, *norC*), and nitrous oxide reductase (*nosZ*) were annotated on the contigs 3, 1396, 3506, and 2576, respectively. These enzymes mediated the sequential reduction of nitrate to nitrogen gas ( $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ ). The analysis of the draft genome was validated by bench-scale experiments for removal of various nitrogenous compounds ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and  $\text{NO}_2^-$ ) from synthetic wastewater. The isolate demonstrated high capacities for removal of soluble organic carbon as indicated by 81% sCOD removal, in addition to 90-95% ammonical nitrogen ( $\text{NH}_3\text{-N}$ ), and nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) removal. The availability of the draft genome sequence of *Citrobacter* sp. may provide insights into the genomic basis of nitrogen removal in denitrifying bacteria with potential application in wastewater treatment systems treating nitrogen-rich wastewater.

**Keywords:** *Citrobacter* sp.; Denitrification; Ammonia assimilation; Draft genome; Gene annotation

## Purification and structure elucidation of bioactive compounds from a potent soil derived actinobacterium

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### Abstract

Emergence of drug resistant pathogens in recent years has resulted in alarming decline in therapeutic options against these deadly microbes. During earlier studies from our lab, actinomycete designated as



colony RI.24 was isolated, characterized using 16S rRNA gene sequencing and screened for bioactive compounds production. Colony RI.24 was detected as strain of *Streptomyces enissocaesilis* with 100% 16S rRNA gene sequence identity. In present study, characterization of bioactive compounds produced by strain RI.24 was undertaken. Cold extraction technique was employed for extraction of bioactive molecules. Minimum inhibitory concentration (MIC) analysis of extracted compounds was performed using micro-dilution method where commercial antibiotic ampicillin was taken as standard. MIC results indicated activity of strain RI.24 in the range of ampicillin (MIC of strain RI.24 0.25mg/mL; MIC of ampicillin 0.025mg/mL). Structure elucidation of extracted compounds was performed by employing LC-MS/MS and <sup>1</sup>H NMR techniques; as a result bioactive compounds daunorubicin, hygromycin B, agecorynin F, indinavir-N-glucuronide and minocycline were identified. On the basis of literature reports and best to our knowledge present investigation reports the above mentioned bioactive molecules for the first time from strain of *Streptomyces enissocaesilis*. In this investigation, PCR detection of non-ribosomal peptide synthetase (NRPS) and polyketide synthase-I (PKS-I) genes was also performed; which resulted in identification of NRPS gene cluster and absence of PKS-I. It may be suggested that detected NRPS gene is involved in the biosynthesis of identified bioactive compounds. Findings of current investigation can be used by the pharmaceutical industries/scientific society for developing new therapeutic options or formulations.

**Keywords:** Actinobacterium; *Streptomyces* sp.; Bioactive compounds; LC-MS/MS and NMR; PKS-I; NRPS

GPM-17

### Functional analysis, protein structure prediction and molecular docking of Chitinases produced from *Streptomyces* sp. strain 130

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#### Abstract

Extracellular enzymes produced from *Streptomyces* are contributing well in various industries. Syncing of traditional microbiological approach with molecular biology helped to improve the quality of products used in industries. *Streptomyces* secretes stable and effective extracellular enzymes as compare to other sources like fungi and other bacteria. But important challenge was to level up the quantity of product to the industrial scale resolved by the cloning of enzyme producing genes into specialized protein expressing system. In our current study, we have screened *Streptomyces* sp. strain 130 for family 18 and 19 chitinase production using SC1F; SC2R and F19F2; F19R sets of primers respectively. Whole genome sequencing of *Streptomyces* sp. strain 130 has been used to identify the characterized and uncharacterized genes producing chitinases. Blastn and "NCBI-conserved domain search tool" were used to find similarity percentage with genes in existing database and enzyme family respectively. Prediction of protein structure was done using In-silico tools, which helped in functional analysis of each enzyme. Molecular docking was done to study the enzyme to ligand interaction, which revealed the binding efficiency of ligand to enzyme. In our future work, we will clone the efficient genes in compatible expression system. Cloning of these genes may help to analyse the activity and stability of individual enzyme. This study may help us to characterize the effective enzymes along with their applications. Use of these enzymes may give an option to replace the toxic and life threatening chemical compounds being used in various industries.

**Keywords:** *Streptomyces*; Extracellular enzymes; Protein structure prediction; Molecular docking

## Deciphering the Microbiomes of Brackish Grown Wildrice Varieties – Pokkali and Kagga from Southern India

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### Abstract

Our current understanding about the plant associated rhizobacteria and their beneficial functions were largely based on studies from terrestrial crop plants. However, little is known from crop plants growing naturally in coastal saline stress soils as in this case wild local saline tolerant rice varieties of southern India remains largely elusive. In this context, Illumina MiSeq platform based 16S rRNA gene based amplicon sequencing targeting the V3-V4 region was performed. The processing of the raw data and downstream analysis were carried out using NGSqc toolkit (Quality check) and QIIME pipeline. The 16S rRNA amplicon sequencing generated 68,90,497 number of high quality reads that were merged into 28,61,801 number of contigs. The alpha rarefaction curve suggests that sufficient sequencing depth was attained to capture the actual microbial diversity within these samples. Further, alpha diversity and shannon index value is higher for the rhizosphere whereas the evenness is higher for the root samples. The Principal coordinate analysis (PCoA) plot representing beta diversity of the samples clustered together for replicates suggesting that there is no much variation between the replicate samples. Initial metagenome analysis revealed that Proteobacteria, Bacteroidetes and Planctomycetes are the most dominant phylum in both pokkali and kagga rice varieties. In addition, the taxa's enriched in the root of Kagga include Firmicutes and Actinobacteria whereas Nitrospirae, Chloroflexi and Acidobacteria were found to be depleted in the root compared to rhizosphere. Similarly in pokkali sample, Verrucomicrobia and Actinobacteria were enriched taxa's where as Chlorobi and Nitrospiraere main depleted in root. As a starting point, studying the diversity of plant microbiota of this ecosystem represents a key step towards improving our basic understanding about the ecology of plant associated microbes in brackish environments. Furthermore, capitalizing these brackish adapted microbes as bio-inoculants may lead for improvised sustainable agriculture applications in coastal saline affected regions.

**Keywords:** Pokkali; Kagga; Metagenome; QIIME; Microbiome

## Establishing Plant Biomass Hydrolyzing machinery of *Klebsiella michiganensis* NDC\_17 for its potential application in Biofuels production

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### Abstract

Plant Biomass hydrolyzing *Klebsiella michiganensis* NDC\_17 was isolated from camel rumen by screening more than 6000 strains for their ability to hydrolyze various cellulosic and hemicellulosic biomasses. The selection process involved three layers of screening where plate-zymography has been used for primary screening and potential isolates were genetically grouped by using RAPD. The selected representative isolates from each group were subjected to substrate specific hydrolysis followed by identification through 16S rDNA sequencing. The selected strain has shown Endoglucanase, xylosidase, galactosidase, arabinosidase and glucuronidase activity as 0.41 U/ml, 0.4U/ml, 0.03 U/ml, 0.02 U/ml and 0.02 U/ml

respectively. The hydrolysis potential of this strain was mapped using genome sequencing; and different CAZymes were annotated using online platform tool dbCAN. The genome analysis revealed additional lytic polysaccharide monoxygenase (LPMO) activity. In recent studies LPMO has been directly related to increased activity of cellulase and hemicellulase thus performing better saccharification of plant biomass and providing more reducing sugar. This has been demonstrated and further supported through saccharification various of agriculture residues.

**Keywords:** Agriculture waste; Rumen; Genome sequencing; LPMO; Saccharification

GPM-21

### Understanding antibiofilm activity of *Lactobacillus rhamnosus* on *Streptococcus mutans*

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#### Abstract

*Streptococcus mutans* is facultative anaerobic coccus commonly found in the human oral cavity being highly efficient at forming biofilms contributing significantly to cariogenesis. Biofilms are complex microbial communities enclosed in a matrix providing resistance to microbes against antimicrobial agents. Previous studies have suggested use of lactobacillus and bifidobacteria as probiotics preventing the biofilm formation by *S. mutans*. Apart from this there is little understanding of mechanisms involved. Quorum sensing, cyclic diguanosine-5'-monophosphate, and small RNAs are the main regulators of every step of biofilm development and dispersal. The objective of this study is to evaluate the inhibitory effect of antimicrobial compounds produced by *Lactobacillus rhamnosus* on *S. mutans* biofilm formation. For this, *S. mutans* was screened by coculturing with lactobacillus species and static biofilm of *S. mutans* was seeded with *L. rhamnosus*. Biofilm quantification was done using crystal violet quantification method. Our results show inhibitory effect with *L. rhamnosus* cells when co cultured with *S. mutans*. Use of cell free supernatant of lactobacillus on *S. mutans* showed similar inhibitory effect suggesting that cell to cell contact is not the prerequisite for inhibition. Since lactobacillus species are known to produce acids which lower the pH of the media and it could be possible that biofilm inhibition is caused by pH change, to investigate this we used pH adjusted conditional medium which also recapitulated the phenotype suggesting that pH was not mediating this effect. We performed phase separation of cell free supernatant and seeded biofilm, where organic phase showed increased antibiofilm activity in comparison to aqueous phase. Our data indicates that lactobacillus species is probably producing a small organic molecule which is responsible for inhibiting *S. mutans* biofilm. To understand which genes are involved in biofilm formation and which are affected by lactobacillus, qPCR was used with lactobacillus treated and untreated *S. mutans*.

**Keywords:** *Streptococcus mutans*; Biofilm inhibition; *Lactobacillus rhamnosus*; Antibiofilm; qPCR

### 3D Structure modelling and comparative study of superoxide dismutase in *Exiguobacterium* species

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#### Abstract

Superoxide dismutase (SOD), considered as a potent antioxidant which catalyse the dismutation of superoxide radical into either ordinary molecular oxygen or hydrogen peroxide. It is known to exert its presence across bacteria to humans. Despite its well-known antioxidant property, its association with various extremophiles in response to various stress conditions is poorly understood. These extremophiles have tendency to tolerate the adverse environmental conditions like temperature, extreme levels of gamma radiation, salt-stress, acidic or alkaline conditions. To gain a more comprehensive understanding about the role of SODs in various stress-responses, we selected *Exiguobacterium* sp., (tolerant towards high doses of gamma radiation, high salt stress conditions and has the inherent ability to grow within a temperature range of -2.5 to 40 C), possesses a free radical scavenging activity. However, unlike most extremophilic microorganisms, biology of *Exiguobacterium* sp. and its adaptability to survive in such extreme environmental conditions is poorly understood. Accumulating evidences from different molecular studies and unusual behaviour of *Exiguobacterium*, prompted us to further study the role of SOD in rescuing such type of stress conditions and their conservation among various extremophiles. The conservation and the prevalence of SODs among 21 representative extremophiles was carried out and a consensus motif was obtained. Further, computational prediction was carried out for the correlation of SOD with the various stress conditions encountered by these extremophiles followed by phylogenetic study. Further, 3-D model structure of SOD for *E. sibiricum* was predicted and validated through various corroboration metrics, including Ramachandran plot, Z-score and normalized qualitative model energy analysis score. Additionally, various physicochemical properties of *E. sibiricum* SOD were calculated using different prominent resources. It is tempting to speculate that SOD might play an important role in rescuing stress conditions thus enabling *E. sibiricum* to grow at high doses of gamma radiation, salt-stress and at a wide range of temperature.

**Keyword:** *Extremophiles*; Superoxide dismutase; *Exiguobacterium* sp.; Homology modelling

GPM-24

### Identification of leukemic genes in patients with Down syndrome using Next Generation Sequencing

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#### Abstract

Down syndrome (DS) is due to an abnormality of chromosome 21 which causes trisomy and is mainly associated with intellectual disability and developmental delay. Apart from these complications, the

most common abnormality in DS is leukemia, which is seen 1 in 800 children with DS. Reported studies suggest that DS children are prone to develop hematological malignancies than the non-DS children, due to the presence of distinct biological entities in DS. We studied the molecular pattern of two DS patients associated with leukemia using Next Generation Sequencing (NGS). To identify the potential leukemic genes in these patients, we extracted genomic DNA from the whole blood and subjected for the NGS. Our study identified defects in potential genes including *KCNE4*, *FLT3*, *IKZF1* and *TP53*. These four genes are known to cause hematological malignancy by having various role of leukemogenesis. For example, the gene *KCNE4* is known to involve in childhood acute lymphoblastic leukemia, *FLT3* is known to involve in the molecular pathology of acute myeloid leukemia, *IKZF1* involves in both childhood and adult B-Cell precursor acute lymphoblastic leukemia and *TP53* is the risk factor for acute myeloid leukemia. Apart from these, there were other mutations identified in this study have been excluded from this report since they are not related to the objective of the current study. For example, mutations in *AUST2*, which is a key gene for the developmental delay, has not been highlighted here. Our current report suggests that molecular defects are associated with the occurrence of leukemia in DS and identification of potential leukemic genes in other DS patients will help in the better management of the disease.

Gene	Reference ID	Defect	DS-L1	DS-L2
KCNE4	rs12621643	Asp196Glu	Heterozygous	Homozygous
FLT3	rs12872889	Asp7Gly	Homozygous	Heterozygous
IKZF1	rs10899750	Arg164Gly	Heterozygous	Heterozygous
TP53	rs1042522	Pro72Arg	Homozygous	Homozygous

**Keywords:** Down syndrome; Leukaemia; Molecular defects; Childhood; Next Generation Sequencing

GPM-25

### An Evolutionary Study of TP53 Gene Paralog of Elephant

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#### Abstract

Tumour protein p53 also known as TP53 or transformation-related protein TRP53 is any isoform of a protein encoded by homologous genes in various organisms such as TP53 (humans) and TRP53 (mice). The homolog is crucial in multicellular organisms, where it prevents cancer formation, thus functions as tumour suppressor. As such p53 has been described as “the guardian of genome” because of its role in conserving stability by preventing genome mutation. The TP53 gene is the most frequently mutated gene (>50%) in human cancer, indicating that the TP53 gene plays a crucial role in preventing cancer formation. The TP53 gene encodes proteins that bind to DNA and regulate gene expression to prevent mutations of the genome. If the TP53 is damaged, tumor suppression is severely compromised. People who inherit only one functional copy of the TP53 gene will most likely develop tumors in early adulthood, a disorder known as Li-Fraumeni syndrome. The TP53 gene can also be modified by mutagens (chemical, radiation or viruses) increasing the likelihood for uncontrolled cell division. More than 50% of human tumors contain a mutation or deletion of the TP53 gene. Loss of p53 creates genomic instability that most often results in an aneuploidy phenotype. Increasing the amount of p53 may seem a solution for treatment of tumors or prevention of their spreading. This, however is not a usable method of treatment, since it can cause premature aging. Restoring endogenous normal p53 function holds some promise. Research has shown that this restoration can lead to regression of certain cancer cells without damaging other cells in the process. The ways by which tumor regression occurs depends mainly on the tumor

type. For example, restoration of endogenous p53 function in lymphomas may induce apoptosis, while cell growth may be reduced to normal levels. So, in order to find the correlation between the homologs of oncogenes, phylogenetic has become a major criteria to explain evolutionary studies as well as their mutation, duplication and diversification. Phylogenetic study also reveals whether the homologs are following Darwin's purifying selection. Here the TP53 gene paralogs are retrieved from ENSEMBL Genome browser and aligned by MAFFT Algorithm where the gaps are removed in GUIDANCE server. Further the aligned structures are proceed to generate phylogenetic tree using Bootstrap method to study the ancestors and then codon-based Z-test is performed using MEGA software. The observations reveals that TP53 gene paralogs of elephant show biasness to purifying selection which leds elephants resistant to cancer.

**Keywords:** Li-Fraumeni Syndrome; Phylogenetics; Bootstrap; Codon-based Z-Test; Purifying Selection

GPM-26

### **Bioprospecting unique niches by metagenomic Approach**

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#### **Abstract**

Metagenomics is a field in genomics used to directly recover the genetic material of microbial communities from environmental samples. It is a valuable tool used to harness the potential of uncultured microorganisms by directly dealing with the genomes of entire communities. Metagenomics has emerged as a powerful tool over the last few decades to study microbial community structure and interactions with a goal to understand their community structure , genetic diversity and ecological roles. A large number of new and novel molecules have been discovered through the use of metagenomics with possible application potential. A major part of the earth's surface has conditions which are extreme owing to one or the other environmental factors like high and low temperatures, salinity, pressure , altitude etc. .Extreme niches could prove to be a rich hub of unique and novel microorganisms which could be harnessed for human welfare. Most of the extreme niches like hot springs, glaciers and brackish lakes are a potential source of significant but untapped microbial diversity encoding novel genes related to tolerance towards different stresses. So Bioprospecting such niches using metagenomics could unravel novel genes useful for various applications. Our research group at SMVDU is engaged in exploring the potential of unique niches of J&K by Metagenomic approach. So far we have studied various niches like Brackish lakes, Hot springs and Glacier samples for community analysis and functional metagenomics with an aim to discover the novel genes of industrial importance. The results obtained so far clearly support the rationale of using metagenomics for harnessing the total microbial diversity associated with these niches. Detailed results will be presented as paper presentation in the conference.

**Keywords:** Bioprospecting; Metagenomics; Extreme niches; Microbial Communities

## High temperature stress alters the expression profiling of Starch synthase I gene in bread wheat during grain development

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### Abstract

High temperature stress during grain development negatively affects yield and yield-related traits in wheat worldwide. Starch accounts for 65-75% of final dry weight of wheat grain and is a major determinant of grain yield. In this study, gene expression of soluble starch synthase I (SSSI) gene was studied by quantitative real time PCR analysis at 7 stages (2, 5, 8, 10, 15, 20 and 25 DAA) during grain development. Four genotypes of wheat *i.e.* two thermo-tolerant (WH 730 and WH 1021) and two thermo-sensitive (WH 147 and WH 711) were evaluated under normal and high temperature (HT) stress conditions. Late sowing was done to create HT stress under field conditions. HT stress altered the expression profile of SSSI gene in the developing grains. Gene expression peaked earlier under HT stress conditions (at 15 DAA) than under normal sown conditions (at 20 DAA) in all genotypes studied. At late stages during grain development, SSSI gene expression fell more abruptly and to a higher level in case of thermo-sensitive genotypes. WH 730 showed maximum tolerance to HT stress while WH 147 was least thermo-tolerant. Altered expression of SSSI gene correlates with decline in grain yield and may be an important determinant for yield reduction under HT stress. This study will help to better understand the molecular basis of yield losses under HT stress and to develop varieties with high yield potential under high temperature stress.

**Keywords:** Gene expression; Yield-related traits; Thermo-sensitive genotypes

## Nutrigenomics: A potential tool for right nutrition and healthy life

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### Abstract

During 20<sup>th</sup> century, nutritional science primarily focused to find out vitamins and minerals and utilizing them to prevent the diseases caused by their deficiency. In developed countries, the nutrition related health problems shifted towards over nutrition, obesity and type two diabetes, thus the focus of modern medicine and that of nutritional science altered accordingly. This requires through understanding regarding action of nutrients at the molecular level. This led to shifting of nutrition research from epidemiology and physiology to molecular biology and genetics and thus nutrigenomics came into existence. It is an important area dealing with utilization of high throughput genomic tools for research in the field of nutrition. It explains individual differences in response to nutrition related disorders and thus depicts effect of food and food constituents on gene expression and thus understands the relationship between nutrition and health. A large number of genetic variations have given a way to enhance the susceptibility to diseases related to diet like Type 2 diabetes mellitus, obesity, some autoimmune diseases, cardiovascular diseases and cancer. Various studies have been reported on the influence of genetic variations on the

relationship of diet and health with implications to vulnerable subgroups and the influence of nutrients on health through changing genome, proteome, metabolome and physiology. Nutrients are dietary signals which influence homeostasis. These are detected by cellular sensor systems which have influence on gene, protein expression and metabolite production. Thus, this novel genome-food interface has remarkable potential implications for advancement of science in future. The understanding of diet and genes interaction may make possible to better manage health and probably delay onset of chronic and age-related diseases. Scientific understanding of nutrigenomics has made it possible to increase commercial development of nutraceuticals and functional foods. This has helped to alter negative health effects of genetic profile of an individual. The food industry has opportunities for improving health by modifying the methods of processing and production as well as by taking advantage of encouraging more positive trends. Thus, nutrigenomics research can be utilized to capitalize on people's health and benefiting food industries using genetic information. In case, precise nutrient regulation of genes distantly related to disease commencement or development is known, new arenas for disease avoidance and potential for treatment may be in forefront in areas of nutritional research and preventive medicine.

**Keywords:** Nutrigenomics; Metabolome; Nutraceuticals; Nutrient; Genetic variations; Genome-food interface

GPM-29

### Thymol Induced Apoptosis mediated through Macromolecular Damage in A549 cells

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#### Abstract

Non-small cell lung cancer accounts for ~ 85% of global lung cancer cases. Current cancer therapies like radiation, surgery and chemotherapy lack specificity and hence lead to side effects. Plant secondary metabolites have proven their tremendous therapeutic potential without any side effects. In this aspect, thymol a monoterpene phenol extracted from *Thymus* species plants was reported to have various pharmacological properties such as anti-inflammatory, antibacterial, antifungal, antiseptic and antitumor properties while its anti-lung carcinoma activity has not been studied. In the present research we reveal the anti-cancer property of thymol against A549 cells through biochemical approaches like MTT, DNA fragmentation, lipid peroxidation, protein carbonylation, Caspase3 assay and proteomics studies like Western blotting. MTT assay indicated that 112µg/ml of thymol was sufficient to induce considerable cell death in 12 and 24h thymol treated cells when compared to the control. The mode of cell death in A549 was found to be through apoptosis where chromatin condensation was clearly visualized in DAPI stained thymol treated A549 cells. Similarly DNA fragmentation pattern in thymol treated A549 cells pointed towards the apoptotic mode of cell death. Thymol was found to significantly enhance ROS production in A549 cells followed by lipid peroxidation, protein carbonylation and activation of Caspase3 which was also evidenced by wide spread cell membrane damage through Ethidium Bromide staining in thymol treated cells than the control cells. To summarize thymol provoked A549 cells to undergo apoptosis through increased ROS level in concordance with membrane, lipid and protein damage. DNA fragmentation and chromatin condensation were other apoptotic indicators accompanied by highly active Caspase3 which is a well known apoptotic mediator. Overall the cytotoxicity of thymol was found to be due to its apoptotic effect on A549 non-small cell lung cancer cells which can be further extended to *in vivo* level.

**Keywords:** Thymol; Lung cancer; A549 cells; DNA fragmentation; ROS



# **INDUSTRIAL MICROBIOLOGY AND MICROBIAL TECHNOLOGY**



# ORAL PRESENTATION



## Combining microbial and chemical approaches for development of novel enhanced oil recovery method

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### Abstract

Water breakthrough, is a common problem faced during secondary recovery process in the oil fields. Oil production, by tertiary recovery processes are therefore increasing over the years as recovery of crude from marginal wells is becoming increasingly important. Hence, new processes are promoted for extraction of crude oil from these challenging reservoirs and harsh environments. Different chemical enhanced oil recovery processes are widely applied nowadays such as polymer flooding to solve the problem of water channelling during secondary flooding. There are a number of polymers and copolymers that have been developed and used in field studies for improving oil recovery, but are sensitive to changes in the reservoir environment. Therefore in the present study changes in rheological properties of different polymer solutions and the potential of using natural polymers for altering wettability of reservoir rocks were evaluated under reservoir conditions. The intrinsic viscosity values of all the samples, except for a novel naturally derived polymer (~21 gm/dl at 65°C) showed significant reduction in their viscosities when subjected under reservoir conditions. This trend was similar to the reduction in molecular weight of different polymers at high temperatures and salinity. The properties of commercially used polymers such as xanthan gum and polyacrylamide also showed less stability compared to novel natural polymer. Contact angle measurements were performed on oil wet reservoir rocks (both carbonate and sandstone) treated with different polymers to evaluate wettability alteration behaviour. The change in the contact angle was maximally noted in the case of novel natural polymer which was found to be 81° near to that of Triton-X-100, a surfactant (79°) taken as control. Qualitative wettability studies (floatation based and two phase separation tests) had also shown similar tendency of novel natural polymer solutions to alter wettability of oil wet surfaces towards water wet. FTIR of the oil wet powdered rock samples treated with polymer solutions were performed and the absorption spectra revealed reduction in peaks of methyl and methylene indicating altering wettability for both carbonate and sandstone rock samples. This changes indicates a stable nature of novel natural polymer, making it a more suitable to be used as a biopolymer for EOR polymer.

**Keywords:** Oil Fields; Enhanced oil recovery; Polymer flooding

OP/IMMT-27

## Development and expression of vectors in the riboflavin overproducer *Eremothecium ashbyii*- RAD 14 like and SPR 3 like genes

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### Abstract

*Eremothecium ashbyii* is a filamentous hemi ascomycete fungus, a natural overproducer of riboflavin and thus an industrially important organism. Since it is important from an industrial point of view, an understanding of the biochemical as well as genetic reasons behind riboflavin overproduction is of interest both from the point of view of fundamental as well as applied research. Keeping this in view molecular tools for genetic manipulation of this organism were constructed using plasmids constructed for the related organisms- *Ashbyagossypii* and *Saccharomyces cerevisiae*. The present study was undertaken with an aim to characterize two candidate genes of *Eremothecium ashbyii*. These are the *S. cerevisiae* *SPR3* gene homologue which is known to play a role in cytokinesis in *S. cerevisiae*. The second candidate gene was the *S. cerevisiae* *RAD 14* homologue which is known to play a role in the Nucleotide Excision Repair pathway in *S. cerevisiae*. The cytokinesis process in yeast is analogous to the process of septation in filamentous fungi. Hence this gene was chosen with a view to aiding in future studies on the regulation of septation and its role riboflavin secretion in *E. ashbyii*. The NER pathway maybe implicated in combating riboflavin toxicity in *E. ashbyii*. Hence this gene was chosen with a view to aiding in future studies. Reporter plasmids constructed in an earlier study were used. These harboured the *E.a* *SPR3* like gene fused to the *LacZ* reporter gene and the *E.a* *RAD 14* like gene fused to the *LacZ* reporter gene. These plasmids were characterized by sequencing followed by homology searches. While the former revealed homology to a family of *S. cerevisiae* zinc finger proteins, *S. cerevisiae* *SPR 3* gene and *Neurospora crassa* *CDC 12* gene involved in cell cycle regulation, the latter showed homology to the *S. cerevisiae* *HOG I* gene which plays a role in the osmotic stress response.

**Keywords:** *Eremothecium ashbyii*; *Ashbyagossypii*; Riboflavin toxicity, Nucleotide Excision Repair

OP/IMMT-28

### Hierarchical macro-porous particles for efficient whole-cell immobilization: Application in bioconversion of greenhouse gases to methanol

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### Abstract

A viable approach for methanol production under ambient physiological conditions is to use greenhouse gases, methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) as feed for immobilized methanotrophs. In the present study, unique macro-porous carbon particles with pore sizes in the range of ~ 1–6 μm were synthesized and used as support for the immobilization of *Methylo cellatundrae*. Immobilization was accomplished covalently on hierarchical macro-porous carbon particles. Maximal cell loading of covalently immobilized *M. tundrae* was 205 mg of dry cell mass (DCM) per g of particles. Among these particles, the cells immobilized on 3.6 μm pore size particles showed the highest reusability with the least leaching and were chosen for further study. After immobilization, *M. tundrae* showed up to 2.4-fold higher methanol production stability at various pH and temperature values because of higher stability and metabolic activity than free cells. After eight cycles of reuse, the immobilized cells retained 18.1-fold higher relative production stability compared to free cells. Free and immobilized cells exhibited cumulative methanol production of 5.2 and 9.5 μmol mg-DCM<sup>-1</sup> under repeated batch conditions using simulated biogas [CH<sub>4</sub> and CO<sub>2</sub>, 4:1 (v v<sup>-1</sup>)] as feed, respectively. The appropriate pore size of macro-porous particles favors the efficient *M. tundrae* immobilization in order to retain better biocatalytic properties. This is the first report concerning the covalent immobilization of methanotrophs on the newly synthesized macro-porous carbon particles and its subsequent application in repeated methanol production using simulated biogas as a feed.

**Keywords:** Greenhouse gas; Immobilization; Macro-porous particles; Methanol; *Methylocella tundrae*

## Potential of newly isolated thermotolerant *Kluyveromyces marxianus* JKSGH-6 in simultaneous saccharification and fermentation of cotton stalk for bioethanol production

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### Abstract

Lignocellulosic biomass (LCB) is a renewable and environmentally friendly feedstock for sustainable production of biofuels and other valuable chemicals. However, its bioconversion faces economy related challenges mainly due to biomass recalcitrance and lower enzymatic conversion during separate hydrolysis and fermentation (SHF) process. Therefore, simultaneous saccharification and fermentation (SSF) is often employed for enhancing the efficiency of bioethanol production by application of thermotolerant ethanologenic yeast. In the present study, simultaneous saccharification and fermentation of pretreated cotton stalk (CS) was attempted for bioethanol production using a newly isolated thermotolerant yeast. Dilute alkali pretreatment (DALP) of milled (40-60 mesh size) CS (10% dry wt.), was carried out at 121 °C for 30 min using NaOH (3%; w/v). Cellulose content of DALP-CS increased from 50% (in native CS) to 73% (w/w) due to lignin removal, which was confirmed by the changes in absorption spectrum during Fourier-transform infrared spectroscopy (FTIR) analysis. The enzymatic hydrolysis of DALP-CS, using commercial enzyme, released 204 mg/g of sugar after 72h. Fifteen thermotolerant yeasts (growing at 42°C) were screened to obtain the best isolate for SSF of DALP-CS. Most potential ethanol fermenting isolate was identified as *Kluyveromyces marxianus* JKSGH-6 by amplification and sequencing of ITS-5.8s rRNA gene. Under SSF of DALP-CS at 42°C the selected yeast produced ethanol titer of 39.4 g/L which showed potential for better ethanol production from lignocellulosic cotton stalk via simultaneous saccharification process.

**Keywords:** Bioethanol; Cotton stalk; Pretreatment; Thermotolerant yeast; Simultaneous saccharification and fermentation

## Bioprospecting halophilic archaea for the production of bioplastic using lignocellulosic biomass

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### Abstract

Polyhydroxyalkanoates (PHA) serve as an important substitute for petroleum-derived plastics because of their close functional analogy and biodegradation quality. Poly-β-hydroxybutyrate (PHB) is the most frequently occurring PHA and constitutes a linear, unbranched homopolymer consisting of (R)-3-hydroxybutyric acid (3HB) units. Among extremophiles, halophilic microorganisms are of much interest because at determined salt concentration, the growth of non-halophiles is prevented, hence allowing the fermentation process without strict sterile conditions and reducing the inherent costs. Hence, halophilic archaea previously isolated from Rann of Kutch, Gujarat, India were screened for PHB production and *Haloarcula* sp. LB14 was selected for further studies based on its ability to accumulate PHB more efficiently. It was used for batch fermentation studies using optimized growth media and physiological parameters. The growth media was comprised of saccharified paddy straw leachate. *Haloarcula* sp. LB14 showed significant growth (158.24 to 328.44 μg mL<sup>-1</sup> protein) on different sugars produced during paddy

straw sand accumulated significantly high amount of PHB ranging from 22.36–39.64 % CDW. The batch process was further unscaled and fermentation parameters were optimized using modified growth media having paddy straw leachate. The optimized parameters for maximum PHB recovery were found to be pH 7.0, air flow rate 1.0 vvm to maintain DO 40%, temperature 37°C and 20% reducing sugar as carbon source. The isolate produced 8.71 gL<sup>-1</sup> CDM (cell dry mass) and 6.24 gL<sup>-1</sup> PHB. The volumetric productivity was found to be 0.0145 g L<sup>-1</sup>h<sup>-1</sup> with 0.71 (w/w) conversion of carbon source. The cost of 1 kg PHB production using optimized media having rice straw leachate was approximately Rs 3383.90. LB14 may only represent a case in point that exemplifies of the potential that hyper saline soils of Gujarat possess for the discovery of novel halophilic archaea able to synthesize PHB.

**Keywords:** Polyhydroxyalkanoates, Poly-β-hydroxybutyrate, Extremophiles, *Haloarculasp*, Straw leachate



# POSTER PRESENTATION



## Optimization studies on polygalacturonase production by *aspergillus* and *pencillium* species isolated from mangrove soils

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### Abstract

In the present study, fifty polygalacturonase producing fungal strains were isolated from mangrove soils of Gilakaladindi and Malakayalanka, Andhra Pradesh. Soil samples were serially diluted and plated on pectin agar media plates for isolation of Fungi. Among the fifty strains, only four i.e two *Aspergillus* and two *Penicillium* species showed maximum polygalacturonase production on pectin agar medium. The identification of fungi was confirmed by after 18S rRNA sequencing analysis. The sequences were deposited in NCBI Gen bank along with accession numbers. They are *Aspergillus nomius* MR 103 (MK192017), *Aspergillusterreus* RR 105 (MG271916), *Penicillium citrinum* RR 101 (KU613360) and *Penicillium griseofulvum* RR102 (KU613362). All the strains were tested for the production of polygalacturonase under submerged fermentation conditions. Enzyme production by *Aspergillus nomius* MR 103 (2.25 IU/mg) was higher at pH 6.5 and temperature of 35°C using sucrose and ammonium sulphate as carbon and nitrogen sources respectively. Among the metal ions tested, Zinc sulphate showed maximum increase in pectinase enzyme activity. Polygalacturonase, an industrial enzyme has been produced from various fungal isolates using solid state and submerged fermentation techniques.

**Keywords:** *Aspergillus nomius*; *Penicillium*; Mangroves; Polygalacturonase

## Ferulic acid from Maize Bran: Extraction, Purification and Evaluation of anti-oxidant & anti-tyrosinase activities

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### Abstract

Ferulic acid (3-methoxy-4-hydroxycinnamic acid) is biosynthesized in plants from caffeic acid by action of the enzyme caffeate O-methyltransferase. Ferulic acid (FA) is a known antioxidant and tyrosinase inhibitor. The antioxidant effect of the acid makes it reactive towards free radicals, while the anti-tyrosinase activity inhibits melanin synthesis in skin. FA together with dihydroferulic acid is a component of lignocellulose, serving to crosslink the lignin and polysaccharides, thereby conferring rigidity to the cell walls. Therefore, an abundantly available lignocellulosic biomass serves as a potential source of FA that can be used as an in-vitro antioxidant as well as an ingredient of skin whitening creams. In the present study, FA was extracted from bran of yellow maize using alkaline extraction method and quantified using HPLC. The FA content in maize bran was found to be 4.5 g/100g of substrate. The FA extracted was purified using Amberlite XAD-4 column and the FA was found to be 92.33% pure. The purified FA was checked for its antioxidant and anti-tyrosinase activity, which were found to be 20.48 μM VCEAC and 3.05 μM KAEAC, respectively. Further, the anti-oxidant and anti-tyrosinase activities of extracted and purified FA from maize bran were at par with the commercial FA from Merck. Thus, maize

bran can serve as a potential source of ferulic acid to be used as a food preservative or as a component in skin creams.

**Keywords:** Ferulic acid; Maize bran; Anti-oxidant; Anti-tyrosinase; HPLC

IMMT-03

### **Jute: a potential biomass for bioethanol production**

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#### **Abstract**

Jute, also known as golden fibre is an important natural fibre crop in India next to cotton in global production and consumption. Jute sticks after retting process can be used as a lignocellulosic feedstock for production of second generation bioethanol which can be a promising alternative for fossil-derived fuels and first generation bioethanol. In present study jute biomass was analyzed and was found to contain 42.52% cellulose, 12.24% hemicellulose and 31.5% lignin. Our focus was on selection of a pretreatment method that could remove maximum amount of lignin and decrystallize the cellulose in jute biomass. Therefore, jute biomass after dewaxing was subjected to various physico-chemical pretreatments viz. dilute acid, alkali, AFEX (Ammonia Fiber Explosion) and steam pretreatments and quantified for the residual holocellulose and lignin. The AFEX pretreated jute contained 51.18% cellulose, 10.98% hemicellulose and 23.51% lignin, while the steam pretreated biomass contained 46.91% cellulose, 11.78% hemicellulose and 23.51% lignin. Furthermore, the structural components in jute biomass after the pretreatments were analyzed by FTIR, XRD and SEM. The result revealed that AFEX and steam pretreatments were best in terms of cellulose enrichment and lignin removal. Overall, the present study gives a clear indication to explore jute biomass for its bioethanol production efficiency. Further, future work is focused on saccharification of jute biomass towards conversion of the released reducing sugars into ethanol.

**Keywords:** Jute; Second generation bioethanol; Saccharification; Holocellulose; Reducing sugars

IMMT-04

### **Production optimization of $\beta$ -cyclodextrin glucosyltransferase enzyme by isolated *Bacillus Species***

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#### **Abstract**

$\beta$ -cyclodextrin glucosyltransferase ( $\beta$ -CGTase) (EC 2.4.1.19) is an industrially valuable enzyme for the production of  $\beta$ -cyclodextrin.  $\beta$ -CGTase belongs to  $\alpha$ -amylase 13 family; they utilize starch as a sole source for the  $\beta$ -cyclodextrin production. Cyclodextrin production has been reported to be

was influenced by starch and nitrogen source as well as source organism. The present study was aimed to isolate and identify  $\beta$ -CGTase producer, manipulating the medium components for their higher production. Optimization of medium components was done by classical optimization method (changing one factor at a time). Various types of starch sources namely soluble, potato, rice, maize, tapioca and wheat starches was used, soluble starch with 8% concentration was found to be best substrate for enzyme production (60U/ml/min). Optimized production medium comprised % (w/v) 8% soluble starch, 2% peptone, 0.06%  $MgSO_4$  and 0.5%  $Na_2CO_3$  increase overall production of enzyme from 60 to 89 U/ml/min. The optimum conditions for the enzyme activity were pH 7 and temperature 60°C. The enzyme production was accelerated in the presence of  $Ca^{2+}$ ,  $Mn^{2+}$ ,  $K^{2+}$ ,  $Fe^{2+}$  ions and strongly inhibited  $Co^{2+}$ ,  $Cu^{2+}$  and  $Zn^{2+}$  ions.

**Keywords:**  $\beta$ -CGTase; *Bacillus* sp; Production Optimization;  $\beta$ -CD

IMMT-05

### Synergistic effect of endoxylanase and phytase in enhancing nutritional properties of poultry feed

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#### Abstract

Xylanases specifically endo-1,4- $\beta$ -xylanases alone and in combination with other enzyme like phytase can be used for improving feed nutrition because xylanases decreased the degree of polymerization in animal feed and breakdown arabinoxylan in feed ingredients and decreased the viscosity of raw materials; whereas phytases hydrolyse phytic acid into myo-inositol and inorganic phosphate which has anti-nutritional properties and cause environmental pollution. In present study, *Myceliophthora thermophila* BJTLRMDU3 endoxylanase alone released higher reducing sugars (23.86 mg/g feed) and soluble proteins (15.07 mg/g feed) from poultry feed at 60°C when 1g feed mixed with 50U of endoxylanase, while lower amount of reducing sugars (19.60 mg/g feed) and soluble proteins (9.90 mg/g feed) were recorded at 37°C. On supplementation of different doses of endoxylanase almost equal increase in release of reducing sugars and soluble proteins was achieved with 100 and 200 U of endoxylanase; when effect of endoxylanase on feed was studied at different time intervals, highest reducing sugars (49.99 mg/g feed) and soluble proteins (17.19 mg/g feed) were released after 36 h at 60°C. When endoxylanase along with different doses of *Aspergillus oryzae* phytase mixed in feed, 15U of phytase with endoxylanase further increased release of reducing sugars (113.01 mg/g feed), soluble proteins (94.87 mg/g feed) and also release inorganic phosphate (36.36 mg/g feed) and when synergistic effect of endoxylanase and phytase on feed was studied at different time intervals maximum release in reducing sugars, soluble proteins and inorganic phosphate was found after 48 h at 60°C.

**Keywords:** Xylanase; Phytase; Poultry feed; Inorganic phosphate; Nutrition

IMMT-07

### Statistically optimized production of fibrinolytic protease from *Bacillus cereus* RSA1

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### Abstract

Fibrinolytic proteases from microbial provenience serve as novel, potent and safe anti-thrombotic agents for the treatment of cardiovascular diseases such as thrombosis. Our study thus demonstrates the screening, enhanced production and validation of microbial fibrinolytic protease. The microbial strain was isolated from junk heaps of different dumping sites of Noida (U.P, India) and initially screened for fibrinolytic activity on sterile skimmed milk agar medium. Fibrinolytic activity of the strains was further evaluated using fibrin plate (fibrinogen, thrombin, agarose, trisodium phosphate) and the highly efficient strain was identified as *Bacillus cereus* RSA1 (NCBI Accession number: MK288105) by 16S rDNA sequencing. The statistical optimization of different growth parameters and optimal level of key variables for enhanced production of fibrinolytic protease was accomplished using Plackett Burman Design (PBD). The growth parameters as carbon and nitrogen sources were optimized with variables glucose, sucrose, casein, peptone, yeast extract,  $K_2HPO_4$ ,  $MgSO_4$  and cultivation parameters including agitation, incubation time, initial pH and inoculum size. Central Composite design in Response Surface Methodology was further employed to study the interactions between the four significant variables (peptone, casein, glucose, and yeast extract) evaluated by PBD. The predicted value for the production of fibrinolytic protease was 32.8 U/ml which was close to the experimental value (30.75 U/ml), thus validating the designed model.

**Keywords:** Microbial fibrinolytic protease; Screening; Statistical optimization; *Bacillus cereus* RSA1

IMMT-08

### Production of docosahexaenoic acid (DHA) in Indigenous strain *Aurantiochytrium* by varying Carbon Sugars: A media engineering perspective

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### Abstract

*Aurantiochytrium* are marine heterokonts, classified as thraustochytrids capable of producing docosahexaenoic (DHA) and eicosapentaenoic (EPA)  $\omega$ -3-fatty acids, have gained attention due to their health benefits and extensive applications in the field of food and pharmaceutical industries. Primarily, DHA is necessary for the brain development in children and also helpful in treating atherosclerosis, rheumatoid arthritis and Alzheimer's disease. However, different parameters can affect the production of very long chain polyunsaturated fatty acids (VLC-PUFAs). Our objective of this investigation was to study the effect of different concentrations of carbon sources on the production of PUFAs, particularly DHA, in *Aurantiochytrium* sp. Our preliminary data shows that the biomass and DHA productivity was maximum in 3% (w/v) glycerol i.e.,  $1.91 \text{ g L}^{-1} \text{ D}^{-1}$  and  $134.96 \text{ mg L}^{-1} \text{ D}^{-1}$  respectively. In this study, we hypothesize that glycerol induces cell growth and also leads to the production of VLC-PUFAs via central metabolic pathways. Also, our qualitative metabolomics has identified nearly 40 metabolites, of which significant fold change was observed in acetic acid and succinate, that seem to play an important role in biomass and lipid content by diverting the carbon flux towards cell growth and fatty acid biosynthesis specifically, PUFAs. In conclusion, *Aurantiochytrium* sp. is capable of producing high amounts of PUFAs and developing media engineering strategies could reduce the cost and increase the production of high value renewables i.e., OMEGA. Our emphasis for future work is to understand the key regulatory pathways, and thus can be fine-tuned by engineering the metabolic genes involved in the production of DHA.

**Keywords:** *Aurantiochytrium*; Carbon; OMEGAs; Glycerol; VLC-PUFAs

## Comparative analysis of saccharification of sugarcane bagasse and rice straw using bacterial xylanase

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### Abstract

Huge amount of agricultural residues generated from sugarcane bagasse, wheat straw, rice straw, paddy etc. are the major concern of environmental pollution because these residues are burned for their disposal which cause air pollution. Approximately 500 million tons (Mt) of crop residues generates annually in India out of which 141 Mt is of sugarcane and 122 Mt is of rice. So, there is demand of some sustainable approaches for their proper utilization for human welfare and industrial applications. In the present study, ecofriendly partially purified xylanolytic enzyme was used for the saccharification of untreated and pretreated sugarcane bagasse and rice straw. After comparative analysis of saccharification, it was found that maximum reducing sugar were liberated from untreated sugarcane bagasse (124.24 mg/g) and Na<sub>2</sub>CO<sub>3</sub> pretreated rice straw (151.65 mg/g) with enzyme dose of 20 U at 60 °C, pH 7.0 after 48 h of incubation time. Generally high amount of reducing sugars have been liberated after pretreatment of lignocellulosic biomass but present study of sugarcane bagasse showed antagonistic results in terms of reducing sugar liberation i.e., high amount of reducing sugar liberated from untreated sugarcane bagasse (124.24 mg/g) as compared to Na<sub>2</sub>CO<sub>3</sub> pretreated (76.23 mg/g). Therefore, production of various value-added fuels such as bioethanol can be accompanied at industrial scale by utilizing these inexhaustible lignocellulosic residues. Furthermore, biophysical characterization of untreated and sodium carbonate pretreated sugarcane bagasse and rice straw using XRD, FTIR and SEM analysis, revealed the modification in structural as well as chemical constituents of respective biomass.

**Keywords:** Sugarcane bagasse; Rice straw; Pretreatment; Sodium carbonate; Saccharification

## Optimization of bio-ethanol production from ammonia pretreated rice straw

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### Abstract

Rice straw is an important, luxuriantly available agricultural residue in nature. It is a potent sugar source for microbial fermentation, therefore, it has been considered as a promising feedstock for various valuable bio refinery products i.e., ethanol in various countries. Pretreatment usually results in disrupting and separation of interlinked constituents of biomass. Ammonia pretreatment is a preferred method to produce an effective digestible biomass for biorefinery industry i.e., bio-ethanol. In present study, saccharification of ammonia pretreated rice straw was optimized by different parameters i.e., enzyme dose (10-100 U), pH (3-7), temperature (30-60 °C) and time interval (3-48 h). Then fermentation of enzymatic hydrolysate (10%) of optimized saccharification conditions i.e., enzyme dose 100U (369.98 mg/g substrate), pH 5.0

(246.33 mg/g substrate), 60°C (224.24 mg/g substrate) for 24 h (345.61 mg/g substrate) were further optimized by *S. cerevisiae* atpH (4-7), agitation speed (100-200 rpm) and temperature (25-35 °C) for bio-ethanol production. Maximum amount of bioethanol was produced at pH 7.0 (20.87 g/L), 150 rpm (19.47 g/L) and 35°C (21.22 g/L). Chemical composition, FTIR and SEM analysis clearly confirmed the structural modification of biomass i.e., retention of sugars and removal of lignin in pretreated biomass.

**Keywords:** Rice straw; Ammonia; Saccharification; Enzymatic hydrolysate; Bio-ethanol

IMMT-11

### Conversion of Atmospheric CO<sub>2</sub> into Calcium Carbonates using Bacterial Carbonic Anhydrase

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#### Abstract

From the past 50 years, the temperature of the earth raised 1 °C and also the level of carbon dioxide (CO<sub>2</sub>) increased up to twenty times in our atmosphere. To reduce CO<sub>2</sub> level, the conversion of CO<sub>2</sub> into calcium carbonate (CaCO<sub>3</sub>) using carbonic anhydrase enzyme may prove eco-friendly and cost-effective. Carbonic anhydrase contains zinc in its active site and converts CO<sub>2</sub> to bicarbonates, which is further converted to CaCO<sub>3</sub> in presence of Ca<sup>2+</sup> ion. CaCO<sub>3</sub> is the one of a product during CO<sub>2</sub> conversion that can be easily separated and used as raw material for various industrial applications such as cement, ceramics, sugar refining, glass, iron, and steel production units. Carbonic anhydrase producing microorganism from ruminant animal saliva and their role in the conversion of CO<sub>2</sub> is an unexplored area of research. In the present study, ten bacterial isolates isolated from cow saliva were screened based on production of a yellow color colony on nutrient agar plate containing *p*-NPA as a substrate. Based on enzyme production, T5 isolate was most potent and selected for further study. The crude enzyme was used for biomineralization-based conversion of CO<sub>2</sub> into calcium carbonate. The CO<sub>2</sub> conversion efficacy of crude carbonic anhydrase was observed to be ~45 mg CaCO<sub>3</sub>/ mg protein. The calcium carbonates precipitates were further characterized using Scanning Electron Microscopy (SEM) analysis, Fourier-transform infrared spectroscopy (FTIR), and X-ray powder diffraction (XRD) analysis.

**Keywords:** Carbonic anhydrase; Conversion; CO<sub>2</sub>; CaCO<sub>3</sub>

IMMT-12

### Lipstatin production from *Streptomyces toxytricini*

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#### Abstract

Obesity, a disorder related to lipid metabolism, at an alarming stage having fifth rank for deaths worldwide. Reticence of pancreatic lipase is the most suitable approach to control obesity. Lipstatin, the only anti-obesity drug which inhibits the pancreatic lipase, synthesized by *Streptomyces toxytricini* as secondary metabolite. *S. toxytricini* is a multicellular filamentous bacterium with complex life cycle. This



work investigated the pellet formation and production of lipstatin at shake flask level. For the analysis of biomass and lipstatin production, the culture conditions were; pH 7.0, incubation temperature 28°C and 220 rpm for broth in incubator shaker. Various strain improvement strategies have been applied for the better growth of *Streptomyces toxytricini* to enhance the production of lipstatin from 900 mg/l to 2.3 g/l at shake flask level.

**Keywords:** Lipstatin; Obesity; Actinobacteria; Streptomyces

IMMT-13

### Understanding the mode of bactericidal action of Enterocin LD3 from food isolate *Enterococcus hirae* LD3 against *Escherichia coli*

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#### Abstract

Enterocin LD3 was produced from food isolated *Enterococcus hirae* LD3. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of enterocin LD3 against *E. coli* were found to be 112 µg/ml and 180 µg/ml, respectively. There was complete loss in cell viability recorded in 2 MIC-treated cells of *E. coli*, whereas untreated cells grew normally up to log 10.0 cfu ml<sup>-1</sup>. The killing was further confirmed by differential staining the dead cells with propidium iodide (PI) and live cells with 4', 6-Diamidino-2-Phenylindole (DAPI) in presence of MIC and 2 MIC of enterocin LD3. The efflux of potassium ion (K<sup>+</sup>) was estimated to be 14 ppm which was further confirmed by increased electrical conductivity 11.56 mS/cm in CFS of MIC-treated cells of *E. coli*. The increased absorbance (OD<sub>260/280</sub>) 0.110/0.075 suggesting release of nucleic acids and proteins in the bacteriocin-treated cells of *E. coli*. The higher infrared absorbance of treated cells at 1094.30 and 1451.82 cm<sup>-1</sup> corresponding to nucleic acids and phospholipids, respectively suggesting its interaction with nucleic acid and membrane of target bacteria. Transmission electron microscopy of the enterocin LD3-treated cells revealed leaking of the cytoplasmic contents ultimately resulting in complete loss of cell walls. Enterocin LD3 demonstrates bactericidal action against target strain by disrupting cell membrane, release of K<sup>+</sup> ion and UV absorbing materials leading to cell death of *E. coli*. Enterocin LD3 also showed the inhibitory action against tested pathogenic bacteria such as *Micrococcus luteus*, *Proteus mirabilis*, *Pseudomonasaeruginosa*, *E. coli*, *Staphylococcus aureus* and *Salmonella typhi*. Enterocin LD3 has the potential to inhibit the growth of Gram-positive and Gram-negative target bacterial strains and based on antibacterial property it will be valuable in food and clinical applications.

**Keywords:** Enterocin LD3; Bactericidal; Intracellular materials; Infrared spectroscopy; Transmission electron microscopy.

IMMT-14

### Extraction of UV Absorbing Compound from Cyanobacteria

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#### Abstract

Ultra violet radiations (UVR) are high energetic radiation that causes harmful effect on living organisms. UV radiation causes more than 90% skin cancer, allergy, burning of skin, DNA and protein damages,

premature skin ageing, photo-degradation of some useful antimicrobial agents such as natamycin. Many chemically synthesized UV protected and UV filters products are available in market for protection from UV radiation, which have negative effect on human life as well as environment. UV absorbing compound such as mycosporine like amino acids (MAAs) are secondary metabolites those have potential to protect from UV Radiation with other additional protective properties such as work against oxidative stress, osmotic stress, thermal stress, photo-degradation of some useful antimicrobial agents such as natamycin. MAAs is synthesized in various organisms such as fungi, cyanobacteria, algae, coral, terrestrial lichens. The focus of our current research work is on production MAAs from different Cyanobacterial species isolate from various regions of Rajasthan. Stress conditions such as high intensity of UV light, rich amount of salt and high TDS condition are known to induce cyanobacterial spp. for the MAAs production. We collected the cultures from different sampling sites and induced these cultures under UV radiation for promoting synthesis of MAAs under stress conditions. The MAAs produced was then identified by UV-visible spectrophotometer and HPLC analysis. The characteristic peak of the specific MAAs was observed between 310-340 nm from induced samples. The future work is focused on production of large amount of MAAs with analysis of the structure and molecular weight of MAAs.

**Keywords:** UV protectant molecules; Cyanobacteria; Mycosporine like amino acids

IMMT-15

### **Assesment of microbes for effective EDT to enhance the paper recycling rate in India**

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#### **Abstract**

Conservation of natural resources is global concern. Paper is a necessity worldwide and data revealed that about 35 % of the trees are cut down annually for paper industries to meet the demand. The scarcity of flora and the conservation of forests policy have prompted the Paper Manufacturing Industries to find an alternative tool. Alternatively, the conventional methodologies use chemicals for paper deinking that causes biomagnification, which leads to addition of toxic moieties to the ecosystem. Current approach enhances chemical discharge thereby affect effluent treatment expenditures, as well harms the flora and fauna in vicinity. On the contrary from last few decades the Enzymatic Deinking Technology has drawn the attention of many industries, researches globally to increase paper recycle rate through green technology (biopulping and biobleaching). The present study aims to explore the utilization of microbial consortium for large scale enzyme production. Bio Deinking involves the dislodging of the ink particles from the paper pulp by the enzymatic action. Current study demonstrates enzyme as underlying principle along with mechanical force to recycle-deink the pulp to form uniform sheets. The aim is to screen potential Laccase, Cellulase and Xylanase for producing microbes from the fruit residues, soil samples and sediments of the river bank from different sites of Rajasthan. Screening of the isolates was done by using selective enrichment. Quantitative screening can be performed to check the enzyme activity. The efficiency of deinked pulp can be determined by the color, brightness, tensile strength, visible ink specks, surface uniformity and fiber strength. The focus is to present a potent alternative as EDT to increase the paper recycle rate in the Nation.

**Keywords:** Enzymatic Deinking Technology; Biopulping; Biobleaching; Biomagnification; Green Technology

## Enhancement of lipstatin production by *Streptomyces toxytricini* by Mutagenesis and precursor Supplementation

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### Abstract

Nowadays, the population of obese individuals are increasing worldwide and it has become a serious health risk for growing population. Lipstatin is an anti-obesity agent which is produced by *Streptomyces toxytricini*. In present study, production of lipstatin was enhanced by mutagenesis and addition of supplements in fermentation medium. Various carbon and nitrogen sources were provided during fermentation at shake-flask level to increase the lipstatin production. Different mutants of *Streptomyces toxytricini* were developed using N-methyl-N'-nitro-N-nitrosoguanidine (NTG) treatment at various concentrations and ultraviolet radiations with variations in exposure time. Biomass production of screened mutants were in range of 4.8-6.9 g/L. Highest lipstatin producing mutants were supplemented with the defatted soya meal, L-Leucine, oleic acid and linoleic acid. Out of these mutant KKD5 was producing highest concentration of lipstatin. Mutant was further validated in 5 L bioreactor.

**Keywords:** Obesity; Mutagenesis; Lipstatin; Fermentation

IMMT-17

## Isolation and identification of antimicrobial compounds from haloalkaliphilic actinomycete *Nocardioopsis sp. Al-HA-5*

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### Abstract

The development of resistance to multiple drugs is major issue in the medication of infectious diseases caused by pathogenic microorganisms. The multidrug resistance is presently targeted on exploration and therefore need to search new bioactive compound. To achieve this conditions and circumstances, there is concern to ameliorate or detect novel class antibiotics that have distinct mechanisms of activity globally. The suitability of secondary microbial products produced from realizable bacterial taxa was significant to discover novel chemicals for the improvement of new therapeutic agents. In this context, our study aimed at exploration of marine actinomycetes as a valuable resource for novel products with antimicrobial agents. The halo alkaliphilic actinomycete *Nocardioopsis sp. Al-HA-5* was isolated from sea shore of Alang coasts (Gulf of Khambhat), Bhavnagar, Gujarat, India by using Starch agar plate containing 5- 10% NaCl and pH 9-10. The typical chalky white colony was observed after 6 days of incubation at 30°C. On the basis of cell and colony morphology, Gram's staining, biochemical reaction and 16s rRNA phylogenetic analysis, the *Al-HA-5* was identified as *Nocardioopsis sp.* The antimicrobial potential of *Nocardioopsis sp. Al-HA-5* was checked against six Gram positive bacteria, eight Gram negative bacteria and three fungi. From which growth of six bacteria was inhibited by *Nocardioopsis sp. Al-HA-5*. The antimicrobial compounds produced by *Nocardioopsis sp. Al-HA-5* were extracted by using ethyl acetate. The Fourier transform infrared spectroscopy (FTIR) and Gas chromatography-Mass spectroscopy (GC-MS) of ethyl acetate extract have been carried out to investigate the bioactive compounds present in crude extract.

**Keywords:** Haloalkaliphilic; Marine actinomycete; Antimicrobial activity; FTIR; GC-MS

## Fermentation of pretreated lignocellulosic biomass hydrolysate by weak acid tolerant industrial yeast strain

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### Abstract

Utilization of the natural reservoir of fuels is constantly increasing which is leading to their depletion in near future. The production of renewable and alternative sources of fuel from plant or agricultural waste can resolve the concern of their depletion. Moreover, it provides the sustainable and promising solution to environment crisis and solves the energy crisis. Prior to their utilization for production of valuable products, pretreatments of lignocellulosic biomasses are required to open up the cell wall components. Commonly used pretreatment methods are physical, chemical, physio-chemical etc. that not only expose the cellulose content, also releases inhibitors. These inhibitors such as organic acids, reduces the overall productivity of a refinery by repressing growth of microbes, decreases ethanol yields and increases fermentation cost. Acetic acid is present in lignocellulosic hydrolysate, is an inhibitory and toxic compound to yeast strains, which contribute to fermentation arrest and reduced ethanol productivity in combination to other toxins. In the present study, a strain of *Saccharomyces cerevisiae* evolved through evolutionary engineering over 50 generations on acetic acid. The developed strain used for fermentation of pretreated rice straw hydrolysate and found more tolerant and resistant to acetic acid.

**Keywords:** Lignocellulosic biomass; Pretreatment; Hydrolysis; Inhibitors; Fermentation

## Bioprospecting of thermophilic nitrilase from hot springs of western Himalayas

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### Abstract

Nitriles are omnipresent organic compounds which are synthesized by cyanogenic plants as cyanoglycosides, cyanolipids as well as added to the environment due to the anthropogenic activities. These compounds are toxic to living organisms, however in nature, there are many microbes which can use them as nutrients or have enzymes which can detoxify them. Nitrilase superfamily of enzymes is mainly responsible for nitrile detoxification in microbial system. They have three types of enzymes viz nitrilases, nitrile hydratases and amidases which can directly or indirectly convert toxic nitriles into carboxylic acid. Nitrilase directly converts organic nitriles into organic acids and ammonia; while nitrile hydratases and amidases work indirectly, by first converting nitrile into amide which further act as a substrate to amidases and transform it into acid and ammonia. These enzymes are also employed in many industries like paint, drugs and pharmaceutical industries. Mesophilic nitrilases/Nhase/amidases are prone to degradation at high temperature which is encountered at industrial level. Thermostable enzymes can be an alternate to this problem and can be either explored in extreme habitats of the nature or can be engineered by their mesophilic counterparts. A few nitrilases from *Acidovorax facilis* 72W, *Bacillus pallidus* DaC521, *Streptomyces* sp. and *Thermotoga maritime* MSB8 have been isolated from volcanic sites; thermal springs have been reported in literature. In present study we are exploring thermostable

nitrilase producing strains present in thermal springs of western Himalayas using enrichment technique as well as metagenomics. Thermophilic microbes possess enzymes which can work at such extreme conditions and may act as a good source of novel enzymes for industrial bioprocess. In past decade many groups have also investigated artificial habitats to search for microbes habituating such environment. These thermostable nitrile degrading enzymes may find their application as catalyst in cyanide/nitrile contaminated site, biosensor for nitrile/amide contamination in food or in various industries.

**Keywords:** Nitriles; Nitrilases; Nitrile hydratases; Amidases; Thermophiles

IMMT-20

### Strain improvement of bioethanol producing yeasts *Kluyveromyces marxianus* JKH5 and *Pichia kudriavzevii* JKH1 for enhancing tolerance towards fermentation inhibitors

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#### Abstract

In the present study, diethylsulphate (DES) was used for mutagenesis of thermo tolerant yeasts *K. marxianus* JKH5 and *P. kudriavzevii* JKH1 for improving their tolerance against fermentation inhibitors (5-HMF, furfural and acetic acid) generated during lignocellulose pretreatment. The obtained mutants were screened on the basis of their improved physiological characteristics viz. degree of multiplication, specific growth rate, doubling time, sugar utilization and ethanol production in dextrose (100 g/L) containing medium. Two mutants, *K. marxianus* JKH5M1 and *P. kudriavzevii* JKH1M2 grew well in the medium containing inhibitors acetic acid 4.8 g/L, furfural 0.8 g/L and vanillin 0.8 g/L and produced higher ethanol (47.5 g/L and 49.4 g/L, respectively) than the parent strains (3.8 g/L and 3.8 g/L, respectively) after 36 h fermentation in synthetic medium containing dextrose. In presence of inhibitors, both the mutants also had better degree of multiplication (3.19 & 3.58), specific growth rate (0.61 h<sup>-1</sup> and 0.25 h<sup>-1</sup>), doubling time (1.13 h and 2.74 h), respectively. Out of the two mutants, JKH5M1 was the superior one, on the basis of better fermentation and growth characteristics and was used for further optimization of the ethanol fermentation process by applying Plackett-Burman (PB) design of experiments. The variables glucose, inhibitor concentration, ammonium chloride and peptone were found to significantly affect ethanol production by mutant JKH5M1.

**Keywords:** Strain improvement; Mutagenesis; Inhibitors; Plackett-Burman (PB) design; Bioethanol

IMMT-21

### Isolation of bioactive compounds from actinomycetes against Gram-negative bacteria

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#### Abstract

With the increasing emergence of resistance to the available antibiotics and due to ineffective antibiotics, it is important to search for new sources for new antibiotics. Global public health faces a desperate situation, due to the lack of effective antibiotics. Most importantly, there is an ever increasing demand for antibiotics against Gram-negative disease causing bacteria. The Gram-negative bacteria are responsible

for many diseases including life threatening diseases like cholera which is caused by *Vibrio cholera*, a Gram-negative bacterium and plague which is caused by *Yersinia pestis*, again a Gram-negative bacterium. Certain types of Gram-negative bacteria have become increasingly resistant against available antibiotics. Actinomycetes are prospective producer of bioactive compounds along with various antibiotics. To focus light on its importance, and availability in the area, we made efforts to study the unexplored biodiversity potentially present in the Shivalik region of Jammu and Kashmir. After screening them for antimicrobial activity, isolates showing potent antimicrobial activity against Gram negative bacteria were selected for the isolation of bioactive compounds. One of the isolates, *Streptomyces* sp. WR-1, showing significant activity against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *E. coli* has been explored for potential bioactives. Efforts are being made to isolate pure bioactive compounds from the bulk culture grown in 5 litre fermenter. Purification and characterization of bioactive compounds and their potential bioactivities are underway.

**Keywords:** Biodiversity; Actinomycetes; *Streptomyces* sp.; Antibiotic resistance; Gram-negative bacteria

IMMT-22

### Synthesis and characterization of thin film to restrict bio-film formation

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#### Abstract

The proposed research work is based on the role of biological applications of various thin films. It is well-known fact that the application of thin films is confined to optics, electronics, mechanics etc. but very limited utilization have been reported toward biological applications. If we follow interdisciplinary approach, then there is wide scope to transform these thin films into biological applications. Metal, metal oxide, nanocomposites, nanoparticles would be the preferred materials for the deposition of thin films. Due to large surface to volume ratio, the desired thin films would be either submicron or nanostructured. There is also an approach to fabricate and design thin films for the preparation of biomimetic surface. It is the priority concern that the deposited thin films should be non-toxic, non-allergenic, structurally and thermally stable, easy to deposit and eco-friendly. Thermal evaporation technique is being used in the formation of Ru doped TiO<sub>2</sub> Thin Films. These coated Thin Films shows restriction towards biofilm formation when subjected to culture medium of *Staphylococcus aureus*. Medical implants and devices are predominantly targeted by microbes and hence become major cause for their failure. Therefore, the main objective of this work is to inhibit the growth and deposition of microbes on any object which further may lead to environmental microbial technology, medical devices, implants, marine and industrial products as well.

**Keywords:** Biofouling; Thin films; Microbes; Implants

IMMT-23

### Optimization of the pyocyanin production from *Pseudomonas aeruginosa* isolated from soil and water

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### Abstract

*Pseudomonas aeruginosa* is known to produce a variety of extracellular pigments. One such pigment is Phenazines which are produced in large amount extracellularly. Phenazine consists of pyocyanin (blue green) which is the main pigment produced by 90-95% of strain of *Pseudomonas*. Pyocyanin has many applications, it can be used as antibacterial agent in medical field and it is also used as a food colorant in food industry. The present study was focused on the production of pyocyanin pigment by *P. aeruginosa* isolated from soil and water. A total of twenty eight *P. aeruginosa* isolates were procured from the lab. Biochemical characterization was done for reconfirmation of the positive isolates. The pyocyanin produced by all the isolates was measured. The three isolates, HS16, HS2 and TS3 showed maximum production 11.008 ( $\mu\text{l/ml}$ ), 9.01 ( $\mu\text{l/ml}$ ) and 8.22 ( $\mu\text{l/ml}$ ) respectively. These three isolates were further used for optimization of the screening media used for the production of pyocyanin. Five different media, Luria Broth, M63, M9, King's A and Super broth were used for optimization. It is found that for HS16 is the best producer with maximum production in Super broth (14.161  $\mu\text{l/ml}$ ). HS2 produced pyocyanin best in King's A (8.732  $\mu\text{l/ml}$ ) and TS3 produced maximum pyocyanin in Luria broth (8.425  $\mu\text{l/ml}$ ).

**Keywords:** *Pseudomonas aeruginosa*; Phenazines; Pyocyanin; Antibacterial agent; Optimization

IMMT-25

### Isolation and characterization of Textile Dye degrading bacteria from textile Dye effluents

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### Abstract

Textile dye industrial waste is one of the most serious problems in the environment. The dye wastes are severely deleterious to surface water bodies. Dyes and dyestuffs find use in a wide range of industries but are of primary importance to textile manufacturing. Wastewater from the textile industry can contain a variety of polluting substances including dyes. Increasingly, environmental legislation is being imposed to control the release of dyes, into the environment. The ability of microorganisms to decolourise and metabolise dyes has long been known, and the use of bioremediation based technologies for treating textile wastewater has attracted interest. The present study was an attempt for the assessment of different physicochemical parameters such as pH, temperature, Electrical Conductivity (EC), Total Solids (TS), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total alkalinity, Total hardness and Chloride content. Therefore, the textile dye effluent degradation was herculean task because the textile effluents contain complex chemicals, highly toxic compounds and heavy metals. The experiment was carried out to degrade the dye effluents by using bacterial isolates from textile dye effluents. Furthermore, the complete degradation of dyes was confirmed by optimization.

**Keywords:** Textile dye industry; Dyes; Dyestuffs; Microorganisms

IMMT-26

### Bioseparation of bovine serum albumin protein using polymeric phase system

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### Abstract

Triple phase system has been currently used as a low cost downstream processing technique for purification of biomolecules. In this study, the partitioning of bovine serum albumin (BSA) was studied using ammonium sulphate/t-butanol triple phase systems and PEG/Salt aqueous two phase system. Among different aqueous phase system, maximum partition coefficient value (2.5) was found for 5% (w/w) PEG concentration, 25% (w/w) tri-sodium citrate salt and pH of 4 units. This high value of partition coefficient indicates a good concentration of BSA in the top phase but the maximum amount of BSA recovered was only 26.67% due reduction in the top phase volume. The precipitation of BSA protein was improved after applying triple phase system by varying volume of t-butanol (0.25ml to 2ml), concentration of ammonium sulphate (20%-50%) and pH (4-7). The precipitation rate of BSA protein increased across interphase layer with increased volume of t-butanol and concentration of ammonium sulphate. The higher rate of precipitation of protein would be observed below the isoelectric point due to accumulation of positively charged amino acid molecules. The maximum amount of BSA protein (98%) was extracted in the interphase at 1ml t-butanol, 50% saturation of ammonium sulphate and pH of 4 units. Thus, Triple phase system can be considered as effective bioseparation technique for recovery of BSA protein in compare to aqueous two phase system. This triple phase system could be used as low cost purification technique to purify BSA protein at a large extent.

**Keywords:** BSA; Triple phase system; Aqueous two phase system; Bioseparation

IMMT-29

### Isolation, screening and enzymatic assay of alkaline cellulase producing fungi from soil of Kapurthala District, Punjab.

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### Abstract

Alkaline cellulase producing fungi were isolated from soil of Kapurthala district Punjab, India on Mandels and Reese media with Carboxy Methyl Cellulose (CMC) and Cellulose powder as sole source of carbon for isolation and screening. Serial dilution of the experimental soil samples using sterile distilled water were used for fungal isolation on agar screening medium. Four (4) out of fifteen (15) different morphological colonies were selected based on their clear zone of hydrolysis using Congo red method and their ability to produce a maximum of 0.55 IU/ml and 3.88IU/ml CMCase and FPase respectively using Dinitro salicylic acid (DNSA) at pH 8.5. High enzyme production was found on both *Alternaria* and *Aspergillus* species. These identified fungi will be optimized for their enzymatic deinking application in pulp and paper industry.

**Keywords:** Alkaline; Cellulase; Enzyme; Kapurthala; Soil

IMMT-30

### Combining genetic manipulation of transcriptional factor *cre A* and genome reshuffling to enhance lignocellulolytic enzyme production in *Talaromyces emersonii*

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### Abstract

The quest for cheaper and better enzymes needed for the efficient hydrolysis of lignocellulosic biomass has placed thermophilic fungi in the limelight for bioprospecting research. Our study provides the first comprehensive insight into secretome of a thermophilic fungus *Talaromyces emersonii*. The exo-proteome analysis of the strain by Q-TOF LC/MS revealed a total of 266 proteins with 121 CAZymes consisting of 94 glycosyl hydrolases belonging to 40 different families. *T. emersonii* was further developed as an industrial workhorse, by employing genetic manipulations to increase the cellulase production capabilities. The study reports the disruption of *creA* gene, a transcriptional regulator that maintains carbon homeostasis by negatively regulating the catabolic genes, and subsequently was transformed resulting in deregulated strain Cre13 that produced high level of endo-glucanase (107U/ml),  $\beta$  glucosidase (32 U/ml), CBH I (5.29 U/ml), FPase (2.04 U/ml), and xylanase (160U/ml) activities. To further enhance the enzyme titres genome reshuffling by intra-specific protoplast fusion followed by diploidization and random mutagenesis was carried out that resulted in developing a hyper-cellulolytic strain YBC18 that exhibited EG (363.63 U/ml),  $\beta$ G (56.41 U/ml), CBH (12.42 U/ml), avicelase (0.92U/ml), FPase (2.81 U/ml) and xylanase (258.04 U/ml) corresponding to respective increase by 4.4, 2.25, 2.17, 3.03, 1.90 and 1.78 folds when compared to the wild type strain. Furthermore, the potential of this strain for efficient hydrolysis of acid-pre-treated rice straw and bagasse was also evaluated.

**Keywords:** *Talaromyces emersonii*; Secretome; Transcriptional regulator; Protoplast fusion; Hydrolysis of rice straw and Bagasse

IMMT-31

### Standardization of process parameters for enzymatic hydrolysis of pine needles by using a statistical approach: Response Surface Methodology (RSM)

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### Abstract

Fermentation of reducing sugars is one of the pathways to produce bioethanol from lignocellulosic forest waste i.e. pine needles. The components of lignocellulosic waste viz. cellulose, hemicellulose and lignin are not readily fermentable for bioethanol production by microorganisms. Therefore, prior to ethanol fermentation, these components of pine needles should be hydrolyzed to fermentable sugars. In general, chemical or enzymatic hydrolysis is the common method used for this purpose. Enzymatic hydrolysis is an environmentally benign process and can obtain higher glucose yield without producing any inhibitory products. In our present study, a mixture of different in-house enzymes (cellulase: xylanase) was used for enzymatic hydrolysis of pine needles and different process parameters were optimized by using response surface methodology (RSM). The process parameters optimized for enzymatic hydrolysis were enzyme dosage, temperature and incubation period. The experiment involved 20 runs of different factors which were considered for optimization. The best optimized conditions obtained were (cellulase: xylanase) at dose of 12.50 ml/g of dry biomass, temperature 43°C and incubation period 72 h yielded 27.52 mg/g of reducing sugars. The appreciable amount of saccharified sugars showed a strong possibility in pine needles as feedstock for bioethanol production.

**Keywords:** Fermentation; Enzymes; Response Surface Methodology; Optimization

### Novelty of debittering enzymes in the production of kinnow beverage

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#### Abstract

Citrus peel is the principle by-product of the citrus fruit processing industry and its disposal is a major problem. The peel being rich in naringin, limonin and carbohydrate content can be used in production of debittering enzymes such as naringinase, limonin dehydrogenase and  $\beta$ -glucosidase. The present study has been undertaken for production of debittering enzymes using citrus peel as a substrate and *Clavispora lusitaniae* yeast. The debittering enzymes from *C. lusitaniae* holds potential for debittering of citrus juices and the bioprocess consists of production and purification and setting up a strong base for bio-enzymatic debittering of citrus juices. The limonin dehydrogenase enzyme has been purified to 1.14-fold with 33.98% recovery, 29.25 IU activity which resulted in 86.43% degradation of limonin in juice by catalyzing oxidation of limonin -A-ring lactone to the non-bitter 17-dehydroxylimonoate. The debittering naringinase enzyme was purified to 1.68 fold, having 24.8 IU activity, reduced 57.93% naringin into non-bitter bioactive compound naringenin. The flavour enhancing  $\beta$ -glucosidase with activity 14.68 IU was purified to 1.18 fold which resulted in the increase in glucose content upto 4.25  $\mu$ g/ml in kinnow juice. The physiochemical parameters in kinnow beverage after 30 days of storage at 4°C revealed maximum reduction in limonin (86.10%) and naringin (94.84%) content but it increased acidity by 51.58% and total sugar content by 53.95  $\mu$ g/ml. The enzymatic debittering is advantageous as it is highly specific, non-toxic, efficient and convenient for large scale commercial debittering of citrus juices and all nutraceutical compounds (flavonoids, flavones, vitamins) formed are retained in the debittered kinnow beverage.

**Keywords:** Limonin dehydrogenase; Naringinase;  $\beta$ -glucosidase; *Clavispora lusitaniae*; Purification; Fermented kinnow beverage

### Novel Yeast Species isolated from the Gut of Bees and their Lipase Activity

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#### Abstract

Ascomycete yeasts are found to be associated with beetles but little is known about their association with the Hymenoptera order of insects. In this study, an attempt was made to study the yeast associated with insect gut. We isolated three novel species from genus *Starmerella* from the gut of different bee species. PIG-3C was isolated from the gut of bumblebee (*Bombus*), PIG-5F was isolated from the gut of stingless bee (*Meliponini*) and PIG-6B was isolated from the gut of black hairy flower wasp (*Scoliasoror*) in Himachal Pradesh, India. PIG-3C and PIG-5F showed the lipase activity which is different from their related species. Lipase activity showed by these yeast isolates suggest their role in nutritional process as it helps in the hydrolysis of fatty acids, phospholipid, triglyceride present in the nectar, tree phloem and

resins. These organic compound stored as fat body in the insects and later used during metamorphosis, latency during pupal and teneral stage of insect development. Based on PCR fingerprinting with nearest isolates, physiological characteristic and ITS/D1D2 sequence of rRNA gene suggested that these are novel species of *Starmerella* clade. Sequence analysis shown that PIG-3C is related to *Candida floriss*, PIG-5F is related to *Candida magnoliae*, and PIG-6B is related to *Candida bombi*. The type cultures are: PIG-3C strain MTCC 12736<sup>T</sup>, PIG-6B strain MTCC 12737<sup>T</sup> and PIG-5F strain MTCC 12738<sup>T</sup>.

**Keywords:** Bee associated yeast; *Starmerella*, *Candida*; ITS/ D1D2 region

IMMT-35

### Enhanced production, characterization and applications of *Pseudomonas melanin*

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#### Abstract

Melanins are poly phenolic dark colored natural pigments produced by most of the biological kingdoms. Based on the structure they are broadly categorized into three groups, Eumelanin or DOPA melanin, Pheomelanin or Cysteinyl DOPA melanin and Allomelanin or DHN melanin. Melanins are known to protect the organisms from harmful and carcinogenic ultraviolet radiations (UVR) and hence they have a huge demand in cosmetic industries. In spite of its abundance in nature, the commercially available natural melanins are limited and only such melanin is that extracted from ink gland of sepia fish. Although a few reports are available from microbial sources, their production at commercial level is not feasible therefore more research required to discover potential microorganisms. The present study focus on the enhanced production, characterization and applications of *Pseudomonas* mediated melanin. Response surface methodology with central composite design was employed for knowing the significant critical process variables for enhanced production of melanin. UV-Vis spectroscopy, Solid state <sup>13</sup>C NMR, FTIR, ESI MS, spectroscopic methods were used to characterize melanin. Purified melanin was used for antioxidant and UV protectant activity. Statistical approach yielded 6.7 g/L of melanin under submerged fermentation conditions. Spectral studies revealed the basic structural constituent of *Pseudomonas* melanin to be 5, 6 Di-Hydroxyl indole carboxylic acid. DPPH assay has shown the free radical scavenging ability of melanin. Potential UV-B protectant activity was exhibited by melanin in a dose dependent manner. Therefore, *Pseudomonas* melanin can act as vital sunscreen ingredient in cosmeceutical industry.

**Keywords:** Melanin; Natural pigment; Antioxidant; UV protectant

IMMT-36

### Detection of extremophilic actinobacteria for extremolytes

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#### Abstract

Extremolytes comprise about 25% of dry cell weight accumulated in microorganisms exposed to various environmental stress conditions. Organic compounds (polyol derivatives -ectoin, hydroxyectoin,

betain and proline); carbohydrates (trehalose and mannose derivatives); negatively charged derivatives of inositol and glycerol; and few U.V radiation protective compounds (scytonemin and melanin) are the extremolytes of greater interest. Extremolytes stabilize and protect cellular membranes, proteins and nucleic acids of microorganisms. These protection molecules are polar and highly soluble in water which consequently leads to protection benefits when applied on human cells. Due to this efficacy, the use of extremolytes in cosmetics and personal care products has various benefits. Extremophiles are the organisms growing optimally in extreme environmental conditions, such as temperature, pressure, radiations, salinity and pH. The extremolytes are normally produced in certain microorganisms under extreme stress conditions to enhance their survival ability. The detection of novel extremophilic microorganisms has enabled the biotechnology industry to innovate extremolytes for the welfare of the mankind. Efforts were made to isolate temperature, salt and p<sup>H</sup> tolerant microorganisms, aiming at thermophilic, halophilic and alkaliphilic extremolytes respectively. A survey was made along the Deccan trap, covering Karnataka, Telangana and Andhra Pradesh harsh geographical areas. Soil samples were collected from the selected harsh habitats and were pre-treated with high range of temperature, salt and P<sup>H</sup> to enrich the isolation of thermophilic, alkaliphilic and halophilic microorganisms. Thus isolated extremophilic bacteria and actinobacteria were identified based on colony, microscopic, biochemical and physiological attributes. The identified organisms were grown in a specific culture medium and induced with a typical extreme physiological condition for the production of respective desired extremolytes. The centrifuged supernatant culture broth after bacterial milking will be subjected to LC-MS, NMR, ESI, FT-IR analytical techniques for their characterization.

**Keywords:** Harsh habitats; Extremophilic microorganisms; Microbial extremolytes

IMMT-37

### **Bacterial Engineers for the Discovery of Petroleum Reservoirs**

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#### **Abstract**

Petroleum products are produced naturally in the form of crude oil and which is later processed in refineries. Discovery of petroleum reservoirs using bacteria is based on the fact that gaseous hydrocarbon such as methane, ethane and butane move upwards from underneath reserves of petroleum by the process of diffusion as studied by (Horvitz,1939) there are many microorganisms which is responsible for highly degradable for any organic matter. Basically this paper describes isolation of microorganisms from soil which is based on bioremediation and biodegradation and their biochemical properties which is prominent application hydrocarbon microorganisms. They are highly degradable microorganism for petroleum based matter.

**Keywords:** Diffusion; Microorganism; Bioremediation; Biodegradation

IMMT-38

### **Isolation, molecular characterization of *Penicillium chrysogenum* for penicillin production**

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#### **Abstract**

Antibiotics are the organic, secondary metabolites produced by diverse microbes having wide range of biological activities. Penicillins and cephalosporins are the natural  $\beta$ -lactam antibiotics produced mainly

by *Penicillium chrysogenum*. Penicillin (PenV/G) has a major commercial and clinical significance and exhibit a diverse antimicrobial spectrum. Penicillins have fascinated more attention for the production of active pharmaceutical intermediate (API), such as 6-aminopenicillanic acid (6APA). 6-APA used for the production of semi synthetic  $\beta$ -lactam antibiotics such as oxacillin, ampicillin, amoxicillin, imipenem, and methicillin and has saved millions of lives. PenV/G was the largest segment of the global antibacterial drug market with a share of 57%. Due to the emergence of new resistant bacteria there is a need of new and efficient antibiotics. In the present study, a total of 80 strains were isolated, morphologically identified and characterized as *P. chrysogenum* on different synthetic media. Furthermore, morphological identifications were authenticated by multilocus sequence typing (MLST) by targeting ITS, Ben A, CaM, NL and LROR genes. Among these, Ben A gene showed better results and differentiated *P. chrysogenum* from *P. rubens* which were not resolved by Vitek MS. Furthermore, metabolic profiling of these strains showed not only the production of PenV/G but also, roquefortine C, chrysogine, meleagrine, sorbicillin which possess important biological activities. Molecular detection of penicillin was determined by targeting their biosynthetic pathway genes respective to Pen V/G biosynthesis and showed positive for all PcbC, Phl, and PenDE genes. Furthermore, PenV/G detection was also performed by conventional TLC and further confirmed by HPLC, LC-HRMS and microbial sensitivity assay.

**Keywords:** *P.chrysogenum*; Multilocus Sequence Typing (MLST); HPLC; LC-HRMS; PenV/G

IMMT-39

### Impact of inoculum size and temperature on the production of fermented beverage from Banana pseudostem core

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#### Abstract

Microbial inoculum and temperature play a very crucial role during production of fermented beverage from banana pseudostem core. Two microbial cultures *Saccharomyces ellipsoideus* and *Lactobacillus acidophilus* were selected and standardized for the fermentation process. The inoculum size was standardized using culture and medium ratio. It was observed in the study that the optimum inoculum size for *Saccharomyces ellipsoideus* was 8%, which significantly did not differ from 12% with respect to pH (3.38 and 3.40 respectively) and 12% would add up to the cost of production. With similar reason, 8% was considered optimum for lactic acid bacteria. Overall acceptability for yeast fermentation at 8% was highest (16.24) on a 20 point hedonic scale. In case of fermentation temperature, *Saccharomyces ellipsoideus* produced highest quantity of ethanol (7 %) at 28 °C, as compared to other temperatures, 30 and 32 °C. It was also found that pH reduced significantly at 28 °C in yeast fermentation (3.37). *Lactobacillus acidophilus* achieved lowest pH and TSS (4.20 and 8.63 °Brix) at 32 °C. Overall acceptability for lactic acid fermentation at 32 °C was found to be highest (12.75).

**Keywords:** Banana pseudostem core; *Saccharomyces ellipsoideus*; *Lactobacillus acidophilus*; Inoculum size

### Optimization and purification of extracellular lipase from *Pseudomonas* sp

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#### Abstract

The bacterial genus *Pseudomonas* is a versatile and well known producer of a number of extracellular enzymes including lipase. Lipases (glycerol ester hydrolases; EC 3.1.1.3) are important enzymes which, due to their ability to catalyze a number of reactions, are receiving considerable interest from both academia and industry. In this study, we carried out the screening of 56 *Pseudomonas* sp for their lipolytic activity. *Pseudomonas plecoglossicida* NAIMCC B: 397 turned out to be the best (28.9 U/ml) lipase producing isolate. In order to optimize the lipase production, ten different cost effective substrates were selected and out of them four best substrates viz. Triton X-100, groundnut oilcake, sesame oilcake, and maltose were used for substrate optimization using Plackett Burman design. Further, response surface methodology (RSM) was applied to the selected substrates supplemented with three nitrogen sources namely Ammonium dihydrogen phosphate, Potassium nitrate, and urea to maximize lipase production. Finally, Triton X-100 (0.8125%), Ammonium dihydrogen phosphate (1.25%), and maltose (0.75%) was finalized based on RSM results which increased the lipase production upto 70.32 U/ml (2.43 fold increase). Mass production of the enzyme was carried out based on the optimized substrate concentration and the enzyme was purified using 70 % ammonium sulphate precipitation, dialysis and anion exchange chromatography. After chromatography approximately forty-two fold increase in specific activity was achieved. SDS confirmed the size of purified lipase to be ~50 KDa in size.

**Keywords:** *Pseudomonas*; Lipase; Plackett Burman analysis; Response surface methodology; Chromatography

### Unveiling diverse array of glycosyl hydrolases in secretome of *Myceliophthora verrucosa* and their purification

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#### Abstract

Thermophilic fungal strains capable of producing thermostable cellulases and xylanases are considered catalytically efficient enzyme candidates for hydrolysis of lignocellulosic biomass. This study reports the production, purification and characterization of cellulolytic and hemicellulolytic enzymes from thermophilic fungus *M. verrucosa*. These secretome Q-TOF LC/MS based analysis of *M. verrucosa* revealed a total of 126 proteins with 57 CAZymes consisting of 30 glycosyl hydrolases (GH), 12 Auxiliary Activity (AA), 7 Carbohydrate Esterase (CE), 4 Carbohydrate-Binding Module (CBM) and 4 Polysaccharide Lyase (PL) proteins. The enzymes produced under solid state fermentation resulted in 81.9, 13.2, 2.0, 4.1 and 435 U/gds of endoglucanase,  $\beta$ -glucosidase, cellobiohydrolase, FPase and xylanase. Further, the

purification of EG,  $\beta$ -G, CBHI, CBHII and xylanase was carried out using ion exchange and gel filtration chromatography. Molecular weights of purified EG,  $\beta$ -G, CBHI, CBHII and xylanase were estimated to be ~42, ~103, ~63, ~35 and ~27 kDa by SDS-PAGE. It was observed that purified CBHI and CBHII exhibited 123.7 %, 137.50% relative activity at pH 6.0, temperature 50° C and 60° C respectively. The optimal activity of purified EG was observed to be at 50° C, pH 6.0 followed by  $\beta$ -G at 50° C, pH 5.0 and xylanase at 40° C, pH 6.0. The enzymatic activities of all the purified enzymes were positively modulated by the presence of  $Mn^{2+}$ ,  $Fe^{3+}$  and  $Ca^{2+}$ . Further the efficacy of these purified enzymes for efficient hydrolysis of acid/alkali treated rice straw/bagasse and oxalic acid treated rice straw/bagasse was also evaluated.

**Keywords:** *Myceliophthora verrucosa*; Secretome; Glycosyl hydrolases; Purification; hydrolysis

IMMT-42

### Isolation of bacterial strains for Polyhydroxyalkanoate (PHA) production from sugar mill industrial effluent

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#### Abstract

Polyhydroxyalkanoates (PHAs) are a class of biodegradable polyesters consisting of 3 – 6 hydroxyalkanoic acids which are produced by variety of gram positive and gram negative bacterial species under nutrient-stress or nutrient-limiting conditions with high carbon and low nitrogen availability. When produced by bacteria PHA serve as a source of energy and as a carbon store and is found as discrete cytoplasmic inclusions in the cell. Attributable to their biocompatibility and biodegradability, PHAs have a huge array of applications in various industries such as biomedical sector including tissue engineering, drug delivery, bio-implant patches surgery and wound dressing. The lipopolysaccharides in Gram negative bacteria's cell membrane co-purify with the PHAs which cause immunogenic reactions in human beings. On the other hand, Gram positive bacteria lack LPS which a positive feature that substantiate intensive investigation into their production of PHAs. Keeping this in mind, the current study was aimed at the isolation of viable and robust gram positive bacterial strain from Sugar mill industry effluent. The isolates were then screened for their PHA production potential. Isolation of 23 bacterial strains was done from the industrial effluent amongst which 5 were identified as putative PHA producers. The isolate having the highest PHA production potential was characterized by performing various biochemical assays. PHA extracted was 75mg/L which was further characterized spectrophotometrically. This viable isolate can now be utilized for commercial production of biomaterials, biomedical industries and bioremediation of industrial effluents.

**Keywords:** Polyhydroxyalkanoates; Biodegradable; Biochemical characterization; Sugar mill effluent; Industrial microbiology

IMMT-43

### Cloning and expression of xylose reductase from *Candida tropicalis* GRA1 in *E. coli* for xylitol production

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### Abstract

Xylitol is a naturally occurring five carbon sugar polyol with potential use in food and pharmaceutical industries. Large-scale xylitol production involves the reduction of D-xylose to xylitol *via* catalytic chemical reaction. The process requires high pressure and temperature, which in turn increases the cost of production. The biological conversion of xylose to xylitol from biomass is highly cost effective owing to the easy availability of low cost substrates. However, an efficient biocatalytic system or microbes with xylose conversion efficiency is lacking. Xylose reductase (Xr) is a key enzyme in xylose metabolism, and catalyses reduction of xylose to xylitol. In the present investigation, xylose reductase encoding gene from *Candida tropicalis* (CtXr) was cloned in pQE30Xa vector under the control of T5 promoter and transformed into expression host *E. coli* M15 strain. The protein expression was induced using IPTG and the over expressed protein was confirmed by SDS-PAGE. The recombinant xylose reductase was purified using Ni-NTA affinity column. The purified recombinant CtXr had a molecular mass of 38 kDa. CtXr showed maximum specific activity of 244 U.mg<sup>-1</sup> at pH 7 and temperature 35°C that requires NADPH as co-factor for its activity. The substrate specificity of xylose reductase was analysed using different substrates like xylose, arabinose, rhamnose, manitol, galactose, glucose and mannitol. The enzyme displayed maximum activity with arabinose (213.9 U.mg<sup>-1</sup>) followed by xylose (192.2 U.mg<sup>-1</sup>) and rhamnose (189.76 U.mg<sup>-1</sup>). This study on biological conversion of xylose using xylose reductase paves the way for future xylitol production in a cost effective manner.

**Keywords:** Xylitol; Xylose reductase; *Candida tropicalis*; Biomass; Polyol

IMMT-44

### Characterization and Application of Silver Nanoparticles Synthesized Using Cyanobacteria Isolated from Extreme Habitat

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### Abstract

Green synthesis of metallic nanoparticles has long been known for its potential role in improving and protecting the environment by reducing the use of toxic chemicals and also their use in biomedical applications. Cyanobacteria are archaic photosynthetic prokaryote and one of the most promising microorganisms to produce valuable bioactive compounds that are capable to reduce silver ions to small sized silver nanoparticles (AgNPs). In the present study, aqueous extract of five cyanobacteria isolated from saline-alkaline Lake were screened for biosynthesis of AgNPs under photosynthetically active radiation (PAR) and their antimicrobial properties were also evaluated. The synthesis of silver nanoparticles was observed within 30 min by all the aqueous extract of cyanobacteria and end point of the reaction was varied strain to strain. Fourier transforms infrared (FT-IR) analysis demonstrated that functional groups such as amine, hydroxyl and methyl groups were present over the AgNPs synthesized by all the isolates, while carboxyl group was also observed in AgNPs of cyanobacterial isolate MDL07. Crystalline nature of AgNPs was confirmed by X-ray diffraction (XRD) analysis. The surface charge of AgNPs of all the isolates was found in the range -20 to -50 mV. The AgNPs synthesized using cyanobacterial isolate MDL01 exhibited strong antibacterial activity against *E. coli* and *Bacillus licheniformis*. Thus, cyanobacterial taxa existing in extreme habitats could be explored more for the synthesis of metallic nanoparticles and can be easily employed for large-scale biogenic production of the silver nanoparticles.

**Keywords:** Metallic nanoparticles; Metallic nanoparticles; AgNPs, MDL07; *Bacillus licheniformis*



## A novel approach for co-production of 3-hydroxypropionic acid and lactic acid from glycerol using co-culture of microbial cells and process optimization

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### Abstract

This work focuses on a novel approach for production of value-added chemicals, 3-hydroxypropionic acid (3HP) and lactic acid (LA), from glycerol by exploiting microbial co-culture and process optimization. The starter culture of *Lactobacillus reuteri* was used to produce LA, and then, the same cells were employed as whole cell biocatalysts for 3HP production in co-culture with *Azospirillum brasilense* cells. The co-culture exhibited significant benefits over the mono-culture of *L. reuteri* in terms of decrease in production time and increase in 3HP titre. This could be attributed to the synergistic functioning of the two microorganisms for efficient conversion of glycerol to 3HP. The process parameters were optimized using Response Surface Methodology (RSM) for maximum production of 3HP. To the best of our knowledge, this is the first study that integrates the whole cell biocatalysis with microbial co-cultures to enhance the production of industrially important bio-based chemicals, 3HP and LA.

**Keywords:** 3-hydroxypropionic acid; Lactic acid; Co-culture; Response Surface Methodology; Process optimization

## Utilization of Cotton Stalk agro residues through SSF using white rote fungi for laccase production

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### Abstract

In the recent past, the demand for enzymes has been increased due to their significant role in moderating the use of agricultural residues. Lignocellulose biomass doesn't have much economic value. In India, the annual yield of Cotton stalk production is about 8 to 10 tons per hectare. In the present study, these agro residues were used as potential substrate for enzyme production by using superior (extracellular enzyme) laccase producing White rot fungus. Laccase production was carried out from (*Pycnoporous*, *T.versicolor* AHO 23, *T. versicolor* HBB 7328). Cultural, morphological analyses of selected strains were carried out. The production was further optimized under various physiological conditions. Among these *T. versicolor* HBB 7328 was the best laccase producer with 540 U/gm enzyme activity. Substrate optimization was also achieved with different parameters such as moisture content, particle size. Moisture content in biomass significantly affects the production rate and preliminary data concludes substrate to water ratio 1:4 was best.

**Keywords:** Laccase; Cotton stalk; SSF; White Rot Fungi

### Evaluation of bioactivity of compound produced from MTCC NSD 10072

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#### Abstract

The wide spread use of synthetic compounds as the antimicrobial compounds show side effect along the long term impact on our immunity or chance of developing the resistance against the immunity or may develop resistance against the particular microbe. So there is a need of such kind of compounds which are biologically active and non-toxic that can be used as antimicrobial agent. To meet the requirement of the present era there is need of such compounds that have biological origin. The research represent the isolation of such compounds and their potential as antimicrobial agent. To full fill the present requirement the compounds were isolated from the *Bacillus cereus* NSD10072 and the compounds were tested against the various microorganisms including the gram positive as well as gram negative. The compounds also tested for their anti-oxidant activity using the DPPH assay. The scavenging effect of metabolite in % scavenging activity tested at the different concentration (1, 0.5, 0.25, 0.12 and 0.06 mg/ml) i.e. 56.78, 51.93, 41.48, 27.61, 17.35 % respectively which is comparable with the ascorbic acid. The compounds show good antimicrobial activity having zone of inhibition 20mm (*E.coli*) and 22 mm for *S aureus* which is higher than the chloramphenicol (20mm). While comparing with standard the metabolite is more effective against *S. aureus*. Further the Nano formulation was done to find out the potential in the form of Nano formulation. The compounds are also active in the form of Nano particles. The study supports that the compound can be further used as an antibiotic or can be used in the pharma industry as a wound healing agent.

**Keywords:** *Bacillus cereus*; Wound healing agent; DPPH assay; Bioactivity; Antimicrobial activity

IMMT-48

### Microbiological activities of bioactive substances produced by Methylophilic *Pseudomonas* species isolated from Lonar lake of Buldhana district, Maharashtra

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#### Abstract

The alkaline Lonar Lake is a unique ecosystem formed by meteor impact, situated in the Buldhana District, Maharashtra, India harbours diversified microbial flora which can detoxify and degrade most harmful pollutant such as methanol. The Methylophilic which degrade harmful methanol can produce bioactive substances having antimicrobial potentials. The two methylophilic *Pseudomonas* species were isolated and characterized by cultural, morphological, biochemical tests and by 16S rRNA gene sequencing and identified as *P. aerogenosa* (DHT2) and *P. hibiscicola* (DHT 11). All these selected *Pseudomonas* species exhibited antimicrobial activities against pathogenic bacteria and data showed that and *P. aerogenosa* were stronger antimicrobial than to *P. hibiscicola*. The Present study provides primary evidence that isolated *Pseudomonas* species were promising sources for production of antimicrobial bioactive substances and represent a new and rich source of secondary metabolites that need to be explored in medical microbiology.

**Keywords:** Lonar Lake; *Pseudomonas* species; Antimicrobial bioactive substances

## Ethanol and xylitol fermentation from sugarcane bagasse derived sugars with multiple cell recycling by *Kluyveromyces marxianus* IPE453

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### Abstract

*Kluyveromyces marxianus* IPE453, a thermophilic yeast was evaluated for its application in a multiproduct biorefinery for alcohols production. Sugarcane bagasse (SCB) was selected as the feedstock for obtaining fermentable sugars pentosan (C5) and glucosan (C6) through acid hydrolysis followed by enzymatic saccharification. The pentose rich fraction was utilized by the yeast for cell generation and xylitol production with an average yield of  $0.315 \pm 0.01$  g/g while the entire glucose rich saccharified fraction was fermented to ethanol with a high yield (~90% of the theoretical) and productivity of  $0.9 \pm 0.08$  g/L/h. A complete material balance analysis with 2 kg of SCB resulted in 125.56 g xylitol and 289.2 g ethanol (366 mL) through multiple cells recycling. Genome analysis by next generation sequencing (NGS) method revealed the complete set of genes associated with strain's sugar transport and fermenting capability. The strain had a genome size of 10.1 million bases (Mb) with a coding length of 7.2 Mb with an exon to gene ratio of 0.98. The complete metabolic map illustrated that the strain possessed a compendium of genes involved in sugar transportation (C5 and C6), a set of alcohol dehydrogenase, xylose reductase, flocculation and thermo stability genes which enabled high temperature fermentation from glucose, galactose, fructose, xylose, lactose, sucrose as well as other low cost sugary, starchy and lignocellulosic biomass derived sugars. In conclusion, the strain was capable of producing both ethanol and xylitol with considerable yield and overall titers making our process advantageous over existing reports on lignocellulosic biomass valorization using yeast biocatalyst.

**Keywords:** Xylitol; Ethanol; Whole Genome Sequencing; *Kluyveromyces marxianus*; Sugarcane bagasse

## Isolation, Screening and Identification of Thermo and Inhibitor Tolerant Yeasts for Enhanced Bioethanol Production

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### Abstract

In recent past, the demand of biofuels (such as bioethanol and biodiesel) has increased due to their significant role in moderating the use of fossil fuels. Lignocellulose biomass conversion process includes a pretreatment step which generates inhibitory compounds affecting fermentation process. Therefore, the fermenting microorganisms should have the ability to tolerate inhibitory concentrations of such compounds generated during biomass pretreatment process. Thermotolerance of yeasts is another desirable trait during bioethanol fermentation via simultaneous saccharification and fermentation process. In the present study, superior bioethanol producing yeasts were explored from samples collected from sugar mills and distilleries. Out of total 150 thermotolerant yeasts isolated, thirty six were growing on yeast

peptone dextrose agar medium supplemented with inhibitory compounds (acetic acid 5g/L, furfural 1.5 g/L, HMF 1 g/L, Vanillin 1 g/L, ethanol 7% v/v). All the primarily screened isolates were evaluated for fermentation in medium having glucose concentration 150 g/L at 42°C. Cultural, morphological were carried out under various physiological conditions and four isolates were capable of producing higher ethanol titers (> 60.0 g/L) and identified by 5.8s rDNA amplification and sequencing as *Pichia kudriavzevii* JKH1, *Kluyvermyces marxianus* JKH4, *K.marxianus* JKH5 and *K.marxianus* JKH7. The maximum ethanol titer produced by the four isolates was 64.7 g/L, 61.75 g/L, 63.26 g/L and 61.75 g/L with productivity 2.6, 2.2, 2.4 and 2.3 g/L/h by strains JKH1, JKH4, JKH5 and JKH7, respectively. All the four isolates demonstrated temperature tolerance up to 45 °C; however, ethanol titer was compromised at 45°C and observed as half than that at 42°C. These thermo and inhibitor tolerant strains have potential for bioethanol production and could be further improved for enhancing their fermentation capabilities in presence of fermentation inhibitors while using undetoxified pretreated biomass slurry.

**Keywords:** Bioethanol; Thermotolerant yeast; Inhibitors; *Pichia kudriavzevii*; *Kluyvermyces marxianus*

# **MEDICAL AND VETERINARY MICROBIOLOGY**



# ORAL PRESENTATION





## Potential antifungal agents for the disruption of fungal cell membrane

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### Abstract

The global burden of fungal infections is continuously growing and in the present day world it has transitioned from a case specific observation to a major cause of high human mortality. An estimated 1.5 to 2 million people die of a fungal infection each year, surpassing those killed by either malaria or tuberculosis. Therefore, novel compounds with innovative methodologies need to be synthesised and evaluated for their antifungal potential. A novel synthetic methodology to access synthetic nucleosides was developed and 21 compounds were synthesised, chemically characterized and evaluated for their antifungal potential against *Aspergillus fumigatus* 3007. The effect of most active compounds on the permeabilization of fungal membranes was examined through confocal laser scanning microscope and the results indicated the fungal membranes to be significantly porous. The production of reactive oxygen species (ROS) was also evaluated and the synthesised compounds increased the ROS levels. To elucidate the antifungal mechanism of the compounds, sterols of treated fungal samples were extracted and analysed using UPLC and the results depicted reduction in ergosterol level in the presence of synthesised compounds when compared to control. The results demonstrated that the mechanism of action is the inhibition of lanosterol 14 $\alpha$ -demethylase, an enzyme involved in ergosterol biosynthesis. Moreover, the direct effect of synthesized compounds on fungal spores was also examined by flow cytometry and a substantial inhibitory effect was observed on fungal spore germination.

**Keywords:** Antifungal; *Aspergillus*; Lanosterol 14 $\alpha$ -demethylase

OP/MVM-50

## Molecular epidemiology of infectious bovine rhinotracheitis in bovines of Chhattisgarh

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### Abstract

Livestock production in Chhattisgarh are largely traditional and subsistence, vast majority being non-descript breed. Bovine population mainly consists of 9.81 million cattle and 1.39 million buffaloes. Infectious bovine rhinotracheitis (IBR) is a disease of cattle and buffaloes caused by bovine herpes virus-1 (BoHV-1). IBR results in infertility, anoestrus, repeat breeding, retained placenta, abortions and abnormal termination of pregnancy. The introduction of IBR into a cattle farm can cause severe economic losses due to weight loss, decrease in milk production and restrictions in the international livestock trade. In the present study, a total of 458 blood samples from cattle (423) and buffaloes (35) were collected from 10 districts of Chhattisgarh. These samples were from different sectors: organized (160), unorganized (298); sex: male (35), female (423); breeds: Cross bred cattle (166), Indian cattle breed (72), non-descript cattle (185); and clinical conditions in animals: with history of reproductive problems viz., infertility, anoestrous, repeat breeding, and abortion (107), without any clinical history (351). Total genomic DNA was extracted from all the blood samples

and was subjected to PCR amplification using BoHV-1 gB gene specific PCR primers (Forward 5'TACGACTCGTTCGCGCTCTC3' and Reverse 5'GGTACGTCTCCAAGCTGCCC3') with an amplicon size of 478 bp. Out of 458 blood samples, 122 were positive for BoHV-1 with an overall prevalence rate of 26.64% . About 26.95% of cattle were found positive in comparison to 22.86% positivity in buffaloes. Sex wise, higher rate of positivity was seen in male (28.57%) than in females (26.48%) population. About 31.33% cross bred, 23.61% Indian breeds, 24.86% non-descript animals were positive for IBR. The positivity didn't varied significantly between apparently healthy animals (26.78%) compared to animals with a history of reproductive problems (26.17%). Overall findings indicate that IBR was highly prevalent in Chhattisgarh, thus warranting a need for implementation of control strategies for IBR in the bovine population of Chhattisgarh.

**Keywords:** Infectious bovine rhinotracheitis (IBR); Bovines; Reproductive problems; Molecular epidemiology; PCR

RF/MVM-07

### Antibacterial-guided isolation of constituents from *Senna alata* root against standard MTCC and clinical isolates

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#### Abstract

Plants are capable of synthesizing an outsized form of secondary metabolites, i.e., alkaloids, coumarins, flavonoids, glycosides, quinones, phytosterols, saponins, tannins and terpanoids that contribute to varied medicinal activities. Chhattisgarh is the twenty sixth state of Indian union; it had a quarter mile of its geographic region coated with forest. Weed flora that are one amongst the necessary a part of the piece of land and *Senna alata* is additionally a weed plant so present investigation deals with the antibacterial activity of ringworm shrub completely different elements viz., flower, fruit, leaf, stem and root of ringworm shrub were collected from completely different agroclimatic zones of Chhattisgarh. Ringworm shrub is employed in inflammatory disease, asthma, cardiovascular disease, erythrocyte anemia, diabetes, hepatitis, skin condition, jaundice, eczema, ringworm, constipation, gastrointestinal disorder, burns, wounds, skin infection, looseness of the bowels and higher tract infection. The bactericide and synergistic activity was tested against 10 MTCC and 5 clinical pathogens by agar well diffusion methodology. The results disclosed that solvent extract of root had high repressing activity against *Listeria monocytogenes* MTCC 1143. The phytochemical screening of the plant disclosed the presence of organic compound, flavonoid, phenolic, glycoside and tannic acid. Purification of root acetone extract of *Senna alata* using thin layer chromatography, column chromatography and high performance liquid chromatography by using a gradient mixture of organic solvent with increasing polarity. To substantiate the presence of alkaloid, atropine in standard compound was characterized on the basis of UV-VIS qualitative analysis, FTIR, LC-MS, H<sup>1</sup> and C<sup>13</sup> magnetic resonance spectroscopic strategies and confirmed compound belong to the alkaloid compound.

**Keywords:** Antibacterial activity; alkaloid; FTIR; LCMS; NMR

***In-vitro* and *In-vivo* assessment of streptomycin and rifampicin therapy: A promising synergistic combination against wound infections of *Acinetobacter baumannii***

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**Abstract**

The damaging effects of antimicrobial resistance have globally manifested themselves as a continuing challenge to human health, costing hundreds and thousands of lives. The emergence of multidrug resistant (MDR) Gram negative bacterial pathogens, particularly carbapenem resistant *Acinetobacter baumannii* poses a major threat to the existing antibiotic arsenal. Global outbreaks of *A.baumannii* in hospital and military bases concomitant with the emergence of highly resistant and virulent isolates, places this pathogen in the category of six most problematic MDR pathogens, by Infectious Diseases Society of America (IDSA). Here, we report the synergistic interaction between the antibiotics, streptomycin and rifampicin which exhibits excellent bactericidal activity against MDR *A. baumannii* clinical isolates. The combination displayed effective penetration and disruption of preformed *A. baumannii* biofilms, thus causing a considerable reduction in the viable cell count. In combination, streptomycin and rifampicin downregulate the expression of *csuA*/BABCDE genes of the type I pilus system, which plays an essential role in *A. baumannii* biofilm formation on biotic and abiotic surfaces, thus acting as a major virulence factor. Studies on the murine wound infection model further established this combination to be a promising treatment option for topical administration.

**Keywords:** *Acinetobacter baumannii*; Drug resistance; Virulence factors; Biofilms; Topical administration; Wound infection



# POSTER PRESENTATION



## Antibiofilm and antibacterial activity of a novel curcumin-molybdenum disulphide nano- hybrids against hypervirulent *Klebsiella pneumoniae*

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### Abstract

The recent emergence of hypervirulent clinical variants of *Klebsiella pneumoniae* (hvKP) emphasized on the urgent need to look for specific and effective therapeutic alternatives against the said pathogen. A new type of MoS<sub>2</sub>-modified curcumin nanostructures (MQCs) has been developed and evaluated against said bacteria to access its potential as anti-bacterial as well as anti-biofilm agents. The curcumin was fabricated with MoS<sub>2</sub> via a seed-mediated hydrothermal method, characterized, and the antimicrobial and antibiofilm activities were evaluated against hvKP as well as other select multi drug resistant bacterial isolates. We investigated the mode of action of MQCs for changes in membrane dynamics using 1, 6-diphenyl-1,3,5-hexatriene (DPH), membrane permeabilization using Sytox green, membrane depolarization utilizing DiSC<sub>3-5</sub> utilizing flow cytometry, confocal microscopy, and steady state fluorimetry. We further investigated in vitro biocompatibility utilizing sulforhodamine B assay, hemocompatibility assay, and lactate dehydrogenase assay, while in vivo biocompatibility was tested utilizing Charles Foster Rats by histopathological screenings. The MQCs inhibited the bacterial growth at a minimal concentration of 0.0156 µg/ml while complete inhibition was evinced at concentration 0.125 µg/ml. At the concentration 0.05 µg/ml, 97% inhibition of biofilm was noted. The MQCs induce a greater susceptibility of the cell membrane to disorganization in terms of acyl shifting (the shielding of the superficial membrane positive and zwitter ion charges) and dynamics (in liquid crystalline phase), which facilitate their self-intrusion across the thickness that eventually lead to the oozing out of the cytosolic contents. MQCs were found non-toxic to the SiHa cells and Charles Foster Rat at dose as high as 1024 µg/ml. The present study reports the development of a safe antimicrobial agent against hvKP as well as other MDR bacterial isolates. The drug may be further evaluated for its use as a broad spectrum antibacterial and antibiofilm agents.

**Keywords:** Quantum dots; hypermucoviscous; Atomic force microscopy; Transmission electron microscopy; Biofilm

MVM-02

## Classification of multidrug resistant *Klebsiella pneumoniae* based on biofilm forming capacity and elucidation of its biofilm chemistry

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### Abstract

The increasing menace of *Klebsiella pneumoniae* as multidrug resistant (MDR) opportunistic pathogen in hospital settings is a worldwide problem. This study was undertaken to classify the *Klebsiella pneumoniae* based on their biofilm forming ability, and for detailed elucidation of biofilm chemistry.

A prospective study on 257 isolates of *Klebsiella pneumoniae* isolated from the clinical samples of ICU, for biofilm formation was performed employing crystal violet assay. Further, antimicrobial sensitivity testing was performed by disc diffusion testing employing select antimicrobial agents. Estimation of total proteins, total sugars, uronates, and acetyl content was done. Besides, confocal microscopic evaluation of the spatial distribution of sugars and proteins were performed using Concanavalin A-TRITC and phalloidin green. Fourier Transform Infrared Spectroscopy (FTIR), and Nuclear magnetic resonance spectroscopy (NMR) were performed on the extracted biofilm matrix while 1-D SDS-PAGE, HPLC, and MALDI MS/MS were performed for isolated protein characterizations. Out of 257 isolates, 32 (12.45%) isolates were non-biofilm former, 71 (27.62%) were weak-formers, and 136 (52.91%) were medium formers while 18 (-7%) isolates were high formers. Resistance was observed to be higher against piperacillin-tazobactam (91.42%), ciprofloxacin (87.14%), imipenem (61.42%) compared to meropenem (54.28%). Total sugar content, uronate content, total acetyl content and total protein content were found to be 2257.207±14.791; 325.3±33.58; 28.42±4.214; and 525.2±2.082 µg/ml respectively. CLSM, FTIR and NMR confirmed juxtapositioning of sugars and proteins in the matrix. SDS-PAGE, HPLC, and MALDI MS/MS indicate 55 different proteins; 22 proteins were related to protein synthesis while 15 were identified related to virulence, eight were related to energy and metabolism while four with capsule and cell wall synthesis. The study indicates presence of significant amount of hydrophobic protein domains, which may be responsible for the stability of the sugar rich biofilm of *Klebsiella pneumoniae*, responsible for its better survival.

**Keywords:** Virulence; Concanavalin A; Galacturonic acid; Glucuronic Acid; Resistance  
MVM-03

**Evaluation of antimicrobial activity of the forest derived soil actinomycetes  
*Streptomyces* sp. PBR21 against  
Carbapenem resistant superbugs**

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**Abstract**

In the present study, an actinomycetes isolate, *Streptomyces* sp. PBR21 was isolated from the soil samples of Pobitora Wildlife Sanctuary of Assam, India. The isolate was identified as *Streptomyces* sp. PBR21 (Genbank accession no. MK981152) based on phenotypic and molecular characterization. The strain PBR21 exhibited promising antimicrobial activity against the Carbapenem Resistant Enterobacteriaceae (CRE) isolates which were isolated from the urine sample of patients suffering from Urinary Tract Infection (UTI). Metallo-β-lactamase (MBL) production by the test UTI pathogens were confirmed by the following three tests namely, Imipenem-EDTA disc potentiation test, AmpC disc test and Hodge test. Further, the test pathogens were evaluated for the presence of metallo-β-lactamase ( $bla_{MBL}$ ) resistant gene. Out of 25 clinical isolates, two isolates namely, AN3 and AN4 were found to be positive for  $bla_{SPM}$  gene and also the positive control *Escherichia coli* ATCC 2469 showed the presence of  $bla_{NDM}$  gene. These two clinical isolates were identified as *Klebsiella pneumoniae* (AN3) and *Escherichia coli* (AN4) based on their molecular characterization by 16S rRNA gene sequencing. Extract of the fermented broth culture of PBR21 was prepared with organic solvent extraction method using ethyl acetate. The extract showed a significant growth inhibition activity against AN3, AN4 and ATCC 2469 (positive control). The ethyl acetate extract of PBR21 showed lowest minimum inhibitory concentration (MIC) of 9.37 µg/mL against *Klebsiella pneumoniae* (AN3), 18.75 µg/mL against both *Escherichia coli* (AN4) and *Escherichia coli* ATCC 2469 (positive control). Scanning electron microscopy (SEM) revealed that treatment of AN3, AN4 and



ATCC 2469 (*positive control*) with the extract of PBR21 destroyed the targeted cells with prominent loss of cell shape and integrity. This study gives an idea that this actinomycetes strain (PBR21) can be a potential antimicrobial agent against Carbapenem resistant gram-negative pathogens. Thus, further study is needed on its bioactive constituents that can be useful in the pharmaceutical sector.

**Keywords:** Actinomycetes; Carbapenem resistant; Metallo- $\beta$ -lactamase; Urinary Tract Infections; Scanning electron microscopy

MVM-04

### Inhibitory effects of Indian herbs on drug resistant Human pathogenic bacteria *Helicobacter pylori*

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#### Abstract

The Problem of antibiotic resistance, as well as the evolution of new strains of disease causing microbes, is of great concern to the global health care community. Our ability to effectively treat disease is dependent on the development of new pharmaceuticals, and one potential source of novel drugs is Indian traditional system of medicine. This study explores the antibacterial activity of aqueous, organic extracts of *test herbs* against different pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli*, *Helicobacter pylori* and *Pseudomonas aeruginosa*. Anti-bacterial activity of all the herbal extracts was measured by Filter paper disc method. The present study demonstrates potentially good antibacterial activity against all the test pathogens. They could be exploited as alternatives to common antimicrobial agents for treatment of bacterial infections in future chemotherapy. There is a growing need to find new medications to be used against *H. pylori*, due to the widespread of this bacterium, its serious pathogenicity and emerging of many resistant strains. As a result, many natural products have been studied to find new effective alternative drugs against difficult to treat *H. pylori* giving special attention to plants used traditionally for a long time against gastrointestinal disorders.

**Keywords:** Antibiotic Resistance; Traditional medicine; Future chemotherapy

MVM-05

### *In silico* analysis of antimicrobial mode of action of bioactive polyketide from *Xylaria* sp.

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#### Abstract

Antibiotic resistance as a survival mechanism of microorganisms poses a greater threat to society from the past few decades. Alongside it encouraged the researchers to develop or find new pharmaceutical agents to combat the antibiotic resistance. Natural product drug discovery gained its importance over synthetic drugs with their minimal side effects. *In silico* analysis or virtual screening of natural product drugs helped to understand the mode of action of their targets in a better possible

way. In the present work, docking studies were performed to analyse the mode of action of an antimicrobial polyketide coriloxin isolated from the mycoendophytic *Xylaria* sp. NBRTSB20. In silico antimicrobial mode of action of coriloxin was predicted using Maestro version 11.2 software against the various targets such as cell wall inhibitors (PDB IDs: 5CG1, 1XFV, 2I80, and 4R7U), protein synthesis inhibitors (PDB IDs: 1OB2, 1QU3, 1JIJ, and 6MIJ), nucleic acid synthesis inhibitors (PDB IDs: 4PRX, 3FFI, 3ZKB, and 3RAE), antimetabolites (PDB IDs: 3TYE, 1AJ0, 3SRW, and 1AI9), and other cytoplasmic targets (PDB IDs: 3QP5, 1EA1, 3JVV, and 3VOB). Bioactive polyketide coriloxin was perfectly docked into all the selected targets and actively interacted. Antimicrobial mode of action of coriloxin was predicted based on the docking scores obtained by Glide docking process. Broad spectrum antimicrobial polyketide coriloxin from *Xylaria* sp. NBRTSB20 tends to actively interrupt the nucleic acid synthesis by interacting with the DNA Gyrase. Along with the nucleic acid synthesis inhibition, coriloxin also interacts strongly with some of the cytoplasmic targets. Virtual screening of selected bioactive metabolites against a panel of antimicrobial targets helps in the prediction of antimicrobial mode of action, which elevates the pharmaceutical significance of the molecule.

**Keywords:** Molecular Docking; Mode of action; Nucleic acid synthesis inhibitors; *Xylaria* sp.; Polyketides

MVM-06

### Conceptual analysis of Soy Protein for Human Nutrition

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#### Abstract

To know Soy protein, is it good for human nutrition, popularize soy foods among human, explore more soy products for human nutrition, explore soy protein as an alternative to meat, explore genetically modified soybeans, explore soy milk as higher protein for human nutrition, understand soybean oil is a higher in protein and good for human nutrition, meal, percentage, analyze soybean flakes, with percentage of soy protein content, evaluate soybean products is it used for proper human consumption, explore Common soybean products include soy sauce, soy milk, tofu, soy meal, soy flour, as a textured vegetable protein, analyze, evaluate is it having higher levels of protein, thiamine, riboflavin, phosphorus, calcium, and iron for human consumption. The study mainly focuses on exploring the Soy Protein and to make the Soy Protein the best replacement for protein diets like meat etc. and also for having some changes in lifestyle especially in food intakes habits for the patients who are diabetic and hyperlipidemic in nature. For this purpose study has been conducted in various laboratories like Krishna Path Lab, Dr Lalpathlabs and cardiology centre like Jalandhar Heart Care centre. Then this data has been analyzed by max, mean and standard deviation and found that there is significant change in health condition of patients having soy protein as food supplement in their diet.

**Key Words:** Meat; Soy Protein; Diet

MVM-08

### Role of Lactic Acid Bacteria in sustainable development of Humans

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### Abstract

Lactic Acid Bacteria (LAB) are considered to be the most promising supplement for enhancing the biological activities of human life. These bacteria have been used as probiotics since years in the traditional as well as modern industrial food fermentation due to its health benefits. The proposed health benefits are blood pressure lowering, prevention of colon cancer, reduction of *Helicobacter pylori* infection, reduction of allergic symptoms, managing lactose intolerance, boosting of immune system, reduction of inflammation and cholesterol; prevention of urogenital infections, antimicrobial effects on pathogenic microorganisms, prevention of osteoporosis, beneficial effects on mineral metabolism and many more. Therefore, the search of novel LAB is the necessity of time. The objective of our study is to evaluate the probiotic potentials of LAB from Human Colostrum. Human Colostrum (HC) was chosen for our study because of its richness in all kinds of nutrients and the presence of large number of bacteria with probiotic potentials. It is also the most essential requirement for proper growth and development of infant. Our study found numerous LAB from HC which fulfilled all the standard criteria for probiotics. After biochemical characterization, the isolates were identified as *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Pediococcus* and *Aerococcus*. On the basis of their resistance against antibiotics and antimicrobial activity, few isolates were found to be very promising that have great potential as a supplement for human consumption. The entire work will briefly describe the importance of LAB in human life.

**Keywords:** Human Colostrum; Lactic Acid Bacteria; Gut Microbiota; Probiotics; Sustainable Development

MVM-09

### Occurrence of fungi causes otomycosis in rural areas

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### Abstract

The aim of this study was to determine etiological agents and predisposing factors of otomycosis. This disease is involved in various age and sex groups. A total 50 clinical specimens are collected from external auditory canal. Clinical specimens were collected by sterile cotton swabs and inoculated on Sabouraud's dextrose agar. Mycological examination of all specimens was done to isolate fungal agents that are involved in otomycosis. In present study *Aspergillus niger* (32.22%) and *Candida* species 15(16.6%) are the major fungal isolate. Higher incidence of otomycosis is predominant seen in male patients between 21-30 years of age. Pruritus is the most common fungal symptoms of otomycosis. In this study high incidence of otomycosis are encountered in rural areas peoples, because due to high humidity, warm and dusty environment. The main focus is to educate the people about predisposing factors (self cleaning) and to aware about treatment methods of otomycosis.

**Keywords:** Aspergillus; External auditory canal; Pruritus; Pre disposing factors; otomycosis

MVM-10

### Therapeutic potential of ethnomedicines against inflammatory disorders

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### Abstract

Generally, plants contain various biologically and medicinally active compounds with structural diversity which are mainly utilized for the treatment of mild or chronic diseases. With negative side effects and low cost, the demand of ethnomedicines is very high at global scale. WHO claims that nearly 80% of world population trust over ethnomedicines, mainly for their basic healthcare needs. At present, novel and re-infectious diseases are growing at a very fast rate with an urgent appeal to find out new antimicrobial compounds having novel way of action. The entry of multidrug resistant strains (MDR) in human society pushes the researchers to get the antimicrobial compounds. Secondary metabolites like tannins, alkaloids, terpenoids, flavonoids of various plants are the rich sources of antimicrobial properties. Various antimicrobial compounds are capable of restricting the growth of MDR strains even at a very low concentration. Many compounds like Chrysin, Baicalin, Apigenin possess anti-oxidant as well as anti-inflammatory properties. The work investigated over these compounds represents their ability to abate the expression of various cytokines and chemokines. These properties make them suitable for various disease models like diabetes, asthma, neurodegenerative diseases, carcinogenesis, etc. Here, we mainly focus on the future development of therapies based on ethnomedicines which are quite promising.

**Keywords:** Ethnomedicines; Antimicrobial; MDR; Baicalin; Chrysin

MVM-11

### Lithium Chloride reduces Newcastle disease virus-induced ER stress

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### Abstract

Newcastle disease is a highly infectious disease of poultry. The causative agent, Newcastle disease virus (NDV) is an enveloped virus with a single-stranded negative-sense RNA genome of approximately 15kb in size. Although several vaccination strategies are already in place, several outbreaks have been reported in vaccinated populations. The lack of therapeutic against NDV makes the development of antiviral drugs essential. Lithium Chloride (LiCl) is a widely prescribed drug for the treatment of bipolar disorder, acute brain injuries, and chronic neurodegenerative diseases. In addition, LiCl has also been repurposed as an effective antiviral for many viral diseases. In the present work, we have analyzed the ability of LiCl to reduce NDV replication *in vitro* and *in ovo*. Our results suggested that LiCl effectively reduces the NDV production in chicken embryo fibroblast cells and embryonated chicken eggs. We also demonstrated that NDV induces the expression of ER stress-related proteins, mainly GRP78, a molecular chaperone required for ER integrity. The results suggested that LiCl protects the cells from ER stress caused by NDV infection.

**Keywords:** Newcastle disease; Lithium Chloride; Chronic Neurodegenerative Diseases

MVM-12

### Isolation, characterization and *in vivo* efficacy of bacteriophages isolated against *Vibrio* sp.

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### Abstract

The annual output in aquaculture has increased from 0.75 million to 9.6 million tonnes in 63 years, with India being the second largest producer globally. Bacterial infections are causing enormous economic loss. The objective of our study was to isolate and evaluate the potential of lytic phages against aquaculture infections. Samples were collected from several water sources belonging to different geographical locations of the southern states of India. A total of 150 different strains of *Vibrio* sp. were isolated from the water samples. Phages were isolated using enrichment method. Phage stability studies, lifecycle using adsorption and one-step growth curve and *in vitro* and *in vivo* (using zebrafish model) phage efficacy were determined. Of the eight phages isolated, two lytic phages (Phage 19 and 57) were studied further. The morphology of the plaques were observed to be circular, transparent and medium-sized. Stability studies revealed that the phages were stable at a wide range of temperatures (-20°C to 60°C), pH (4-11), solvents (chloroform, phenol, ethanol, isopropanol) and detergents (SDS, Tween 80, CTAB). One-step growth curve revealed that the phages 19 and 57 had an adsorption time of 18±1 and 15±1 mins and the latency period of 25±3 and 21±1 min with a burst size of 120±20 and 50±5 phages respectively. *In vitro* study (MOI=0.5) showed that within 12 h the bacterial load was reduced by five and seven log units by phage 19 and 57 respectively. The *in vivo* study showed that the bacteriophages were able to increase the survival rate of *Danio rerio* up to 45% and 40% by phages 19 and 57 respectively within 48 h of treatment. Our study indicated that phages are an excellent source of biocontrol agents against *Vibrio* sp. With the implementation of phages in therapy economic burden due to bacterial infections can be reduced.

**Keywords:** Aquaculture infections; Antibiotic resistance; Bacteriophages; Biocontrol agents; *Vibrio* sp.

MVM-13

### Characterization of Chicken Chemerin and its potential use as an antiviral against Newcastle disease virus

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### Abstract

Chemerin, also known as retinoic acid receptor responder protein 2, is an adipokine secreted by white adipocytes. It is also expressed by different tissues such as white and brown adipose tissue, liver, kidney, lung, pancreas etc. Chemerin is shown to modulate blood pressure, immune responses, angiogenesis, adipocyte differentiation and carbohydrate metabolism. Newcastle disease virus (NDV) is the causative agent of an infectious disease in poultry. In the present study, we have cloned and characterised the chicken chemerin using a eukaryotic expression system. Our results showed effective reduction of NDV in the presence of chemerin both in chicken embryo fibroblast and embryonated chicken eggs. The reduction of NDV post chemerin treatment was supported by a decrease in viral gene as well as protein expression. We are trying to understand the underlying mechanism of anti-viral effects of chemerin against NDV using bioinformatics and other molecular biology approaches.

**Keywords:** Chemerin; Adipokine; Newcastle disease virus; Angiogenesis; Adipocyte

## Herbonaturals for the treatment of fungal dermatophytic infection

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### Abstract

Dermatophytosis is a common infectious disease induced by fungi which is recognized as dermatophytes. The term 'keratinolytic' is used for fungi inculcated with the capabilities to attack and use keratin with the aid of enzymes. Nail, skin cells, and hair are the parts of human body which are rich in keratin content. Dermatophytes are group of fungi that infect keratinized tissues of human and animals. The group consist of three different genera namely, *Trichophyton*, *Microsporum*, *Epidermophyton* and several species within each genera. Although these infections are treatable and many commercial drugs are available that can be applied topically (Tinactin, Naftin, Butenafine) on the infected areas but they have very low efficacy and high probability of relapse. To increase the efficacy of treatment, the patient are given supplementary oral medicines (fluconazole, itraconzole, ketoconzole) for prolong duration that leads to hepatotoxicity. There is an urgent need of formulating some Bio-Derm medicine that process better efficacy without any associated side effect on human body. The aim is evaluate the potential antifungal plants against human pathogenic dermatophytes. In this present study, involve the usages of leaves of plants (*Acacia nilotica*, *Catherenthus roseus*, *Lawsonia inermis*, *Thuja occidentalies*, *Tagetes erecta* and *Ricinus communis*) and by extractions of their methanol i.e. extract and testing them against *Aspergillus flavus*, *Cladosporium* sp., *Fusarium* sp., *Drechslera* sp., *Penicillium* sp., Isolated for affected sites from human their phytochemicals like alkaloids, flavonoids, tannins, Sponoin and phenol were isolated & tested for antifungal activity with respect to Fluconazole. Plants under testing exhibit antifungal activity against all the dermatophytes fungi significant control of test fungi were exhibited by *Acacia nilotica*, *Catherenthus roseus*, *Lawsonia inermis*, *Thuja occidentalies*, *Tagetes erecta* and *Ricinus communis* anti-dermatophyte activity. Best results were shown by Flavnoid of *Ricinus communis*, tannin of *Lawsonia inermis* and *Ricinus communis*.

**Keywords:** Dermatophytes; Phytochemicals; Antifungal activity

## Protease cleavage of Bovine Adenovirus (BAdV) -3 protein VII (pVII) in transfected cells.

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### Abstract

The final maturation step for the production of infectious progeny adenovirus requires proteolytic cleavage of six adenoviral proteins by adenovirus protease. However, it is not clear if proteolytic cleavage of each of these proteins is essential for the production of progeny adenovirus. Bovine adenovirus-3 (BAdV-3) is a non-enveloped, double-stranded DNA virus, classified as member of *Mastadenovirus* genus. One of proteins encoded by BAdV-3 designated as pVII is a basic abundant core protein, which appears to be cleaved by BAdV-3 protease. Here, we report that BAdV-3 pVII is

cleaved by BAdV-3 adenovirus protease at potential consensus protease cleavage site. The cleavage of pVII appears conserved in other members of *Mastadenovirus* genus. To determine the requirement of pVII protease cleavage for production of progeny virus, we are constructing a recombinant BAdV-3 expressing pVII containing mutated protease cleavage site. Analysis of this mutant BAdV-3 should provide evidence regarding the requirement of proteolytic cleavage of pVII for production of infectious progeny virions.

**Keywords:** BAdV-3; pVII; Protease cleavage

MVM-16

### Development and application of a novel colorimetric isothermal amplification assay for fast detection of drug resistance in *Plasmodium falciparum* malaria

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#### Abstract

*Plasmodium falciparum* malaria remains one of the major public health concern. Sulphadoxine and pyrimethamine (SP) have been used as long-acting partner antimalarial drugs in artemisinin combination therapy (ACT) for falciparum malaria. The emergence and increasing spread of SP resistance in malaria-endemic areas have become a challenge for the control of malaria. Presently, many strains of *P. falciparum* are SP resistant due to a point mutation in codon A437G of the *Pfdhps* gene and codon S108N allied with *Pfdhfr* gene. The existing molecular methods for antimalarial drug resistance detection are time consuming, sophisticated and require trained personnel with standard laboratory infrastructure. Moreover, their use is limited and challenging to implement in resource limited settings. Isothermal amplification based approaches open new horizons in diagnosis and drug resistance detection of various infectious diseases. In this study we developed an instrument free colorimetric loop mediated isothermal amplification (LAMP) approach for visual and fast detection of SP drug resistance. The optimization has been done on the well characterized 80 *P. falciparum* isolates including wild type (3D7) and mutant (DD2) control strains. The amplified positive product is differentiated by visual color change reaction, and the specificity of developed assay was further confirmed by RFLP and gold standard sequencing method. This developed colorimetric assay is easier to access, less time consuming, highly sensitive (7 parasite/ $\mu$ l), with good specificity and stability and demonstrated a robust, rapid and user-friendly alternative for visual detection and monitoring of SP resistant mutants directly in clinical samples of *P. falciparum* malaria. Moreover, this approach has the potential to be applied even in resource limited settings and also in laboratories unfamiliar with PCR or other molecular methods, and in future, this can be helpful for the better management of anti-malarial policy.

**Keywords:** *Plasmodium falciparum*; Drug resistance; Loop mediated isothermal amplification (LAMP).

## Recent advances in periodontal microbiology

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### Abstract

Microbial members of the subgingival plaque community play a major role in the initiation and progression of periodontal diseases. Majority of these bacteria are anaerobic in nature and several anaerobic systems have been used for their cultivation. Among them anaerobic jars are the most popular and are routinely used for the detection of periodontal pathogens from clinical samples. Despite best efforts, a significant portion of oral microbes have not yet been cultivated and several hypotheses have been put forth to explain this anomaly. This has led to renewed efforts to cultivate the oral bacteria so far identified only by their molecular signatures resulting in improvisation of existing culture techniques and devising novel methods of isolation. Several devices have been used on environmental samples successfully: One method called "minitraps" has been successfully adapted to oral cavity and has shown great promise in isolation of not yet cultivated oral bacterial species. These newer techniques are sure to shed more light on the role of microbes in the etiology of periodontal diseases.

**Keywords:** Anaerobes; Culture; Diffusion Chamber; Minitrap

## Prevalence and induction of multiple drug resistance in Gram-negative Bacilli

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### Abstract

Multi-drug resistant pathogens and antibiotic toxicity are two greatest challenges faced by today's medical world. The inappropriate use of antibiotic dosage has led to the increased resistance in Gram-negative bacilli (GNB). Present investigation reports the prevalence and induction of drug resistance mechanisms among the gut microflora. Sewage and human stool samples were collected from different locations and individuals of Indore city respectively. GNB were isolated from these samples. They were biochemically characterized and analysed for drug resistance mechanisms. Majority of the isolates; 36% of the total were *E. coli*, *Pseudomonas sp.* was the next abundant organism i.e. 18% and 12% of the isolates were *Vibrio sp.* Remaining 34% isolates were *Klebsiella sp.*, *Acinetobacter sp.*, *Citrobacter sp.*, *Enterobacter sp.*, and *Aeromonas sp.* Among the lactose fermenting GNB 82% of the isolates exhibited resistance to commonly used drugs which is an alarming situation. Much serious concern is the occurrence of ESBL (Extended Spectrum of Beta Lactamase), MBL (Metallo Beta Lactamase) and HL Pse (High Level Penicillinase) resistance mechanism in 53% of the isolates. Non-fermenting GNB were investigated for MBL resistance mechanism, 27% of the isolates emerged as drug resistant. Some isolates exhibited possible carbapenem impermeability. A strain of *Klebsiella* which was initially sensitive to beta lactam drugs developed marginal resistance in 24 hours when co-cultured with resistant *E. coli* strain. Complete expression of resistance gene was observed in 48 hours and the organism exhibited (HL AmpC  $\beta$ ) High Level AmpC  $\beta$  lactamase resistance mechanism.



*Pseudomonas* isolate PS1; which was originally sensitive to Piperacillin-Tazobactam, was found to acquire resistance within 24 hours when exposed to drug concentrations lower than MIC. This indicates that free availability of antibiotics and of drug resistant microbes in environment naturally increase the drug resistance mechanisms besides human intervention.

**Keywords:** Multi-drug resistant; GNB; Beta lactam; Resistance Mechanism; Antibiotics.

MVM-19

### Acid stress based *Mycobacterium fortuitum* surrogate model for *Mycobacterium tuberculosis* persistent infection

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#### Abstract

One-third of the global human population harbours *Mycobacterium tuberculosis* in latent form with the potential to reactivate and infect, imposing major challenge to eradicate tuberculosis. Absence of a holistic model is a major bottleneck in management of potential latent form of tuberculosis. Literature review and bioinformatics analysis based on presence as well as alignment of major genes involved in latency led to short-listing of fast-growing, pathogenic, Non-Tuberculous mycobacterium - *Mycobacterium fortuitum*, as a probable model organism. Latent mycobacteria reside inside granuloma as Non-Replicating Persistent (NRP) state, where acidic stress conditions are prevalent as part of host immune response. Hence, to mimic this acidic stress in vitro, *M. fortuitum* was subjected to acidic conditions varying from pH 7.0 to pH 3.0. Analysis of viable count under different pH conditions resulted in constant CFU of *M. fortuitum* under acidic stress, indicative of an NRP state. Electron microscopy showed a reduction in the size of *M. fortuitum* bacilli to coccobacillary form. Further, loss of acid fastness, resistance to commonly used antimycobacterials, and reduction in metabolic activity under acidic stress confirmed the NRP state of *M. fortuitum*. Molecular validation of acid-induced *M. fortuitum* NRP state through qRT-PCR showed up-regulation of NRP state marker genes namely *icl*, *sigH*, *pcaA*, *narK2* and *narX*. Up-regulated genes in *M. fortuitum* NRP state have been known to play important *M. tuberculosis* latency. Thus, we established *M. fortuitum* as a model organism that mimics latent *M. tuberculosis* infection and can be utilised for studies related to *M. tuberculosis* persistent infection, target identification and rapid screening of potential lead molecules.

**Keywords:** *Mycobacterium tuberculosis*; Acidic stress; *Mycobacterium fortuitum*; Latent infection; In vitro model

MVM-20

### The microbiological progression and peculiarities of probiotics combative/militant to Lactobacilli species seeding/rooting Bacterial vaginosis

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### Abstract

The advance clinical manifestation of the microbial infections in women includes diagnosis and treatment of many lower reproductive tract infections especially in developing countries where there is a serious need for development. Bacterial Vaginosis which is termed as a serious microbiological disease have the attributes of the shift in the normal flora from the dominant species to a poly-microbial flora. The causative factor of the disease is the largest known genus *Lactobacillus* which consists of about 50 different discovered species. The microbiological action towards the disease includes variables like Bacteriocin and Probiotics. The development of the strain includes the involvement of the techniques like RAPD for the identification and classification of the desired strain for the treatment. The media used for the studies include MRS Broth and agar, TGYE media for the plating. This indicates positive results in the medical technology and proves to be a promising treatment for the reproductive tract infections caused in human population.

**Keywords:** Bacterial Vaginosis; Probiotics; Bacteriocin; *Lactobacillus*; Reproductive tract infection

MVM-21

### Repurposing screen identifies a combination of Verapamil and Metronidazole as antibacterial and antibiofilm agents for multi-drug resistant *E. coli* and *Salmonella*

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### Abstract

Pathogenic *E. coli* and *Salmonella* are important Gram-negative bacterial pathogens worldwide. Presently, long-term use of antimicrobial leading the emergence of multi-drug resistant (MDR) *E. coli* and *Salmonella*. Further, biofilm formation ability of both *E. coli* and *Salmonella* also significantly associated with their increased survival and persistence in hosts. Drug-repurposing of approved drugs is a fast track approach for the drug development process. Since in the present study we have screened the combinations of FDA approved drugs as antibacterial and antibiofilm agents against poultry and vegetables/fruits-origin MDR *E. coli* and *Salmonella* strains. A combination screen of 30 non-antimicrobial FDA approved drugs using a bacterial growth inhibition assay led to the identification of Verapamil and Metronidazole as active antibacterial agents for MDRE. *coli* and *Salmonella*. Further analysis revealed that the combination of Verapamil and Metronidazole was potent biofilm inhibitor against MDR *E. coli* and *Salmonella*. Here, we provide *in vitro* evidence of the combination of Verapamil and Metronidazole as antibacterial and antibiofilm agents against MDR Gram-negative bacteria. In conclusion, the present study highlights the therapeutic potential of non-antimicrobial drugs Verapamil and Metronidazole to be repurposed to treat chronic infections caused by MDR *E. coli* and *Salmonella*

**Keywords:** *E. coli*; *Salmonella*; Multi-Drug Resistant; Biofilm; Drug Repurposing

MVM-22

### Gut microbiome alteration in mice exposed to multi-metal mixture

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### Abstract

Exposure of animals and humans to different metal components through contaminated drinking water can result in a wide range of gut microfloral changes. The gastrointestinal tract microflora has a distinct capacity to deal with the increased load of ingested metals. However, heavy metals may have harmful effects on gastrointestinal tract microflora. In this study changes in the population of intestinal microflora in healthy mice were examined following exposure to mixture of toxic heavy metals (mostly present as groundwater contaminants) through drinking water. Five experimental groups of swiss male albino mice were exposed to the metal mixture at 0, 1, 10, and 100 times the mode concentrations (the most frequently occurring concentration) of the individual metals in drinking water for 30 days. The control group was given water at a concentration equal to the maximum permissible limit (MPL) of individual heavy metal. Fecal contents were aseptically collected periodically and bacterial counts were performed. The fecal microflora in the control group was represented by bacteria of the genera *Bacillus cereus*, *Lactobacillus* spp., *Escherichia coli*, *Enterococcus* spp. and *Proteus* spp. As the result of dysbiosis induced by heavy metal mixtures, a sharp decline in the population of all microbial species in the fecal contents was observed. Significant changes in fecal metabolic profile were also observed with respect to control group. Thus, intervention in established gut microbiome provides significant insight on the link between gut microbial ecosystem, metal toxicity and its relevance to host health.

**Keywords:** Gastrointestinal tract; Microflora; Heavy metal; Toxicity

MVM-23

### Characterization of antibiotic resistance and multi-drug resistance in sewage and clinical samples

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### Abstract

The aim of this study was to isolate and characterize antibiotic resistant and multi-drug resistant isolates from sewage samples and clinical wound samples. Sewage samples were serially diluted and plated on nutrient agar. Colonies obtained were tested for resistance to different antibiotics by well diffusion assay. Resistant cultures were plated on differential media for tentative identification. The isolates were subjected for MIC test to selected antibiotics by micro dilution broth method and were further grouped by RAPD PCR. Sewage samples (n=5) were collected from different areas in Mysuru, Karnataka. Out of 41 isolates, 26 were found to be resistant to antibiotics (n=6) tested by well diffusion method. The isolates showed resistance to ampicillin, cefotaxime and rifampicin and intermediate resistance to gentamicin, chloramphenicol, and tetracycline. The cultures were subjected for Gram staining and were found to be Gram-negative rods and cocci (20) while some of the isolates (6) were found to be Gram-positive cocci. The isolates were tentatively identified as *Escherichia coli*, *Enterobacter* spp., *Staphylococcus aureus*, *Klebsiella* and *Micrococcus* spp. The minimum inhibitory concentration (MIC) values were found to be above the breakpoint values described by CLSI for selected isolates. Total DNA was isolated and RAPD-PCR was carried out to group the isolates which showed different banding pattern belonging to 5 different species. The results indicate the dissemination of antibiotic and multi-drug resistance in pathogens which could act as reservoirs for transfer of these genes against potent antibiotics making treatment difficult. Currently, further molecular characterization of the resistance mechanisms and use of bacteriocin coated nanoparticles and bacteriophages against resistant cultures are envisaged.

**Keywords:** Antibiotic; Resistance; Multi-drug resistance; Well- Diffusion; RAPD

### A rapid diagnostic kit for determination of MIC in clinical isolates of *E.coli*.

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#### Abstract

A total of 551 samples were collected from 26 hospitals of seven cities in Central India. The Clinical samples consisted of Pus (158), Urine (108), Blood (115), and urinary catheters (169). Total 298 clinical isolates were isolated from the samples that included, *Pseudomonas*, *Klebsiella*, *Enterococci*, *Proteus*, *Staphylococcus*, etc. The antibiogram of *E.coli* was determined using Disc diffusion method, MIC was determined using E Test and Agar dilution tests. The organisms were found to highly resistant to beta lactum group of antibiotics. A rapid microtiter based test was conducted to determine the MIC of the samples for ampicillin. The method consisted of MH broth, gelling agent and antibiotic in concentration same as that of E Test in increasing concentration per well. The wells were inoculated with culture (0.5 McFarland units) and incubated for 12 hours at 37°C. The chromogen was added and plates were incubated for 1 hr at 37°C. The colouring agent was added and the visual development of colour was observed till the antibiotic concentration allowed the growth of *E.coli*. The antibiotic concentration that inhibited the growth showed no colour change in medium and was considered as MIC. The test was statistically analysed and found to have PPV of 99.8%, NPV 95%, Sensitivity 99%, Specificity 98%. The results were obtained in 13-14 hrs much earlier than the traditional methods produced results. The method helps to start early treatment of antibiotic.

**Keywords:** *E.coli*; Antibiotic resistance; Clinical bacterial isolates; Micro titre plate test; MIC

### Prevalence and induction of multiple drug resistance in Gram-negative Bacilli

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#### Abstract

Multi-drug resistant pathogens and antibiotic toxicity are two greatest challenges faced by today's medical world. The inappropriate use of antibiotic dosage has led to the increased resistance in Gram-negative bacilli (GNB). Present investigation reports the prevalence and induction of drug resistance mechanisms among the gut microflora. Sewage and human stool samples were collected from different locations and individuals of Indore city respectively. GNB were isolated from these samples. They were biochemically characterized and analysed for drug resistance mechanisms. Majority of the isolates; 36% of the total were *E.coli*, *Pseudomonas sp.* was the next abundant organism i.e. 18% and 12% of the isolates were *Vibrio sp.* Remaining 34% isolates were *Klebsiella sp.*, *Acinetobacter sp.*, *Citrobacter sp.*, *Enterobacter sp.*, and *Aeromonas sp.* Among the lactose fermenting GNB 82% of the isolates exhibited resistance to commonly used drugs

which is an alarming situation. Much serious concern is the occurrence of ESBL (Extended Spectrum of Beta Lactamase), MBL (Metallo Beta Lactamase) and HL Pase (High Level Penicillinase) resistance mechanism in 53% of the isolates. Non-fermenting GNB were investigated for MBL resistance mechanism, 27% of the isolates emerged as drug resistant. Some isolates exhibited possible carbapenam impermeability. A strain of *Klebsiella* which was initially sensitive to beta lactam drugs developed marginal resistance in 24 hours when co-cultured with resistant *E. coli* strain. Complete expression of resistance gene was observed in 48 hours and the organism exhibited (HL AmpC  $\beta$ ) High Level AmpC  $\beta$  lactamase resistance mechanism. *Pseudomonas* isolate PS1; which was originally sensitive to Piperacillin-Tazobactam, was found to acquire resistance within 24 hours when exposed to drug concentrations lower than MIC. This indicates that free availability of antibiotics and of drug resistant microbes in environment naturally increase the drug resistance mechanisms besides human intervention.

**Keywords:** Multi-drug resistant; GNB;  $\beta$ -lactam; Resistance mechanism; Antibiotics

MVM-26

### Molecular identification and characterization of uropathogens isolated from clinical urine samples

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#### Abstract

Urinary tract infections (UTIs) are the most common bacterial infections globally that can occur at any stage of life of an individual. These infections lead to high morbidity and an economic burden of nearly \$2.14 billion throughout the world. UTI is mainly caused by bacteria of which uropathogenic *Escherichia coli* (UPEC) accounts nearly 80-85%, followed by *Staphylococcus* and a few other species that account for the rest 10-15%. More recently, these infections have become a cause of major concern globally, because of an alarming rise in the emergence of resistance to commonly used antibiotics among the uropathogens. The present investigation is part of a long-term study initiated with an aim to find alternate therapeutic measures to counteract this rapid emergence and spread of multi-drug-resistant (MDR) bacteria. As a first step in this direction, uropathogens were isolated from urine samples of patients suffering from mild to severe UTIs. Isolation was carried out by standard dilution plating method using MacConkey agar and eosin methylene blue (EMB) agar media. The antibiotic resistance pattern of 40 purified isolates was evaluated by subjecting them to antibiotic sensitivity test against 24 standard antibiotics used for the treatment of UTI; seven uropathogens were found to exhibit resistance to more than 75% of these standard antibiotics. The isolates were also evaluated for ESBL activity, and it was observed that out of a total of 40 samples, 18% isolates were ESBL producers. For identification, all the 40 isolates were characterized using microscopic, and standard biochemical tests, and more than 50% of them were predicted to belong to the genera *Escherichia coli*. Two selected isolates which exhibited almost 100% resistance to the tested antibiotics were subjected to molecular identification by PCR amplification of 16S rRNA gene using universal primers. Based upon BLAST and MEGA 6.0 analysis of the amplified product, the isolate was confirmed to be *Escherichia fergusonii* and *Shigella flexneri*. Further characterization of the isolates for their biofilm-forming ability is underway

**Keywords:** Antibiotic resistance; ESBL; MDR; Uropathogens; UTIs

## Studies on bacterial colonization of urinary catheters and strategies for its prevention

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### Abstract

UTI accounts for an estimated 25-45% nosocomial infection, out of which 90% are associated with urinary catheter, called Catheter Associated Urinary Tract Infections (CAUTI). The present study aimed to study bacterial colonization of indwelling urinary catheters and development of strategies for its prevention. Microbial contamination of indwelling urinary catheters was investigated. Biofilm forming ability of the isolates was determined by Tissue Culture Plate method. Prevention of biofilm formation by *Pseudomonas aeruginosa* was determined by treating the catheter with some agents. Total of 560 urinary catheter samples were collected from different hospital of Amravati city, Maharashtra, India and processed for isolation and identification of pathogens. It was found that approximately 93% catheterized samples were contaminated with different uropathogens.

In the study, 22 different uropathogenic species and 2940 strains were isolated from 560 urinary catheters. The most prominent uropathogenic bacteria isolated was *Pseudomonas aeruginosa* (472), followed by *Candida albicans* and *Escherichia coli*. Out of total isolates, 2000 isolates were biofilm producer. The sterilized new Foley urinary catheters were used for the coating with different antimicrobial agents to prolong the durability and prevent biofilm formation in the urinary catheter. *Pseudomonas* species was introduced as the culture. Each day catheter section was studied for the presence of *Pseudomonas* species by the standard procedure. During the study it was found that coated catheter resist more against bacterial attachment than uncoated catheter. In uncoated catheter (control) the biofilm formation by *Pseudomonas aeruginosa* was observed in 3 days. The highest inhibition of biofilm was observed with triclosan, ceftazidime+ CuNps and Copper nanoparticles. It prolonged the attachment for 24, 21 and 19 days respectively.

**Keywords:** CAUTI; Urinary catheter; Biofilm; MIC; MBEC

## Study of susceptibility and resistance of antibiotics in oxic and anoxic conditions using facultative anaerobes isolated from human- gut

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### Abstract

Facultative anaerobes are the most common cause of human infections of anoxic part of human body including deep wound, vagina, periodontal pockets, human-gut, genitourinary tract and lungs etc. Generally evaluation of susceptibility and resistance in facultative anaerobes performed in aerobic conditions due to less cumbersome handling and quick growth in aerobic condition. Variation in antibiotic susceptibility and resistance pattern in facultative anaerobes for same antibiotics in anaerobic and anaerobic conditions leads to failure of antibiotic treatments of anoxic pockets of wounds in animal

and human. In current study we have tested the susceptibility and resistance pattern of members of 19-genera of bacteria isolated from human gut for 22-different antibiotics from six classes in aerobic vs. strict anoxic conditions. A preliminary result obtained during screening was re-verified using minimum inhibitory concentration (MIC) assay in triplicates. Our data indicated that amongst the selected members 10 showed similar pattern of susceptibility and resistance in both the conditions while 9 gave substantial difference in susceptibility and resistance. Therefore, learning the exact behaviour of the antibiotic in aerobic vs. anaerobic conditions is 'imperative for infections related to anoxic part of human body for successful therapy.

**Key words:** Minimum inhibitory concentration; Facultative anaerobes; Antibiotic susceptibility

MVM-29

### Isolation of bacteriophages against antibiotic resistant Uropathogens

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#### Abstract

After years of improper and overuse of antibiotics, antibiotic resistant strains of bacteria have emerged on a global scale and represent a worldwide public health threat. Predominantly, new antibiotics are not making progress at the same rate as the antibiotic resistant strains. This upheaval of emergence of antibiotic resistance has shifted the focus towards substitutes of antibacterial agents, ones that cannot be resisted by the same genes that render bacteria resistant to antibiotics. Bacteriophages or bacterial viruses that specifically kill bacteria can be seen as a potential alternate to treat and prevent infectious diseases in humans and animals. The narrow spectrum and specificity allow phages to be used as harmless agents to treat infections. The major advantage of phage therapy is its host specificity and their use as co-therapy with antibiotics. Efficiency of phages increases with the use of antibiotics along with them. Considering the above mentioned advantages, phages can be seen as a potential alternative to the antibiotics currently in use for treating urinary tract infections (UTIs). In the present study, using enrichment culture method, the authors have isolated five different bacteriophages from sewage soil samples against clinical uropathogenic *Escherichia coli* (UPECs) procured from different clinical pathology laboratories in Sonapat district in Haryana. The antibiotic sensitivity profile of all the UPECs was evaluated for different standard antibiotics currently being used for the treatment of UTIs, and many of them were found to be resistant to several antibiotics. The isolated bacteriophages were purified and their lytic activity against UPECs was tested using spot test method. Transmission electron microscopy (TEM) analysis of one of the phage reveals that it belongs to Siphoviridae family. Further characterization of phages is underway and the most potent phage will be selected for whole genome sequencing analysis.

**Keywords:** Antibiotic resistance; *E.coli*; Phage therapy; UPECs; UTIs

MVM-30

### Isolation of phenotypically hemolysis negative mutant of hemolytic *Vibrio alginolyticus* ATCC17749

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### Abstract

*Vibrio alginolyticus* is a halophilic Gram-negative bacteria naturally found in temperate marine and estuarine environments. Few recent studies indicated it as an opportunistic human pathogen. The ability of this potential human pathogenic organism to capture host iron resource expected to be essential for their effective virulence. In this study, ability of *V. alginolyticus* to acquire iron from few iron containing compounds as sole source of iron were tested. We found, under iron limited growth condition, hemin (iron rich compound of human host), Ferrioxamine (hydroxamate group of siderophore) and ferrous sulphate (inorganic iron source) stimulated growth of *V. alginolyticus* as a sole source of iron. Utilization of hemin, a heme iron derivative can an important virulence associated adaptation for *V. alginolyticus* evolving into opportunistic human pathogen. Utilization of hemin suggests their potential in using host heme iron released through cell lysis. Therefore, we tested for their hemolytic ability *in vitro*, and *V. alginolyticus* ATCC 17749 turned out to be hemolytic on blood agar plate. *V. alginolyticus* ATCC 17749 has two circular DNA molecules containing approximately 4854 genes. We have created random mutant library with more than 10X coverage for the genes by the transposon insertional mutagenesis via conjugation mating. These mutants are being screened for loss of hemolytic activity on blood agar plate and so far four hemolysis negative mutant on blood agar plate were isolated from the library. Those four mutant were grown on two different broth medium and degree of salinity and the spent culture filtrate were subjected to hemolytic assay, which confirmed their hemolysis negative phenotype. Expectedly the transposon induced gene disruption lead to the loss of hemolytic phenotype in the isolated mutants, which need to be identified and characterized in future to understand the putative role of hemolysis in *V. alginolyticus* virulence.

**Keywords:** Heme; Heme utilization; *Vibrio alginolyticus*; Insertional mutagenesis; Hemolytic activity

MVM-31

### Green synthesis, characterization of Ag/ZnO nanocomposites & its effect on xanthine oxidase activity *in vitro*

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### Abstract

Nanotechnology has emerged as one of the fastest growing areas of science and technology in the last decade. Silver nitrate, silver sulfadiazine has been in use for the treatment of burns, wounds, and other bacterial infections. This property led to silver (Ag) being incorporated into various bactericidal applications. The purpose of present study was to synthesize silver and zinc oxide nano composites, their characterization and evaluate their effects on xanthine oxidase activity. Transmission Electron Microscopy study revealed that the Ag/ZnO nano composites was well dispersed and predominantly spherical in shape, while some of the nano composites was found to be having structures of irregular shape with the diameter in the range, 25-70nm. The activity of xanthine oxidase was inhibited substantially in the presence of synthesized Ag/ZnO NCs when compared to the native enzyme activity of xanthine oxidase. The Ag/ZnO nano composites, at concentrations 0.5, 1.0, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 mg/ml showed 9, 16, 34.5, 49.5, 56.5, 64.5, 72, 81.5, 92, 95.5 % activity, respectively. Nano composites can affect the activity of enzymes when incubated with them so this is a promising field for future research. Proper understanding of such interaction is important to study deeply as these materials are used in drugs and medicine.

**Keywords:** Nanotechnology; Xanthine oxidase; Green synthesis



## Current Concept on Dental Plaque and the Microbiology of Periodontitis and Dental Caries

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### Abstract

The oral cavity like other parts of Gastrointestinal tract possesses its natural microflora. Teeth provide hard non-shedding surface that allow accumulation of large masses of microorganisms (Dental plaque and Dental biofilm), mainly in stagnant areas. Due to its presence on non-shedding surface and because of its primary ecological role in dental caries and periodontitis, dental plaque has always been focus of dental research. Oral cavity is continuously bathed with saliva has pH range (6.75-7.25) that focuses growth of many microorganisms.

**Keywords:** Microflora; Dental biofilm; Dental plaque; Periodontitis; Dental caries

## Virulence factors, Drug Resistance pattern, and Biofilm formation Ability of Group B Streptococcus isolated from Indian Population

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### Abstract

*Streptococcus agalactiae* (Group B streptococcus), is a common commensal organism that colonizes the gastrointestinal and vaginal flora and causes severe infection in neonates, adults, and immunocompromised individuals. In case of other pathogenic microorganisms such as *S. aureus* and *P. aeruginosa*, the formation of biofilms has been associated with infection and generation of antibiotic resistance by decreasing the antibiotic penetration rate and mediating bacterial gene expression. A growing degree of research reported that colonizing GBS may act as a source of serotype diversity, virulence factors, and also provide information on increased resistance to antibiotics. In case of *S. agalactiae*, mechanism of biofilm formation and its association with antibiotic resistance have never been investigated in detail. Therefore, the present study is an attempt to understand the association between biofilm formation and antibiotic resistance. As we performed antibiotic resistance, the result determined that these isolates have increased resistance to erythromycin, clindamycin, ofloxacin, gentamicin and penicillin (which is highly used intrapartum prophylaxis antibiotic). As GBS has the ability to switch from planktonic stage to biofilm formation which is considered to be an important factor for the establishment of infection as well as providing resistance to antibiotics. However, the study on how GBS initiates disease and form biofilm upon infection is lacking on comparison with other gram-positive pathogens. The current study is focused on understanding the mechanism of biofilm formation and to establish a correlation between biofilm formation and antibiotic resistance. Recently, we determined *in vitro* resistance of GBS isolates to different antibiotics for the determination of correlation between antibiotic resistance pattern and biofilm formation. The presence or absence of virulence factors in pathogenic bacteria such as GBS is an important variable that decide the course of illness. These findings may provide a deeper insight into the antibiotic resistance mechanism by biofilm formation in *Streptococcus agalactiae*. Would this be the

possible outcome, the involved virulence factors could constitute new therapeutic and preventive targets against this important human pathogen.

**Keywords:** *Streptococcus agalactiae*; Biofilm; Antibiotic resistance; GBS

MVM-34

**Detection and antibiotic sensitivity pattern of *Gardenerella vaginalis* isolated from vaginal secretion of pregnant women of Bundelkhand region**

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**Abstract**

Bacterial vaginosis is an emerging health issue associated with many gynecological complications and various adverse pregnancy outcomes such as premature rupture of membranes, preterm deliveries, chorioamnionitis and even post-partum endometritis. A cross-sectional study was conducted on 112 symptomatic pregnant women of various reproductive age groups and including different trimesters, attending a Medical College of Bundelkhand Region, to detect *Gardenerella vaginalis* in bacterial vaginosis and to study antibiotic susceptibility of the isolates by Kirby-Bauer disc diffusion method against different antimicrobial agents. Amsel's criteria and Nugent scoring method was the clinical criteria performed for the prevalence of bacterial vaginosis. Culture microscopy was performed on blood agar and mac-conkey agar. Antimicrobial resistance is one of the major treatment threats against bacterial vaginosis. The study revealed the isolates were highly resistant (62%) to most commonly used drug Metronidazole, which is also drug of choice in UTI. All the isolates were found to be sensitive to impenem, meropenem and clindamycin. High drug resistance was also seen with ampicillin, only 19 % isolates were susceptible to ampicillin. Hence, antimicrobial sensitivity pattern of the organism should be in routine observation to lay better line of treatment for clinicians and patients.

**Keywords:** Bacterial vaginosis; *Gardenerella vaginalis*; Amsel's criteria; Nugent scoring; Antimicrobial sensitivity; UTI

MVM-35

**Antibacterial potential of green synthesized silver nanoparticles and their effect on mobility patterns of multidrug resistant *Pseudomonas aeruginosa***

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**Abstract**

*Pseudomonas aeruginosa* is a ubiquitous Gram-negative opportunistic pathogen associated with nosocomial infections. It exhibits variety of virulence factors including lipases, exo polysaccharides, type IV pili etc. Biologically synthesized Nanoparticles (NPs) have been reported to possess antimicrobial activity. In this study we synthesized silver nanoparticles using a plant *Syzygium cumini* which has also been reported earlier for its antibacterial activity. Thus, we explored the combinatorial effect of both in the form of synthesized NPs and optimized its different parameters

(reaction time, pH, extract concentration, temperature and salt concentration). We characterized these NPs through transmission electron microscope (TEM), UV-Vis spectrophotometer and Fourier-transform infrared spectroscopy (FTIR). We investigated the antibacterial activity, MIC and MBC of NPs against *Pseudomonas aeruginosa*. Further, we assessed its motility patterns by different assays like swarming, swimming and twitching using different concentrations of AgNPs. We concluded that synthesized NPs were  $\approx$  7-10nm in size and showed maximum absorbance at 420 nm. MIC of the NPs were achieved at 7 $\mu$ g/ml whereas it showed MBC at 25  $\mu$ g/ml. These NPs showed a marked reduction in swarming, swimming and twitching motility of *Pseudomonas aeruginosa* above 35  $\mu$ g/ml. Further, kinetics of these NPs was done with varying concentrations and time to prove its efficacy against this multidrug resistant *Pseudomonas aeruginosa*. This study highlights the importance of 'Green or Biologically synthesized' silver nanoparticles as an effective approach to overcome infections caused by multidrug resistant *Pseudomonas aeruginosa*.

**Keywords:** Nanoparticles (NPs); *Pseudomonas aeruginosa*; Motility; *Syzygium cumini*

MVM-36

### Assessment of soil transmitted Helminths prevalence and intensity among school children in five districts of Gujarat

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#### Abstract

Soil-transmitted helminthic infection (STH) is one of the neglected tropical diseases that affects nearly 2 billion people globally with majority in Asia. Chronic STH infections adversely affect physical and mental development of children and diminish work capacity and productivity of adults. We estimated prevalence and intensity of STH infections (round worm, whip worm and hook worm) among school children in Gujarat state as per WHO recommended school based sentinel site methodology. A total of 415 students were enrolled from 7 sentinel sites in 5 districts during December 2015. Morning stool samples were collected and examined by Kato-Katz method. Potential risk factors like socio-economic status, use of household toilets, hand washing practices, usage of footwear and eat/play with soil etc were studied. 132(31.8%) children were found positive for STH infection, of which *Ascaris lumbricoides* accounted for 31.5% and *Trichuris trichiura* 0.3%. Hook worm was not detected in any of the stool samples. Intensity of infection was observed to be mild in all positive samples with EPG (eggs per gram of stool) less than 4999 for *A. lumbricoides* and 999 for *T. trichiura*. Study results indicate poor personnel hygiene and sanitation as key factors for occurrence of STH infection. The study provided necessary technical input to the government strategy of periodic mass deworming through administration of albendazole and in advocating other prevention and control measures for control of STH infections in the state.

**Keywords:** Soil Transmitted Helminths; *Ascaris lumbricoides*; *Trichuris trichiura*; Deworming

## Characterization of G-quadruplex motif in Ni<sup>+2</sup> permease system of *Helicobacter pylori* as potential drug target

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### Abstract

*Helicobacter pylori* infects the human stomach and causes *H. pylori* infection. Approximately 50% of the humans are infected by this bacterium which can result in peptic ulcer, MALT lymphoma and even adenocarcinoma. As a result of growing antibiotic resistance, treatment methods based on antibiotics is becoming ineffectual as an antibacterial therapy. Keeping its high infection rate and severity of the infection in mind, it is of the utmost importance to find alternate methods for the eradication of this infection. The non-canonical DNA secondary structure such as G-quadruplex is well distributed in virtually every organism present on earth and plays a vital role in various biological processes and has also been found to be a drug target for anticancer therapy. However, these secondary structures have not been explored much in human pathogenic bacteria. In the present work, we report presence of highly conserved G quadruplex motif within in the nickel transporter genes (nixA, niuB1, niuB2, and niuD) of the *Helicobacter pylori* genome. Nickel is required in *H. Pylori* as a cofactor to urease enzymes which is crucial for the survival of this bacterium in the stomach lining and can be targeted through ligands to counter the *H. pylori* infection. Bioinformatics analysis was performed to identify such PGQs containing gene and CD spectroscopy and electrophoresis techniques were employed to confirm the in-vitro formation of stable G-quadruplex structure. G-quadruplex coupling ligands examined for their therapeutic potential has determined that they effect the stability of the G-quadruplex structures which then lead to the downregulation/halted expression of genes, resulting in the death of the cells. MTT assay followed by G4 specific compound treatment on cells shows a concentration dependent cell death. Treatment of cells with ligands and its subsequent qPCR against the Nickel transporter genes has also shown altered expression levels of the genes suggesting the effect of the ligands in cell physiology. Further investigation of this ligand-quadruplex interaction is expected to provide more insight into the ligands potentiality as a therapeutic target.

**Keywords:** *Helicobacter pylori*; G-quadruplex

## Do biofilms and antimicrobial resistance in *E. coli* contribute to its adaptability under simulated gut condition

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### Abstract

Emergence and spread of antibiotic resistance among pathogenic *E. coli* is a serious problem worldwide. Their ability to form biofilm and possess antibiotic resistance traits under *in vitro* gut condition needs greater understanding. This study aimed at identifying the possible relationship between antibiotic resistance and biofilm formation by *E. coli*. 92 *E. coli* isolates were screened for their resistance pattern to 10 antibiotics by Kirby-bauer disk diffusion method. The biofilm forming ability was assessed by microtiter plate method and crystal violet staining. Selected isolates were also checked for their ability to form biofilm under simulated *in vitro* gut condition. Of the 92 isolates, 79(86%) isolates were resistant to cefotaxime, most of the isolates were sensitive to chloramphenicol 77(84%). 79(85%) were multidrug resistant and 13(15%) sensitive to most antibiotics. In biofilm assay 10(11%) isolates formed strong biofilm that included (6 MDR isolates and 4 MDS isolates), 12(13%) of the isolates were moderate biofilm formers that included (10 MDR and 2 MDS strains). 30 (32%) of the isolates were weak biofilm formers that included (27 MDR and 3 MDS strains) while the remaining 40 (44%) failed to form biofilm (36 MDR and 4 MDS strains). 18 isolates that included (7 strong, 6 moderate, 3 weak formers and 2 non biofilm formers) were selected for their ability to form biofilms under *in vitro* gut conditions that included parameters such as bile, NaCl, low iron and high iron. On exposure to bile all the isolates showed increased biofilm forming ability. Biofilm formation varies under different gut environmental conditions. The study suggests that biofilm formation varies under different gut environmental conditions. The study also showed antibiotic resistant and sensitive isolates had the tendency to form biofilm. The ability of sensitive & resistant isolates forming biofilm in the gut would enable greater contact and transfer of resistance genes from resistance to sensitive isolates.

**Keywords:** Antimicrobial resistance; Biofilm; Multi Drug Resistant (MDR); Multi Drug Sensitive (MDS) strains

MVM-40

### Evaluation of antimicrobial properties of stem of *Tinospora cordifolia* (giloy)

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### Abstract

In developing countries, including African and Asian countries, the burden of infectious diseases is increasing day by day. The excessive or overuse of antibiotics to combat microbial diseases led the development of antibiotic resistance in these pathogenic microbes and eventually leads to development of multiple drug resistant (MDR) in pathogens and adverse side effect in humans. Therefore, finding of an alternative to antibiotics is of high demand in modern research. In this regard, traditional medicines are obvious choice to be used as an alternate for antibiotics. It has been well documented that medicinal plants not only exerts beneficial effects on whole body physiology but they do also have antibacterial property and thus are of better choice as an alternate for antibiotics. In current study, we have investigated the antimicrobial activity in stem extract of medicinal plant *Tinospora cordifolia*. We have demonstrated that the stem extracts (both methanolic and aqueous extracts) have significant antimicrobial properties against Gram -ve bacterium *E. coli* and Gram +ve *B. subtilis*. The growth inhibition studies, we observed that the stem extract significantly reduced the growth of both *E.coli* and *B.subtilis* as compare to control samples treated with solvents only. Further identification and purification of these antimicrobial compounds from *T. cordifolia* stem could be used as an alternative of antibiotics to treat bacterial infections and may reduce the antibiotics burden.

**Keywords:** Multiple drug resistant; *Tinospora cordifolia*; Antimicrobial Activity

## *Candida auris* and its epidemiology

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### Abstract

Fungi have emerged as a significant cause of human infections, especially in immune-compromised individuals. Rather of being commensals to humans, *Candida* species is the fourth most common cause of invasive fungal infections. In the last decade, a novel *Candida* species namely *Candida auris* has emerged with a serious global health threat. This pathogen is a deadly superbug which has capability to cause nosocomial infections. This yeast pathogen result in consistent candidemia and can invades the central nervous system, bloodstream, and various internal organs. *Candida auris* found to be allied with high mortality rate. Furthermore, this organism frequently exhibits multidrug-resistance and displays an unusual sensitivity profile. Commercialized identification techniques that are employed for the testing of *Candida auris* frequently misdiagnosed *Candida auris* as *Candida famata*, *Candida haemulonii*, and *Rhodotorulaglutini*. This misidentification leads to treatment failure which complicates the management of candidiasis. But now it can be accurately identify using PCR, Real Time-PCR, and MALDI-TOF. Genomic epidemiology has identified four genotypes of *Candida auris* from different geographical regions which are genetically dissimilar. An international study named as SENTRY isolate collection reveals that *Candida auris* came to existence in 2009. Since 2009 pathogenic incidents of *Candida auris* have been reported throughout the world. Till date, *Candida auris* has been progressively recorded from more than 30 countries.

**Keywords:** *Candida auris*; Nosocomial infection; Epidemiology; Candidiasis; Multidrug resistance

## Therapeutic approaches to conquer virulence of pathogenic strains of *Staphylococcus aureus*

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### Abstract

*Staphylococcus aureus* is the most significant human pathogenic bacteria that cause a wide range of clinical manifestations. Nosocomial infections caused by *Staphylococcus aureus* comprise those affecting the skin and soft tissue, lower respiratory tract, bloodstream, as well as ventilator-assisted pneumonia and central venous catheter-associated bacteraemia. The increasing prevalence of Staphylococcal infections leads to serious health consequences that are intensified by the inadequacy of novel classes of antibacterial drugs. This article describes various therapeutic approaches to conquer virulence of pathogenic strains of *Staphylococcus aureus* that includes monotherapy, combination drug therapy and phage endolysins therapy. The primary targets for antibacterial drug action in staphylococci are (i) the cell envelope, (ii) the ribosome and (iii) nucleic acids. Various other new targets are arising from modern drug discovery programs. Now most of the strains of *Staphylococcus aureus* are resistant to ample of antibiotics. To fight such resistant strains, combination therapy and phage endolysin therapy are emerging option. Combinations of more than one antibiotics or combination of adjuvant with antibiotics are prominent as a propitious therapeutic approach. Phage endolysin therapy also presents as a possible alternative to conventional antibiotic therapies. Endolysins display high antibacterial activity, specificity and minor chances of developing resistance making them promising approach to combat pathogenic strains of *S. aureus*.

**Keywords:** *Staphylococcus aureus*; Antibiotics; Monotherapy; Combination drug therapy; Phage endolysin therapy

## Endophytic actinobacteria as a potential source against emerging multiple drug resistance (MDR) human pathogens

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### Abstract

Multiple drug resistance (MDR) pathogens is one of the most threatening condition which is affecting the people worldwide by acquiring resistance to almost all antimicrobial drugs that are present in the market. In recent years, the uses of bioactive compounds for treating various disease conditions have high demand in pharmaceuticals. The bioactive compounds produced by endophytic microorganisms associated with traditional medicinal plants not only helps in growth promotion of plants but are also considered as a potential leads that are active against human pathogens. The endophytic microorganisms produce some novel antibiotics that are effective against MDR pathogens. In the present study, different endophytic strains were isolated and screened for their antimicrobial activity against MDR pathogens such as *Staphylococcus aureus* (ATCC-BAA-44); *Pseudomonas aeruginosa* (MTCC-10145); *Klebsiella pneumoniae* (ATCC-BAA-2814); *Enterococcus faecalis* (ATCC-51575); *Candida albicans* (ATCC-64124); *Streptococcus pneumoniae* (ATCC-10015); *Micrococcus luteus* (ATCC-10240); *Bacillus subtilis* (ATCC-11774); *Saccharomyces cerevisiae* (ATCC-2601); *Salmonella typhimurium* (ATCC-51812); *Escherichia coli* (ATCC-10536)]. Fifty-five endophytic strains were isolated and screened for their antimicrobial activity. The endophytic isolates that show potential activity against more than 2 MDR pathogens or at least for one MDR pathogen (MIC1-10mg) were selected. Further three endophytic isolates were selected and grown in ISP1 broth at 28°C in a shaker incubator for 7-10 days. The filtrates of the grown cultures were extracted using methanol. The antimicrobial activity of the methanolic crude extracts of all these selected strains were done using agar well diffusion method. Among all endophytic isolates one isolate (code-111) showed the MIC of 1 mg against *S. aureus* and inhibition of 7 other MDR pathogens. The best isolates from the study will be selected and grown at large scale for the development of antimicrobial formulation.

**Keywords:** Multiple drug resistance; Actinobacteria; Antimicrobial activity

## Amoxicilin incorporated hydrogel membranes for wound dressing applications

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### Abstract

Wounds provide a favourable environment for colonization of microorganisms which may delay healing process. The primary objective of wound care is to prevention wound from microbial infections. Hydrogels are three dimensional, hydrophilic, polymeric network and have ability to swell and retain a significant fraction of water or biological fluid within its structure but will not dissolved in water or biological fluid. Hydrogel are advantageous for wound dressing application due to its resemblance with natural soft tissues

and inherent biocompatibility which can be partially attributed to their soft, flexible nature and high-water content. Hydrogels leave no residue, are malleable and improve re-epithelialization of wounds. Hydrogels incorporated with antimicrobial agents such as amoxicillin may use for wound dressing application. Amoxicillin have potential antimicrobial activity against pathogenic microorganism, and have high potential to control microbial species in a wound and to minimize the opportunity for infection. The target site of amoxicillin is inhibition of synthesis of peptidoglycan chain. The peptidoglycan chain is important component of cell wall and provide strength and rigidity to bacterial cell. Amoxicillin binds to penicillin-binding proteins (PBP's) located on the inner membrane of the bacterial cell wall and inactivate them. This interrupt bacterial cell wall synthesis and result in the weakening of the bacterial cell wall and cause cell lysis. The objective of present work is to fabricate amoxicillin incorporated hydrogel membrane and evaluation of its antimicrobial activity against bacteria *E. coli*. Hydrogel membranes were prepared using 5% acrylamide and 1% agar using glutaraldehyde as cross-linking agent by freeze thawing method. Amoxicillin (1mg/ml) is incorporated in the hydrogels before casting as membranes. The fluid absorption capacity of amoxicillin incorporated hydrogel membranes was observed 49.67% after 4 hours to 80.02% in 24 hours. The antimicrobial efficacy of hydrogels were evaluated by zone of inhibition test. The zone of inhibition was observed 3.80mm, 2.72mm and 0.23mm after 24 h, 48h and 72 h respectively. The results demonstrated optimum fluid absorption capacity and strong antimicrobial activity of amoxicillin incorporated hydrogel membranes. The fabricated hydrogel membrane can be effectively used in the management of infected wounds.

**Keywords:** Amoxicillin; Hydrogel; Wound dressing; Antimicrobial activity

MVM-45

### Antimicrobial activities of various Thiazolidine derivatives

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#### Abstract

Fungal and bacterial infections have augmented at an alarming rate presently, which is becoming a challenge to researchers. There are a large number of fungal and bacterial species that cause relentless infections in humans. The most commonly encountered fungal pathogens are *Candida albicans* that causes candidiasis a common infection of the skin, oral cavity, esophagus, gastrointestinal tract, vagina and vascular system of humans and *Aspergillus spp.* that causes a wide spectrum of infections ranging from Aspergilloma, Invasive pulmonary aspergillosis (IPA), Chronic necrotizing pulmonary aspergillosis and Allergic bronchopulmonary aspergillosis (ABPA) and bacterial pathogens that includes *Salmonella spp.* causes fever and gastrointestinal tract infections, *Escherichia coli* is responsible for diarrhea (often bloody) which may or may not be associated with nausea, fever, vomiting, and abdominal cramps and *Staphylococcus spp.* that causes skin and soft tissue infections. The antimicrobials used to treat these infections have restricted success due to emergence of multidrug resistance to most widely used antifungal and antibacterial chemotherapies such as azoles, allylamines, polyenes, echinocandins, penicillins, tetracyclines, cephalosporins, quinolones and lincomycins. So researchers have synthesized various novel compounds which can be used to treat these microbial infections. Thiazolidine derivatives are one group of such novel compounds, thiazolidine is a five membered ring which consists of a thio group at first position and an amine group at third position and these molecules are known to have various types of biological activities like antiviral, anti-tubercular, anticancer, and have shown promising antibacterial and antifungal activities.

**Keywords:** *Candida albicans*; *Aspergillus*; *Salmonella*; *Staphylococcus*; *Escherichia*; Thiazolidine



## Dramatically enhanced antibacterial activity of pediocin decorated silver nanoparticles: A double edge weapon against food spoilage and multidrug resistant bacteria

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### Abstract

Despite current advancements in modern medicine, bacterial infections remain a major cause of mortality and socioeconomic burden for millions of people worldwide. This is aggravated by the fact that antibiotics are losing efficacy because of the rising resistance of microorganisms to these substances. As a result, the number of multidrug resistant bacteria is rising day by day, unfortunately, the potential new antibiotics introduced into the clinical market has declined significantly. Therefore, there is an urgent need to develop novel non-antibiotic strategies which combine the feasibility of high antibacterial activity and low tendency to induce resistance at cost-effective rate. To address this problem, as an antimicrobial agent, silver nanoparticles bio-functionalized with an antimicrobial peptide (pediocin; Pd-SNPs) were designed and developed a double edged weapon with enhanced antibacterial function to act against Gram-positive food borne pathogen *Listeria monocytogenes* and multidrug resistant clinical Gram-negative *Vibrio fluvialis*. The synthesized particles were characterized by various biophysical techniques. The nanoparticles were found to be homogenous, spherical, mono-dispersed and capped with multi-layered protein shell. The results revealed that antibacterial efficacy of bio-functionalized nanoparticles (Pd-SNPs) has distinctly enhanced biocidal effects over citrate capped silver nanoparticles (CSNPs) as Pd-SNPs exerted 10-fold (*Vibrio fluvialis*) and 13 folds (*Listeria monocytogenes*) higher antibacterial activity. The functionalized Pd-SNPs were found stable even after pasteurization and highlight the possibility of use of these “nano-biotics” in food industry. Mechanistic studies based on morphological effects on the membrane damage deciphered the importance of capping of silver nanoparticles with pediocin. We also assessed the ability of *L. monocytogenes* to develop resistance against Pd-SNPs vis-à-vis pediocin. Following 10 repeated passages of *L. monocytogenes* cells to the MIC levels of Pd-SNPs, the MIC of Pd-SNPs against *L. monocytogenes* remained constant indicating that bacterial resistance to the Pd-SNPs did not develop. Hemolytic and cytotoxicity effects of the bio-functionalized nanoparticles was also evaluated which suggested that Pd-SNPs can be used as biocompatible bactericidal agent. This study will provide effective and food grade antimicrobial agents for food preservation and therapeutic purposes.

**Keywords:** Multidrug resistance; Bio-functionalized silver nanoparticles; Antimicrobial peptide; Bacteriocins; Biofilm inhibition

### Antimicrobial activity of cave actinobacteria from Krem dam, Meghalaya, India

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#### Abstract

This work reports on the assessment of antimicrobial activity of cave-dwelling actinobacteria isolated from sediment samples of Krem Dam, a limestone cave near Mawsynram, Meghalaya, India, which is an under-explored environment. Isolation of actinobacteria was performed using different pre-treatment methods and selective media. The isolates were characterized morphologically, biochemically, chemotaxonomically and by 16S rDNA sequence analyses. Screening of the isolates for antimicrobial activity was performed by cross-streak and agar-well diffusion assay methods against three Gram-negative, three Gram-positive bacteria and two candidal species. The isolates were also screened for presence of biosynthetic gene clusters related to antibiotic synthesis viz. PKS-I, PKS-II and NRPS. Forty-eight isolates were obtained which were putatively identified as actinobacteria based on micro-morphology. Biochemical, chemotaxonomic and 16S rDNA sequence characteristics showed almost all the isolates were *Streptomyces*, except for two, which were identified as *Amycolatopsis*. Three isolates showed antibacterial activity against *E.coli*, one isolate showed activity against *Pseudomonas aeruginosa*, 15 isolates showed activity against *Bacillus subtilis*, 11 isolates showed activity against *Staphylococcus aureus* and *Micrococcus luteus*, 9 isolates showed activity against *Candida albicans* and 6 isolates showed activity against *C. tropicalis*. The cross-streak method scored more number of bioactive isolates. 29.2% of the isolates were detected with PKS-I gene, 66.7% were detected with PKS-II gene and 31.3% were detected with NRPS gene. 41.67% of the isolates showed bioactivity and were also mostly detected with at least one of the biosynthetic gene clusters. Culture-dependent cave actinobacteria from Meghalaya were dominated by *Streptomyces* and were potentially bioactive against bacterial and candidal strains. The PKS and NRPS gene clusters seemed to play a role in bioactivity of the isolates. The bioactive isolates can be further investigated to assess their activity over a wider range of microbial strains and their bioactive compound production can be optimized for characterization and further investigation.

**Keywords:** Cave actinobacteria; Krem Dam; Meghalaya; Foldscope; Antimicrobial

### Role of coconut husk in the management of Oral bacteria

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#### Abstract

Oral diseases like dental caries continue to be most important global oral health problems. Aim of the present study was to evaluate the antimicrobial activity of Coconut husk in the management of oral bacteria. Aqueous and Ethanolic extract of Coconut husk, Neem leaves, Guava leaves, Ginger and Garlic were evaluated against total 10 (ten) oral bacterial pathogens i.e. *Staphylococcus aureus* (n=5) and *Escherichia coli* (n=5) by agar well diffusion method. Antibiotic resistance profile of the oral bacteria was carried out by disc diffusion method. Coconut husk was found effective against 20% of *E. coli* while Garlic and Neem Aqueous extract were effective against 40% *E. coli* each. Garlic Ethanolic extract was effective against 20% *E. coli*. However, Aqueous Garlic and Neem extract

were effective against 40% and 20% *S. aureus* respectively. Ethanolic Garlic and Neem extract were effective against 20% *S. aureus* each. All *S. aureus* were resistant to Trimethoprim and Methicillin while all *E. coli* were resistant to Aztreonam. Phytochemical analysis showed that Flavonoids were present in Garlic, Neem and Guava extracts. Tannins were present in Coconut husk, Neem and Guava. Glycosides and Terpenoids were present in Coconut husk. Alkaloids were present in Coconut husk, Ginger and Guava. Ninhydrin were present in garlic, Neem and Guava extract. It is concluded that coconut husk is beneficial with antimicrobial effect on oral cariogenic bacteria. It may open a new path to fight against dental caries by incorporating the active antimicrobial agents of coconut husk into modern oral care systems such as tooth pastes or mouth washes. It may provide benefits to mankind for better oral health.

**Keywords:** Coconut husk; Oral bacteria

MVM-51

### Suppression of Sarco/Endoplasmic Reticulum Calcium-ATPase impairs Paramyxovirus Replication

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#### Abstract

Sarco/endoplasmic reticulum calcium-ATPase (SERCA) is a membrane bound cytosolic enzyme that is known to regulate the uptake of calcium into the sarco/endoplasmic reticulum from the cytosol. Here, we show for the first time that SERCA can also regulate paramyxovirus [*Peste des Petits Ruminants virus (PPRV)* and *New castle disease virus (NDV)*] replication. Treatment of Vero cells either with SERCA specific inhibitor (Thapsigargin) at a concentration that is nontoxic to the cells significantly reduces PPRV and NDV replication. NDV and PPRV were shown to induce SERCA expression which could be blocked by Thapsigargin. It was observed that Thapsigargin specifically inhibits viral entry and subcellular localization of the viral proteins. On sequential cell culture passage (P=70) in presence of Thapsigargin, NDV only acquired a partial resistance whereas PPRV showed no resistance to Thapsigargin. As compared to the P0 and P70-Control, the fusion (F) protein of P70-Thapsigargin virus exhibited a unique mutation at amino acid residue 104 (E104K), whereas no Thapsigargin-associated mutations were observed in HN gene. To the best of our knowledge, this is a first report describing that SERCA regulates any virus replication and a rare report suggesting that virus may acquire resistance even in presence of an inhibitor that targets a host factor. The study will contribute in understanding paramyxovirus replication and suggesting SERCA (host factor) as a potential target for antiviral drug development.

**Keywords:** Sarco/endoplasmic reticulum calcium-ATPase (SERCA); New castle disease virus; *Peste des Petits Ruminants virus (PPRV)*; HN gene; Paramyxovirus



# MICROBIAL DIAGNOSTICS



# ORAL PRESENTATION





## Rapid pathogen identification and phenotypic antimicrobial susceptibility testing

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### Abstract

Unavailability of rapid diagnostics for pathogen identification and antimicrobial-susceptibility-testing has been the significant factor contributing towards unnecessary/inappropriate use of antibiotics resulting into emergence/spread of antimicrobial-resistance (AMR) among pathogens. We are developing a syringe compatible, single-use, affordable and user-friendly combination of device that can capture bacteria from whole-blood/urine samples, enrich, culture, identify and establish their antimicrobial susceptibility profile(s) for multiple drugs simultaneously within 90-minutes. The proposed solution would help in guided clinical-decision making and minimize empirical use of antibiotics and emergence/spread of AMR. This would ultimately reduce duration and cost of treatment as value proposition to the customers.

**Keywords:** Rapid pathogen capture; Micro culture; Rapid antimicrobial susceptibility testing



# POSTER PRESENTATION



## Comparative evaluation of multi-targeted LAMP and multiplex PCR for rapid diagnosis of osteoarticular tuberculosis

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### Abstract

Tuberculosis (TB) is a major health problem worldwide. In developing countries like India, extrapulmonary TB (EPTB) constitutes ~15-20% of TB cases. Notably, osteoarticular TB (OATB) accounts for 10-18% of total EPTB cases, the major cause of osteomyelitis. Diagnosis of OATB is quite difficult due to paucibacillary nature of specimens and localization of disease at sites that are difficult to access. Several conventional tests *i.e.* smear microscopy, culture and histopathology are utilized in diagnosing OATB but these tests have certain limitations. Over the last two decades, nucleic acid amplification tests (NAATs) such as polymerase chain reaction (PCR) targeting IS6110, *mpt64* (Rv1980c), *pstS1* (Rv0934), *devR* (Rv3133c), etc. have been employed for diagnosis of OATB, but they often lead to false-positive results. Other NAATs such as loop-mediated isothermal amplification (LAMP) are employed in diagnosing pulmonary TB (PTB) cases and the WHO has endorsed LAMP for PTB diagnosis, however, its utility to detect EPTB cases, including OATB is uncertain. We recently developed LAMP using multigene target approach, *i.e.* *mpt64* and *pstS1* to detect *Mycobacterium tuberculosis* (*Mtb*) DNA in body fluids of suspected OATB cases and results were compared with multiplex PCR (M-PCR) targeting *mpt64* + *pstS1*. Detection limits of both *pstS1* and *mpt64* were determined to be 10 fg/ $\mu$ L in *Mtb* H<sub>37</sub>Rv purified DNA by LAMP, which was 10-fold higher than M-PCR. Moreover, LAMP revealed sensitivities of 90% and 75.4% in confirmed (n=10) and suspected (n=57) OATB cases, respectively with specificities of 96.9% in non-TB controls (n=33). Strikingly, sensitivity attained with LAMP in total OATB cases (n =67) was significantly higher ( $p < 0.05$ ) than M-PCR (77.6 versus 58.2%). Overall, our LAMP is sensitive, specific and cost-effective test without requiring a thermocycler. After validating the efficacy of LAMP in different geographic regions, this test can be translated into a commercial kit.

**Keywords:** NAATs; LAMP; Multiplex-PCR; *Mycobacterium tuberculosis*; Osteoarticular TB

## *In vitro* and *In silico* anti-mycobacterial evaluation of some plant compounds against dormant form of *Mycobacterium smegmatis*

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### Abstract

The dormant phenotype acquired by *Mycobacterium tuberculosis* during infection poses a major challenge in disease treatment, since these bacilli show tolerance to front-line drugs. A hypoxia dormancy model (MSD) as described by Wayne produced phenotypically dormant *Mycobacterium smegmatis* after 10 days of incubation under conditions of low oxygen, low pH, and nutrient limitation. Bacilli in this model exhibited the classical dormancy traits, including loss of acid fastness and most importantly, tolerance to front-line drug rifampicin. In this model, dormant *M. smegmatis* was treated with rifampin,

metronidazole and phytomolecules. Three phytomolecules including Betulinic acid, Icariin and Ursolic acid were evaluated against non-replicating form of *M. smegmatis*. Betulinic acid, Icariin and Ursolic acid killed dormant bacilli, as shown by lack of regrowth in solid and liquid media. Furthermore, *in silico* validation for anti-mycobacterial activity of phytomolecules against drug targets in dormant bacilli was also performed. Further investigation of dormancy-active hits was carried out using *in silico* approaches druggable targets in dormant bacilli, ultimately leading to new therapeutics able to reduce lengthy anti-TB therapy.

**Keywords:** Tuberculosis; Dormancy; Drug targets; Phytomolecules

MD-03

### **Recombinant outer membrane rOmp28 protein-based sandwich ELISA for rapid detection of *Brucella* species**

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#### **Abstract**

Brucellosis infection is most debilitating, re-emerging, economically important bacterial zoonosis and affecting globally 800,000 cases per year with 30-40% of chronic infections. It is endemic in several states of India with variety of hosts including humans. The causative bacterium is gram-negative alpha-proteobacteria, facultative-intracellular cocco-bacilli. The incidence of infection is pre-dominated by *Brucella melitensis* and *Brucella abortus*. In our present study, we have developed double-antibody sandwich ELISA (S-ELISA) based detection assay using polyclonal antibodies (pAb) generated against intact whole cell (WC) and recombinant (rOmp28) antigen of *Brucella*. The S-ELISA was initially standardized with WC pAbs at 10 µg mL<sup>-1</sup> of capture rabbit IgG and at 50 µg mL<sup>-1</sup> of mice IgG as detection antibody with limit of detection at 10<sup>3</sup> CFU mL<sup>-1</sup>. For increasing the sensitivity of assay, detection antibody was replaced with rOmp 28 mice IgG at 100 µg mL<sup>-1</sup> with limit of detection at 10<sup>2</sup> CFU mL<sup>-1</sup> with P/N ratio is 1.1 as compared to rOmp28 based S-ELISA which is 0.9 and 0.6 for detection of *Brucella abortus* and *Brucella melitensis* respectively. Optimal concentration of capture and detection antibody was standardised with P/N ratio of 11.8 (OD 0.59±0.118) and 6.3 (OD .411±0.029) for both *Brucella melitensis* and *Brucella abortus*. The western blot characterization of pAbs with rOmp28 protein antigen was performed and found to be immuno-reactive for assay optimization. The pAbs generated against *Brucella* whole cell, sonicated, cell envelope and rOmp28 antigens were also evaluated for effective capture antibody at P/N ratio of 4.4 > 4.2 > 4.1 > 2.4 respectively. For assay validation and cross-reactive study, *Brucella* WC Ag were spiked in clinical and non-clinical samples and detected WC *Brucella* in human sera followed by bovine sera, cow milk and urine. No cross-reactivity was observed with closely related bacterial species. The developed S-ELISA can be effectively used for detection of *Brucella species* from different clinical and environment matrices.

**Keywords:** *Brucella*; Brucellosis; Sandwich ELISA; rOmp28; Whole Cell Detection.

MD-04

### **Computational identification of novel targets for molecular diagnosis of *Burkholderia pseudomallei*, the causative agent of Melioidosis**

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### Abstract

Melioidosis is a fatal infectious disease caused by the gram-negative, intracellular, aerobic bacterium *Burkholderia pseudomallei*. This disease is endemic and mostly under-diagnosed in Indian subcontinent and diagnosis of Melioidosis is always a challenge for the physicians. Specific, sensitive and cost-effective assays are required to replace the time taking culture method for diagnosis of Melioidosis. In this study, a novel *B. Pseudomallei* gene was identified by *in-silico* analysis. The whole genome sequence of *B. pseudomallei* K96243 (Accession no. NC\_006350.1) was extracted from NCBI (<http://www.ncbi.nlm.nih.gov>) and blast server was used to infer the unique sequences within the genome. Primers were designed by identifying highly conserved regions among the strains of *B. pseudomallei* using ClustalW programme. Conventional PCR assay was standardized for *B. Pseudomallei* specific 363 basepair region (from 1918466 to 1918828 bp) of BPSL 1655 gene. Genomic DNA was isolated from *B. pseudomallei* NCTC 13392 and 2 fold serial dilutions were prepared for the determination of limit of detection (LOD). The amount of DNA was converted to genome equivalent (GE) copies based on the *B. pseudomallei* K96243 genome size of 2.25 Mb (No. of GE copies =  $(6.022 \times 10^{23} \text{ copies/mol}) \times (\text{DNA amount in grams}) / (7.25 \times 10^6 \text{ bp} \times 660 \text{ g/mol bp})$ ). The primer set was tested against 18 different isolates of *B. pseudomallei* (four standard strains, four soil isolates and ten clinical isolates), 03 other species of *Burkholderia* genus (*B. mallei*, *B. cepacia* and *B. gladioli*) and 20 other closely related species. The limit of detection (LOD) of this conventional PCR assay was found to be  $3.99 \times 10^3$  CFU/ml. This set of primer was positive for 18 different isolates of *B. pseudomallei* in this assay and also had no cross reactivity with other species of *Burkholderia* genus and closely related species. This PCR assay can further be evaluated with clinical samples for specific detection of Melioidosis.

**Keywords:** *Burkholderia pseudomallei*; Melioidosis; Molecular Diagnosis; Computational Biology; Polymerase Chain Reaction

MD-05

### Anti-MRSA evaluation of *Pestalotiopsis microspora* an endophytic fungus of *Dillenia pentagyna* Roxb

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### Abstract

The frequent use of antimicrobial agents worldwide has led to the development of antibiotic resistance in human pathogens. *Staphylococcus aureus* has been recognized as an important cause of disease around the world and it has become a major pathogen associated with both hospital and community-acquired infections. The introduction of penicillin greatly improved the diagnosis for patients with severe staphylococcal infections, but after a few years of clinical use, resistance appeared due to production of  $\beta$ -lactamases. The second generation of penicillin (methicillin) was introduced to the clinic in 1959, and just in two years by 1961 strains of methicillin resistant *Staphylococcus aureus* (MRSA) had emerged. Since natural bioactive compounds produced by fungi are known to inhibit wide range of pathogens, they can serve as potential source of novel antibiotics. In this study endophytic fungus *Pestalotiopsis microspora* was isolated from the surface sterilized bark of an endangered tree species *Dillenia pentagyna* Roxb. The fungus was cultured in potato dextrose broth for 21 days. The culture filtrate was extracted with ethyl acetate to get crude bioactive metabolites. This crude extract of *P. microspora* showed activities against all test bacterial strains including MRSA except *Escherichia coli*. The partial purification of ethyl acetate extract by Thin Layer Chromatography (TLC) has shown three major anti-MRSA fractions. The Minimum Inhibitory Concentration (MIC) of the crude extract was determined  $20 \mu\text{g/mL}$  and  $30 \mu\text{g/}$

mL for methicillin sensitive *Staphylococcus aureus*(MSSA) and MRSA respectively. Minimum Lethal Concentration (MLC) was determined 40µg/mL and 70µg/mL against MSSA and MRSA respectively. The future work is to obtain the anti-MRSA compounds by column chromatograph and characterize them by HPLC, GCMS and NMR spectra. Furthermore recovery of host origin compounds from endophytes may prevent the overexploitation of endangered species of medicinal plants.

**Keywords:** *Staphylococcus aureus*, Penicillin,  $\beta$ -lactamases, *Pestalotiopsis microspora*, *Dillenia pentagyna*, Minimum Inhibitory Concentration

MD-06

### **A hypothesis of rapid diagnostic test for differential detection of *Plasmodium falciparum* and non *P.falciparum* malarial infection**

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#### **Abstract**

Conventional methods presently available for malarial diagnosis have been tedious; even extortionately priced modern stellar techniques are wanting in precision. Rapid Diagnostic Tests (RDTs) being economical and lucid, represent the best prospect for malarial diagnosis and control. In recent years, *Plasmodium* enzymes like Lactate Dehydrogenase (LDH), Aldolase(s) and Histidine Rich Protein (HRP) have been used as antigen markers in these RDTs. However, the discovery of a new malarial biomarker *Plasmodium* Glyceraldehyde -3- Phosphate Dehydrogenase (pGAPDH), a metabolic enzyme that promotes Kupffer cell transversal, presents an opportunity to scale-up the quality of parasite based diagnostic methods, owing to its abundance relative to other such biomarkers. *Plasmodium* sporozoite GAPDH facilitates Kupffer cell infection by binding to human CD68, and is expressed in the ring, trophozoite, and schizont stages of malarial infection. This article proposes a novel RDT to mediate the differences between *P. falciparum* and non *P.falciparum* infections by detecting species-specific peptides of the GAPDH enzyme. This is achieved by identifying *P.falciparum* specific surface epitopes (CADGFLIGEKKVSVFA and CAEKDPSQIPWGKCQV), and the surface epitope common to all the other species of *Plasmodium* (CKDDTPIYVMGINH) by monoclonal antibodies (IgY) raised against the a forementioned respective epitopes and further isolated from the yolk of immunized chicken. Detection is achieved by employing blue latex bead labelled antibodies i.e, anti-pGAPDH whole protein antibodies, the F<sub>c</sub> region of which is complementary to the paratope of primary antibody immobilized on the control line. On the basis of lateral flow immunochromatography, antigens, if present in the blood sample are captured by primary antibodies immobilized by adsorption on a nitrocellulose membrane. This hypothesized RDT may outdo its contemporaries because of fewer false-positive results - pan malarial epitopes differ from human GAPDH by ~50% - and improved sensitivity. This RDT could be a stepping stone in the path towards the collective augmentation of malarial diagnostic devices.

**Keywords:** Malaria; Rapid Diagnostic Test; Immunoassay; Differential Diagnosis; GAPDH

MD-07

### **Absolute quantitation of oral bacteria involved in oral cancer by real-time PCR**

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### Abstract

India carries approximately 34% of the global oral cancer burden and mortality rate of 41%. The major risk factor of oral cancer is the common habit of tobacco chewing. Along with tobacco chewing habit, there are few other factors, along with new risk factors that are being studied globally. One of them is the involvement of certain bacteria in progression towards various cancers, including oral cancer. Next-Generation Sequencing (NGS) studies have identified few bacterial species and the changes in their abundance in healthy and diseased populations. Most of these reports are based on 16S rRNA gene diversity analysis. The present study showed a method for absolute bacterial population quantification from oral cavity rinse samples. The method is real-time qPCR assay and based on the fact that certain genes are present in one copy per cell, and we can correlate the copy numbers of these genes with cell number in a sample. This method is more accurate than the common NGS 16S rRNA gene-based approach because the 16S rRNA gene is present in more than one copy per cell. The method involves the establishment of a linear correlation between qPCR assay result and cell numbers of a known bacterial species. Consequently, the qPCR assay was performed with significant oral bacterial species. The assay and quantification was performed on oral rinse samples of oral cancer/tobacco chewers and healthy subjects. The obtained bacterial quantification correlated well with the previous reports. The qPCR method shown here is an efficient, faster and resource-friendly method. This can be used to quantify bacterial population in cancer or diseased subjects, and even in samples from different environmental sources.

**Keywords:** Real time qPCR; Absolute quantification; Bacterial quantification

MD-8

### Bacteriocin of *Lactobacillus plantarum* in bio-preservation of indigeneous foods

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### Abstract

In this study, the efficacy of bacteriocin of *Lactobacillus plantarum* was tested in various food items. *Lactobacillus plantarum*, *Micrococcus luteus* and *Staphylococcus aureus* were grown in MRS and Nutrient broth medium, respectively. The growth of bacterial strains were determined in the form of optical density and found OD<sub>600</sub> 1.04 in *L. plantarum*, 1.5 in both indicator strains *Micrococcus luteus* and *Staphylococcus aureus*. The bacteriocin was purified through ultrafiltration using 3kDa membrane filter and collected the retentate and permeate. The retentate and permeate were showed 18 mm and 17 mm against *M. luteus*. The retentate was concentrated and showed 18 and 25 mm zone of growth inhibition against *S. aureus* and *M. luteus*, respectively. The effects of bacteriocin on organoleptic properties of food items such as mango pickle, homemade bread, commercial bread, grapes, moong bean sprouts, milk and fruit juice were tested. It was observed that 18.38 to 183.8 µg/ml of bacteriocin was sufficient to inhibit the growth of microorganism on respective food items. Thus, bacteriocin of *L. plantarum* may be used as natural preservative for various food items available.

**Keywords:** *Lactobacillus plantarum*; Bacteriocins; Milk; Pickle; Food preservatives

### Study of urinary tract infection in HIV patient and its correlation with complete blood count in Nasik

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#### Abstract

HIV patients are immune-suppressed patients, so they are prone to many opportunistic infections like Tuberculosis, Meningococcal infections, Parasitic & Bacterial infections of stool, etc. Urinary Tract Infection and pyrexia are important opportunistic infections amongst the HIV patients. Clinicians miss this many times. To rule out Urinary Tract Infection, urine culture along with antibiotic sensitivity is important among investigations. Pyrexia also alter the complete blood count picture particularly white blood cells. White blood cells are the important initial infection marker. In selected HIV patients with PUO (Pyrexia of unknown origin), we carried out urine culture and antibiotic sensitivity test, if culture is positive and complete blood count on a automated blood cell counter to correlate the obtained results. We studied 19 cases of PUO in HIV positive patients in Nasik. Among these 12 samples (63.15%) show no growth, and remaining 7 samples showed growth (36.84%). Positive samples shown *E.coli* in 3 samples, *Staphylococci* in 2 samples, and *Pseudomonas*, *Klebsiella* in 1 sample each. The antibiotic sensitivity is good in all positive culture patients. WBC count in complete blood count reflects; increase in WBC count in 2 cases, Normal in 13 cases, and Low in 4 cases. In differential WBC count Polymorphs increased in 3 cases while normal in 16 cases.

**Keywords:** Pyrexia; *Staphylococci*; Antibiotic sensitivity

### Study of seminal infection among an infertile male population in Nasik, and its effect on sperm quality

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#### Abstract

Semen is considered as the surrogate marker for male fecundity while assessing infertility in men. There are several reasons why a male could be suffering from infertility whether primary or secondary. The role of inflammation or infection of male accessory sex glands are very important or the potential effects that these conditions may have on male fertility. Sperm Bacterial contamination is quite frequent and could contribute to the deterioration of the sperm quality of infertile men. Since the ejaculate is a mixture of secretions derived from the urogenital tract and the male accessory glands, seminal culture identifies the present of germs in any section of the seminal tract. On the basis of clinical and experimental research, it is found that there is association between isolation of bacteria from semen and deterioration of spermatogenesis and spermatozoa function which can ultimately lead to infertility. Semen samples were assessed macroscopically and microscopically.

It includes semen volume, semen color, pH, viscosity, sperm concentration, sperm motility, sperm morphology, microscopic examination of leukospermia and semen culture. To study the microbiota associated with semen of non fertile men and also their effect on semen parameter in Nashik city. Total of semen analysis and semen culture of infertile men showed that out of 48 semen samples, 17 samples were infected. The most common infective organisms isolated on culture come out to be *Staphylococcus* species and *Enterococcus faecalis*, *E. coli*, *Streptococcus* spp., *Klebsiella* spp., *Acinetobacter lwoffii*. Out of 17 infected samples 9 samples were oligospermic, 2 samples were azospermic and 7 samples were normospermic.

**Keywords:** Leukospermia; *Acinetobacter lwoffii*; Azospermic; Normospermic

MD-11

### Study of Urinary Tract Infections in Diabetic and non-diabetic patients in Nasik and its correlation with Complete Blood Count

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#### Abstract

Diabetes is a group of metabolic disorders characterised by high blood sugar levels over a prolonged period. As of 2017 an estimated 425 million people had diabetes worldwide. This represents 8.8 % of the adult population with equal rates in men and women. Trend suggests rate will continue to rise. UTI (Urinary Tract Infections) among Diabetic patients is common. Presence of sugar in urine promotes bacterial growth. The patient may have diabetic neuropathy, lazy bladder that does not empty completely. Even the defective immune factors predispose the infections. Various impairment in the immune system may all the factors contribute to the pathogenesis of UTI in diabetic patients. UTI causes considerable morbidity in diabetic and if complicated can cause severe renal damage and life threatening infections. The escalating anti-microbial resistance causes recurrent UTI. In this study we collected blood and urine samples from 40 patients in Nasik city and blood was checked for CBC and urine was investigated for routine examination, culture and antibiotic sensitivity. Out of 40 Patients 21 patients were diabetic and 19 were non diabetic. Among these 21 diabetic patients 13 were suffering from UTI (61.9 %) and the pathogens for UTI we isolated were *E coli*, *Klebsiella*, *Staphylococcus*, *Streptococcus*. Among 19 non-diabetic patients, 5 patients (26.3 %) were suffering from UTI. The isolated pathogens from these samples were *E coli*, *Staphylococcus* and *Pseudomonas* spp. White blood cells help the body to fight against infections so the increased WBC count may be observed in UTI patients. 8 UTI patients among the 24 patients were showing increased WBC count.

**Keywords:** Diabetes; Urinary tract infections; *Klebsiella*; Antimicrobial resistance

MD-12

### High level expression and purification of recombinant capsular fraction 1 antigen, a biomarker for detection of *Yersinia pestis*

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### Abstract

*Yersinia pestis*, a responsible entity for one of most devastating disease the plague, is Gram negative, non motile, non endospore forming bacterium. Capsular fraction 1 protein (F1 antigen) was secreted by *Y. pestis* as a capsule-like surface antigen and demonstrated to be a qualified marker for identification of this bacterium. In the present work, we have cloned and expressed recombinant F1 protein of *Y. pestis* in *Escherichia coli*. The expression conditions including cultivation media were optimized to enhance the protein expression. The recombinant F1 protein was purified employing affinity chromatography under denaturing conditions and further difiltered. The protein purity and its reactivity were confirmed employing SDS-PAGE and Western blot, respectively. The antibodies were raised against purified F1 protein in rabbit model was evaluated by ELISAs. Our results showed that the F1 protein could be used as an antigen for detection of *Y. pestis*.

**Keywords:** *Yersinia pestis*; Caf 1; F1

MD-13

### Isolation and Identification of Endophytic Fungi from *Curcuma longa* L.

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### Abstract

Plants are pool of large number of microorganisms known as endophytes. Endophytes colonize the internal tissues of the plants beneath the epidermal cell layers, without causing any apparent harm or symptomatic infection to their host. They are believed to confer fitness to their respective host plant. The study of endophytes is alluring because of their particular habituation, hyperdiversity and is easy to study. Recent research shows that there are various biological activities performed by endophytic fungi that pave way to new methods of utilizing them. The present investigation includes the isolation of endophytic fungi from *Curcuma longa* L. popularly known as Turmeric. The sample was collected from Haridwar district of Uttarakhand. A total of six endophytic fungal species were isolated from healthy plant parts including leaf, stem, root and rhizome and identified on the basis of their morphological characterization.

**Keywords:** Endophytic fungi; Biological activities; *Curcuma longa* L.

MD-14

### Isolation and Identification of Endophytic Fungi from *Aloe vera* L.

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### Abstract

The choice of host plant is of critical importance when working with endophytic fungi. The exploration of endophytic fungi is still an emerging field and all plants seem to harbour fungi. Endophytic organisms are those that live internally in apparently healthy and asymptomatic hosts. Endophytes appear to be ubiquitous; indeed, no study has yet shown the existence of a plant species without endophytes. Therefore,

exploring valuable endophytes and understanding its interaction is a prospective area of research. The present investigation aim is to isolate the endophytic fungi from *Aloe vera* L. and to identify them further. The *Aloe vera* sample was collected from Haridwar district of Uttarakhand state. Three endophytic fungi were isolated from healthy leaf of *Aloe vera* and identified on the basis of their morphological characterization.

**Keywords:** Endophytes; Endophytic fungi; *Aloe vera* L.

OP/MD-15

### Development of LAMP based diagnostic assay for early detection of *Rhizoctonia solani* causing sheath blight of rice

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#### Abstract

Rice is a staple crop for most of the Asian populations mainly India, China and Japan. *Rhizoctonia solani* AG<sub>1</sub> I-A is a well known and one of the most devastating pathogens of rice. Heavy damages have been reported due to sheath blight disease caused by *R. Solani* and ultimately commercial losses. It is a soil born pathogen and its early and cost effective detection can help the farmers to undertake appropriate management strategies. In the present study, a loop mediated isothermal amplification assay has been developed to detect *R. solani*. The genomic region of target was a polygalacturonase gene (cell wall degrading) which is essential for *R. solani* during host tissue invasion. The LAMP assay in conjugation with rHTTP method (a rapid method to prepare PCR ready template from plants) could detect the pathogen from live diseased samples within an hour. The assay can detect upto one ng of pathogen DNA. With the help of this LAMP assay, *R. solani* was detected within 24 hours of its induction of disease in sheaths of rice plants in a pot experiment. The presence of *R. solani* could also be detected in soil DNA isolated from rice field using the optimized assay. The ease of performing this assay makes it applicable in field diagnostic studies which could be of direct benefit to the farmers. This is the first report of such a diagnostic assay for this devastating pathogen.

**Keywords:** *Rhizoctonia solani* AG<sub>1</sub> I-A; LAMP; Soil DNA; Polygalactouronase

MD-16

### Drug-DNA interaction on combed DNA fibres

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#### Abstract

DNA combing is a technique by which single DNA molecules are combed over coated glass surfaces. Combed DNA fibres can be used for studying drug-DNA interaction but no study has taken place where drug-DNA interactions were observed by fluorescent microscope other than atomic force microscope. We optimized several combing solutions over different coating surfaces for its use in several different applications. Platinum cancer drugs form adducts with DNA molecules, such interactions can be studied by using cancer drug in solution form on combed DNA fibers. Combing solution optimized by us was having high retention, higher fluorescent intensity, and lower background on coated glass surfaces. While optimizing the combing solution it was taken into consideration that the combing solution constituents don't hinder adduct formation by anticancerous drugs to bound combed DNA fibres. Observ-

ing adducts of DNA on combed DNA fibres is easier as compared to previous studies where such studies were performed on DNA combed over mica surfaces. In our study DNA adducts with anticancerous drugs such as Cisplatin were observed by fluorescent microscope and is the first report where adducts were visible without the help of atomic force microscope. We can thus use DNA combing technology to study effect of anti-cancerous drugs on DNA, also effect of different drugs on DNA can be studied by using our combing solution optimized especially for such purpose.

**Keywords:** Atomic force microscope; DNA fibres; Anticancerous drugs

MD-17

### **A Chemical Genetic approach in *E. coli* results in generation of highly sensitive whole-cell Biosensor for Tetracycline class of antibiotics**

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#### **Abstract**

Historically, antibiotics have been used at sub-therapeutic dosage as growth promoting agents for livestock and poultry farming. The long term usage of antibiotic in food animals is linked with significant antibiotic resistance development in bacterial pathogens. Tetracycline group of antibiotics are frequently used in livestock & poultry sector due to their broad spectrum of antibacterial activities and low comparative cost. Considering the amounts of tetracyclines used worldwide, and their inimical effects, the monitoring and mitigation of these xenobiotics is of paramount importance. Whole cell biosensors play an important role in monitoring of environmental pollutants, but do not deal with the removal of these harmful agents. Here, we describe a counter strategy which deals with the biosensing as well as riddance of tetracycline category of antibiotics from the environment. Tetracycline inducible promoter regulating Green Fluorescent Protein expression platform was used to monitor the different levels of tetracyclines in the environmental samples. To enhance the sensitivity of our biosensor, one of the active efflux pump was knockout by homologous recombination system. The genetic knockout increased the sensitivity of detection many folds and allowed us to detect the tetracycline below its Maximum Residual Limit (MRL). Magnetic activated carbon nanofibers were synthesised and employed to concentrate tetracyclines from large volume of environmental samples. Nanofibers loaded with pollutants were coupled with our whole cell biosensor which exclusively monitored the level of tetracyclines and translated it into the expression of GFP. This is one of the first reported system which deals with sensing as well as mitigation of tetracycline from the environment with a lower limit of detection of 10 ng/ml.

**Keywords:** Tetracycline; Whole-cell Biosensor; Knockout; Green Fluorescent Protein; Magnetic Activated Carbon Nanofibers

MD-19

### **Isolation and Identification of *Campylobacter* spp. from Acute Diarrhea Patients by Conventional and Molecular Methods in Bhopal.**

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### Abstract

*Campylobacter* spp. is one of the most significant causes of bacterial gastroenteritis worldwide, especially under 5 years of age in most of the countries. The most frequently isolated strains of this bacterial genus are *Campylobacter jejuni* and *Campylobacter coli*. Prevalence of the *Campylobacter* diarrhea in India ranges around 3 to 16% depending on various geographical locations. Aim of this study is to evaluate the prevalence of *Campylobacter jejuni* and *Campylobacter coli* among the patients of acute diarrhea in AIIMS Bhopal and Primary health centres of Bhopal. During the period of study, 123 stool samples were collected from the patients of acute diarrhea. Stool samples were cultured on selective media at microaerophilic condition followed by gram staining and biochemical analysis; simultaneously stool samples were subjected to multiplex PCR after DNA extraction. Out of 123 stool samples, 6 stool samples were found to be positive for *Campylobacter jejuni* by culture as well as PCR. Sensitivity, Specificity and accuracy of PCR were calculated by the comparison with culture by using medcalc diagnostic test evaluation calculator. Through this study all the cases were found to be in concordance with culture followed by biochemical analysis. Recent prevalence of the *Campylobacter* diarrhea in this area is 4.88%. As culture is a standard method of *Campylobacter* detection and appropriate to use it as gold standard in the laboratory and in statistical analysis. The results from this study indicate that PCR is also an accurate and a specific method for the detection of *Campylobacter* in human stool sample with additional benefits having rapid diagnostic ability as compared to culture. PCR can detect and identify *Campylobacter* to the species level and the results are obtained on the same day. Based on the results of this study, we can use culture as well as PCR for the detection of *Campylobacter*.

**Keywords:** *Campylobacter*; Gastroenteritis; Prevalence; Culture; PCR





# **MOLECULAR MICROBIOLOGY**



# ORAL PRESENTATION



## Methylation dependent transcriptional control by GcrA coordinates cell cycle and cell shape in *Caulobacter crescentus*

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### Abstract

The dimorphic Alphaproteobacterium *Caulobacter crescentus* is a model to study prokaryotic development and cell cycle. In *Caulobacter* the combined action of chromosome replication and the expression of the DNA methyl-transferase CcrM at the end of S-phase creates a cyclic alternation between a full-to hemi-methylated chromosome. This transition of the chromosomal methylation pattern affects the DNA binding properties of the transcription factor GcrA that controls several key cell cycle functions. However, the molecular mechanism by which GcrA and methylation are linked to transcription is not fully elucidated yet. Using a combination of cell biology, genetics and in vitro analysis, we studied how GcrA integrates at the transcriptional level the transition of methylation in several S-phase promoters. We demonstrated in vitro that transcription of *ctrA* from its P1 promoter in its hemi-methylated state is activated by GcrA, while in its fully methylated state GcrA had no effect. In addition, GcrA and methylation are involved in the pre-divisional cells for a peculiar distribution of *creS* transcripts, encoding for the intermediate filament like protein crescentin responsible for the characteristic shape of the *Caulobacter* cells. This gene is duplicated right after the onset of chromosome duplication and the two hemi-methylated copies are spatially segregated, towards the opposite poles of the pre-divisional cell. Our results indicated that GcrA and methylation transcribed only the copy in which the methyl group is on the coding strand. In vitro transcription substantiated the finding showing that the transcription output from one form of hemi-methylated *creS* promoter is higher in a GcrA dependent manner. As several of the cell cycle regulated genes are also under the influence of methylation mediated and GcrA dependent transcriptional regulation, this could be a mechanism involved in maintaining their transcription dosage during the S-phase.

**Keywords:** *Caulobacter*; DNA Methylation; Cell Cycle; Transcription Regulation; Crescentin

OP/MM-16

### Morphogenesis in cyanobacteria: Mechanistic insight of maximizing the fitness

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### Abstract

Cyanobacteria are Gram-negative photosynthetic bacteria which produces oxygen during photosynthesis similar to the higher plants. In recent years, these organisms have shown their potential to be utilized in the sustainable development program to fulfil the energy and food demands of our society. Cyanobac-

teria have metabolic chassis to efficiently capture the greenhouse gas (GHG) CO<sub>2</sub> from the atmosphere and convert it to various industrially important metabolites. Additionally, the biomass produced by cyanobacteria could be utilized for the production of CO<sub>2</sub> neutral biofuels. However, for successful utilization of cyanobacteria and/or algae in the sustainable development program, higher biomass production per unit of area is essential for the economic viability of such ventures. Being a photoautotroph, cyanobacteria are subjected to fluctuating environmental conditions such as varying quality and quantity of light together with salinity, temperature, pH and nutrient availability. These environmental fluctuations greatly reduce biomass production by compromising the fitness of the organisms. Therefore, fine-tuning of the photosynthetic apparatus together with other parameters under fluctuating environmental condition is critical for the maximum production of biomass. We study morphogenesis and its molecular mechanism(s) in cyanobacteria that increase the fitness of the organism under fluctuating environmental conditions. In my presentation, I will be sharing some of the recent work done in my laboratory on morphogenesis in cyanobacteria and show some recently developed mutants which possess phenotypes that increases the fitness of the organism.

**Keywords:** Cyanobacteria; Bioenergy; Morphogenesis; Photosynthesis; Mutagenesis

OP/MM-19

### Enolase phosphorylation by Ser/Thr protein kinase, PrkC contributes to spore germination in *Bacillus anthracis*

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#### Abstract

*Bacillus anthracis*, an etiological agent of anthrax, follows a complex transitional program from vegetative cells to infectious spore formation. These spores are metabolically dormant and their germination is essential for a successful host infection. Bacterial spore acquires its molecular composition in the form of 'phenotypic memory', which helps it to germinate during the favourable environmental conditions. However, the exact control mechanism of bacterial metabolism during the transitional phases remains elusive till date. Here, we attempted to understand the role of metabolic enzymes in the spore germination process. Our results indicated that a glycolytic enzyme, Enolase (Eno) acts as a major contributor to the spore revival phenomena. The protein levels of Eno are maximum at early vegetative stages and get progressively diluted during the later phases of growth. Artificially induced expression of Eno in the engineered strains showed decreased germination efficiency. Further, we found that a bacterial serine threonine protein kinase, PrkC regulates Eno expression and controls its catalytic activity and localization through phosphorylation. Additionally, the major phosphorylation sites found on Eno, have been seen to contribute in the protein catalysis. Thus, based on our extensively validated results, we propose that Eno is a constituent of spore memory whose protein levels and function are regulated by the bacterial signal transduction protein, PrkC. Overall, PrkC and Eno work as joint gears to "fine-tune" the overall spore germination programming.

**Keywords:** Signaling; Phosphorylation; Phenotypic memory; Metabolism; *Bacillus*

## ***Helicobacter pylori* Pathogenesis: Role of Inflammasome Activation and Regulation**

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### **Abstract**

*Helicobacter pylori* (*H.pylori*) is a gram-negative, micro-aerophilic helically-rod shaped bacterium that colonizes the human gastrointestinal tract. It is the **causative agent** of a number of gastrointestinal disorders and is present in the stomach of about two third of the world population. It is also a leading cause of gastric inflammation which leads to cancer. The toxins such as CagA is primarily responsible for inflammation whereas other factors such as urease, VacA, HopQ, BabA and SabA are responsible for its survival in acidic environment, adherence and cellular damage to host tissue. The molecular mechanisms associated with its pathogenesis and persistent infection is not completely understood. The prolonged infection results in chronic inflammation, oxidative stress and DNA damage. Inflammasomes are multimeric protein complexes which get assembled in cytoplasm after getting stimulated with Pathogen Associated Molecular Patterns (PAMPs)/Damage Associated Molecular Patterns (DAMPs) or crystals and further activates cellular caspases which enhance inflammation via pro-inflammatory cytokines. The mechanism of inflammasome activation by *H. pylori* is not studied extensively and very few virulence factors such as UreB, CagA, FlaA and VacA and their role in inflammasomes is established. Our research group is trying to establish the mechanism of inflammasomes regulation and associated pathways through which *H. pylori* regulates inflammasome activation. Using human gastric (AGS) and human monocytic (THP-1) cell lines, we are also exploring whether Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) produced by the immune and epithelial cells damage the host cells thereby resulting in oxidative stress which leads to cellular damage and is ultimately responsible for the *H. pylori* associated fatal complications during its pathogenesis.

**Key Words:** *Helicobacter pylori*, Inflammasome, IL-1 $\beta$ , Virulence factors, and Caspases

OP/MM-24

### **Role of an orphan response regulator in promoting virulence of *Xanthomonas oryzae* pv. *oryzae* on rice**

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### **Abstract**

Two component systems (TCSs) are one of the most prevalent ways by which bacteria sense, respond, and adapt to various changes in their environment. We are using Rice-*Xanthomonas oryzae* pv. *oryzae* (Xoo; the causal agent of rice disease called bacterial blight) pathosystem to understand the role of TCSs in plant-microbe interaction. Genome sequence analysis revealed that Xoo strains are equipped with large repertoire of TCSs. TCS constitute a signal transduction mechanism which is basically formed by two proteins: a membrane bound histidine kinase sensor and a cytoplasmic response regulator. Sensor histidine kinase responds to an environmental change or stimulus by phosphorylating a cognate response regulator which triggers changes in cellular physiology or gene expression in order to enhance adaptability. A system level mutagenesis in an Indian Xoo strain BXO1 led to identification of several virulence genes encoding for response regulators. Here, work will be presented indicating importance for an orphan response regulator (encoded by BXO1\_007010 gene) for Xoo virulence on rice. This re-

sponse regulator contains the CheY-like receiver domain and is required for *in planta* growth as well as migration, suppression of host defense response, induction of non-host resistance, optimum expression of type 3 secretion system, EPS production, motility, biofilm formation and survival under specific stress conditions.

**Keywords:** Two component system; *Xanthomonas oryzae* pv. *oryzae*; Virulence; Plant Immunity; Signal Transduction

OP/MM-30

### QDR genes have impact on the intra-cellular glucose levels in pathogenic fungi *Candida albicans*

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#### Abstract

Multiple drug resistance (MDR) has become a global widespread phenomenon with multiple clinical and non-clinical implications. The ATP-Binding Cassette (ABC) proteins and the Major Facilitator Superfamily (MFS) transporters are the two major types of efflux pumps that have been widely associated with the development of MDR. The yeast *S.cerevisiae* homologous QDR (Quinidine Drug Resistance) MFS transporters have been identified conferring resistance and involved in pathogenicity in several candida species. Considering the importance of QDR transporters in the virulence and/or pathogenicity of *C.albicans*, the present study was conducted to focus on other metabolic pathways where QDR genes might play a significant role and thus contribute to survival and/or colonization of *Candida* in human hosts. The transcript profiling of the knockout strains show the differential expression of many of the sugar transporter genes, which mainly are high affinity glucose transporters. Glucose accumulation assay results revealed significantly higher accumulation of glucose in the QDR knockout strains with corresponding less glucose accumulation in the QDR overexpression strains compared with their respective controls. Among the knockout strains, QDR123 (triple knockout) strain displayed the highest accumulation of glucose. In conclusion QDR genes seem to affect cellular glucose levels, which can have important role in fungal pathogenicity.

**Keywords:** Opportunistic fungi; Drug resistance; QDR transporters; *Candida albicans*

RF/MM-15

### Development of real-time loop-mediated isothermal amplification assay for the detection of *Bacillus anthracis*, the causative agent of anthrax

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#### Abstract

*Bacillus anthracis* is a gram positive, non-motile, spore forming bacterium. It is a tier 1 agent that causes anthrax, a zoonotic disease which primarily affects animals but can also infect humans who are in close contact to infected animals. Its spores can persist in soil and water for years and can be aerosolized. High virulence of *B. anthracis* is due to toxins and poly D glutamate capsule encoded by plasmids pXO1 and pXO2, respectively. Plasmids are the most promising target for anthrax detection as its chromosome



shows high sequence similarity with other closely related bacteria. A sensitive and specific method to detect *B. anthracis* is important for anthrax risk management and control in animal cases to address public health issues. LAMP assay is a novel technique that amplify target DNA at constant temperature with high sensitivity and specificity. A LAMP assay targeting *lef* gene of *B. anthracis* harboured by pXO1 plasmid was optimised with assorted reagents. Specific primers were designed using primer designing software (Primer explorer version 4). Assay conditions (time & temperature) and optimum concentration of reaction components (betaine, MgSO<sub>4</sub>, KCl) were optimized for the assay. The target was amplified at 65 °C for 1 hour and the assay could detect as low as 10 fg of genomic DNA and as few as 10 copies of target DNA harboured in a recombinant plasmid. The amplification was monitored in real-time by turbidimeter and visual detection was also accomplished after adding SYBR green dye under normal and UV light. The developed LAMP assay was found to be highly sensitive and did not cross-react with closely related *Bacillus* spp. and other bacterial strains used in the study. This assay however needs to be further validated for detection of *B. anthracis* in environmental and clinical samples.

**Keywords:** *B. anthracis*, LAMP assay, lethal factor, Biological weapons

RF/MM-18

### Methane oxidizing bacteria (MOB) from Indian rice ecosystems: Diversity and Cultivation

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#### Abstract

Methane is the second most important greenhouse gas contributing to global warming. Rice cultivation is one such anthropogenic source of methane, which contributes to about 9% to the total methane emissions in the world. Methanotrophs inhabiting the rice rhizospheres can oxidize about 20% of the produced methane. Thus, to control the methane emissions from such ecosystems, it is important to study the indigenous methanotrophs. Very few methanotrophs have been cultivated from tropical rice fields and other terrestrial and aquatic habitats. In the cultivation independent analysis, we found that clades of uncultured bacteria existed especially within gamma proteo bacterial methanotrophs enhancing the need and scope for cultivation of methanotrophs. We cultivated and isolated pure strains of methanotrophs from Indian rice fields. A member of a novel genus and species *Methylocucumis oryzae* was described by us. In the present study, we report the isolation and characterization of two strains belonging to putative novel taxa from the genera *Methylomonas* and *Methylomicrobium*. Strain Kb3 (*Methylomonas* sp.) and strain RS1 (*Methylomicrobium* sp.) were isolated from the rice rhizospheric samples of InKb (Kalbhorwadi, Indrayani variety) and InM (Malegaon, Indrayani variety), respectively. Both of these represented putative novel species as they shared maximum 16S rRNA gene homology of less than 98.6% with the nearest type species, *Methylomonas methanica* S1 and *Methylomicrobium album* BG8, respectively. Kb3 cells are bulbous rod shaped that form pink watery colonies on agarose plates. Strain RS1 is pleomorphic and forms translucent white colonies on agarose plates. Both these strains are motile and utilize only methane or methanol as source of carbon and energy. Metagenomic analysis of the soil samples based on the V3-V4 region of the 16S rRNA gene revealed sequences similar to the genera *Methylomonas* and *Methylomicrobium*. Thus, culturing new species of these genera from a tropical rice rhizosphere habitat is an important step.

**Keywords:** Methane; rice ecosystems; methanotrophs; isolation; metagenomics

## Repertoire of oxidative stress tolerance genes from different ecological habitats using functional metagenomics

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### Abstract

Microorganisms have the ability to respond to impromptu alterations in the environment for their survival. Oxidative stress is one of the major challenges that they encounter due to transient exposure to ambient oxygen. Over the past decades, much has been learned about the mechanisms by which these reactive oxygen species i, e.  $O_2^-$  and  $H_2O_2$  can poison bacteria and about the strategies by which bacteria strive to defend themselves. They possess different stress tolerant genes, such as catalase and superoxide dismutase, to protect themselves against oxidative stress by ROS. However, under extreme environmental condition this basic protection is not sufficient to cope with oxidative stress; hence bacteria require additional mechanisms to counteract the ROS. Other global responses enable bacteria to survive the stress period. Metagenomics is a promising approach to identify novel mechanisms evolved for stress resistance in different ecological habitats. Using functional metagenomics approach, we identified yet uncharacterized genes responsible for oxidative stress tolerance in microbial communities from various ecological habitats. Metagenomic libraries were constructed and transformed in *E. coli* DH10B and screened for stress tolerance. Twenty five clones that conferred increased resistance in *E. coli* were identified and sequenced, some encoding for well-known proteins previously related to stress resistance such as alkaline hydrogen peroxidase and RecA, whereas others were proteins not previously related to this function in microorganisms including hypothetical proteins. Furthermore, eleven of the retrieved novel genes were cloned and over-expressed in *E. coli* and they also conferred stress resistance. The functional mechanism of the unique genes is being characterized by overexpression, purification and biochemical assays.

**Keywords:** Metagenomics; Oxidative stress; Functional Metagenomics; Novel mechanism; Protein expression

## A novel small noncoding RNA AbsR28 regulates stress adaptation in *Acinetobacter baumannii* ATCC 17978

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### Abstract

During its lifecycle, bacteria faces various environmental and internal challenges which can be perceived as stresses. Bacteria have developed several genetic machineries to survive, adapt and counteract these stresses. Bacterial chromosomes encode a number of small regulatory RNA (sRNA) that may interact with target mRNA and regulates its expression. In the last few decades, sRNAs have been shown to be involved as key players in stress responses. Our group have identified 31 novel sRNA in *Acinetobacter baumannii*, a nosocomial multidrug resistant pathogen, but the targets of most these sRNAs are currently unknown. AbsR28 is one of these novel sRNA and we are trying to understand the role of AbsR28 in stress response. Methods: Deletion mutant of AbsR28 was created by

homologous recombination. In silico analysis was performed for target prediction and expression of major targets were checked by qRT-PCR. Results: The mutant showed defect growth at high temperature. The expression of a two-component system (TCS), known to regulate envelop stress, was down regulated in mutant. Further, to understand the molecular mechanism of AbsR28 regulation, we have checked the importance of Hfq (a chaperon often required for binding to target mRNA) for AbsR28 stability. AbsR28 seems to function Hfq-independently and regulates envelop stress adaptation in *A. baumannii*.

**Keywords:** *A. baumannii*; Stress adaptation; Multi-drug resistance pathogen; Two-component system (TCS)d



# POSTER PRESENTATION



## Combinatory effect of selected antibiotic drugs with citrus flavonoid against Methicillin Resistant *Staphylococcus aureus*

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### Abstract

Naringenin, found in citrus plants, is a bioactive flavonoid known to exhibit potential antimicrobial activity. To elucidate this further, we designed a study to investigate the antibacterial effect of Naringenin alone and in combination with  $\beta$ -lactam antibiotics (Amoxicillin, Oxacillin & Cloxacillin) against the Methicillin resistant *Staphylococcus aureus* strains. The effect of naringenin on the expression of penicillin-binding protein (PBP2a) encoded by *mecA* gene was also studied. We employed the broth micro-dilution method and checkerboard dilution test to explore the anti-MRSA activity of Naringenin. Naringenin showed good anti-microbial activity with a value of 125 $\mu$ g/ml, 15 $\mu$ g/ml and 162.4 $\pm$ 106.77 $\mu$ g/ml against reference MRSA, MSSA and MRSA-isolates respectively. The average MIC against clinical isolates (n=49) for the three combinations (Amox. + Narin.; Oxa. + Narin. And Clox. + Narin) was found to be 22.69 $\pm$ 13.24, 17.61 $\pm$ 9.67 and 13.28 $\pm$ 9.89  $\mu$ g/ml respectively. Expression of Penicillin Binding protein 2a (PBP2a) protein was investigated by western blotting method in the presence of naringenin alone and in combination with  $\beta$ -lactam antibiotics. The susceptibility of MRSA toward  $\beta$ -lactam antibiotics was augmented in the presence of naringenin. There was suppression of expression of PBP2a when MRSA (ATCC 43300) was treated with the combination of naringenin and  $\beta$ -lactam antibiotics implying that the combination of naringenin and  $\beta$ -lactam antibiotics intensifies their antimicrobial effect against MRSA. The combination of oxacillin and cloxacillin with naringenin was found to have synergistic effect whereas amoxicillin showed additive effect when used in combination with naringenin. The present study also demonstrated that the mechanism of action of naringenin is dependent on the inhibition of PBP2a.

**Keywords:** MRSA; Flavonoid; Naringenin; Antibiotics; PBP2a

## A Polyclonal Sera against Trimethyl Chitosan Nanoparticles Facilitates

### Detection of Phytopathogenic Fungi

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### Abstract

The objective of this research was to provoke a tool for the first-line detection of fungal infection in plants or any other fungal contamination in consumables. Chitin is one of the most abundant fungal cell wall

polysaccharide that's naturally deacetylated to chitosan when the pathogenic fungi infects the host. During infection this deacetylation process is serve to represent the pathogenicity mechanisms to protect the fungi from plant extracellular chitinases because chitosan is a poor substrate for chitinases. Thus, detection of chitosan could be potentially aid in the detection of fungal contamination. Previous studies have reported that chitosan is a poor immunogen hence, we used trimethyl chitosan nanoparticles (TMC) as an antigen to enhance the immunogenicity. We used several phytopathogenic fungi strains for the study. Mice were immunized with TMC nanoparticles to generate a polyclonal sera. This sera could provide an enhanced humoral immune response and generate a rich and heterogenous repertoire of antibodies against chitosan/TMC. The binding affinity of the sera with fungal cell wall was analyzed by various techniques such as ELISA, langmuir isotherm, confocal microscopy and ITC (Isothermal Calorimetry) and it was found that the polyclonal sera were able to detect chitosan in the fungal cell wall. Interestingly, it was found that the detection specificity varied among the strains in proportion to the chitin content of their cell wall. In ELISA, *Fusarium oxysporium* was detected with the highest affinity while *Trichoderma resei* was detected with the least affinity. To ensure the specific and high binding capacity we performed the adsorption isotherm and ITC, additionally confirmed by confocal microscopy. The notion of generating this antibody repertoire against chitosan, utilizing methylated derivative of chitosan as nanoparticles is a novel attempt that could prove to be beneficial in the detection of human fungal pathogens involved in the different fungal infections.

**Keywords:** Chitosan; Chitin; Fungus; Cell wall; Polyclonal sera

MM-04

### **Insights into the zinc homeostasis of *Bacillus anthracis* by characterization of its zinc uptake regulator**

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#### **Abstract**

Zinc plays paramount roles including catalytic, structural, and regulatory. Apart from the varying Zinc levels in the external environment, pathogenic bacteria encounter Zinc-inconstancies within the host. Zinc-dependent proteins are also known to manifest virulence in pathogenic bacteria. Hence, for their successful survival Zinc homeostasis is imperative. Zinc uptake regulator (Zur) is a transcriptional regulator connoted in maintaining Zinc homeostasis in pathogenic bacteria. Zinc homeostasis has been marginally scrutinized in *Bacillus anthracis*, a bioterror and causative agent of fatal disease anthrax, with no decipherment of Zur. BAS4181 is annotated as Zinc-specific transcriptional regulator, further substantiated by computational and experimental analyses. BAS4181 gene (bazur) lies in a three-gene operon. The residues critical for Zinc and DNA binding were delineated by homology modelling and sequence/structure analysis. Purified BaZur prodigiously exists in dimeric form, indicated by size exclusion chromatography and blue native-polyacrylamide gel electrophoresis. Computational and manual strategies were employed to decipher the putative regulon of BaZur. The DNA binding ability of BaZur to the promoter regions of the regulon candidates was ascertained by electrophoretic mobility shift assays. Most of the regulon genes lacked functional annotation hence comprehensive in silico analysis was employed, revealing their role in Zinc uptake, mobilization and as Zinc chaperones. BaZur was found to exert negative regulation on most of the regulon genes. A downregulation was observed in the expression of bazur under Zinc-excess and marked upregulation under Zinc-depleted environment, adding credence to its negative autoregulation. Moreover, an increase in the transcript levels of the regulon genes upon Zinc-



depletion connoted their role in combating hypo-zincemic conditions. It's proposed that under conditions of Zinc feast, the BaZur represses the expression of its regulon genes and under Zinc famine BaZur exists in non-Zinc form, causing their derepression. Thus, this study provides an insightful investigation of Zur and Zinc homeostasis in *B. anthracis*.

**Keywords:** Zinc homeostasis; *Bacillus anthracis*; Zinc uptake regulator; Regulon; Transcriptional regulator

MM-05

### **Molecular cloning of the *UbiA* Gene encoding PHB decaprenyl transferase from *Agrobacterium tumefaciens* to enhance CoQ10 yield**

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#### **Abstract**

Coenzyme Q (CoQ), a naturally occurring molecule is present in all biological membranes predominantly located in the hydrophobic domain of the phospholipid bilayer of inner membrane system of the mitochondria where it plays role in essential electron transport chain. CoenzymeQ10 has immense health benefits to be considered as Nutraceutical or bioceutical. For industrial production of CoQ10, use of microbes as cell factory has been found to be an attractive and viable method. Natural and genetically engineered microorganisms like *Agrobacterium tumefaciens*, *Paracoccus denitrificans*, *Rhodobacter sphaeroides* and *Escherichia coli* are commercially exploited to produce Coenzyme Q10. *UbiA* is enzyme that catalyzes a key step of linking para-hydroxybenzoate and isoprenoid for CoQ10 biosynthesis. For enhancing CoQ10 yield, cloning of *ubiA* gene was carried out. The *ubiA* gene from *A. tumefaciens* was isolated, which encodes PHB decaprenyl transferase was cloned in *Escherichia coli* (*E. coli*) and will be characterized. With gaining knowledge of regulatory mechanisms, enzymes, precursors involved in biosynthetic pathway of UQ (ubiquinone) and by modulating this pathway through genetic engineering of hosts, such as *Escherichia coli* there might be enhanced UQ production at commercial level.

**Keywords:** CoQ; *Agrobacterium tumefaciens*; Nutraceuticals; PHB decaprenyl transferase; *UbiA*

MM-06

### **Structural and functional distinctions associated with a novel identified *hipBA*<sup>Xn3</sup> Toxin-antitoxin (TA) module from *Xenorhabdus nematophila*: *Insilco* approach**

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#### **Abstract**

In the whole world, antibiotic persistence problem is an intimidating remark and bacteria are continuously adopting new persistence mechanisms by which they are emerging, thriving and spreading globally. Bacterial toxin-antitoxin (TA) modules are one of the best investigated mechanisms for the formation of persister cells. This is the first report to study an *insilico* approach towards chromosomal *hipBA*<sup>Xn3</sup> TA module from *Xenorhabdus nematophila*, a species-specific mutualist of entomopathogenic nematode *Stein-*

*ernema carpocapsae*. This TA module was identified on the chromosome of *X. nematophila* ATCC 19061 (NCBI Refseq NC\_014228) and located at position 2834500-2836018 bp on the positive strand of chromosome under XNC1\_operon 0534 locus tag. Further, it consists of a 1251 bp *hipA<sup>Xn3</sup>* toxin gene which encodes for a 416 amino-acid residue protein and a 276 bp *hipB<sup>Xn3</sup>* antitoxin gene which encodes for a protein having 91 amino acids. As no crystallographic structure is available so far, therefore structural models for both proteins were deduced by threading approach and validated by VERIFY-3D program as well as Ramachandran plot analysis. Results showed that almost 96 % residues were falling into the most favored and allowed regions. Various ligands which may bind with HipA<sup>Xn3</sup> toxin and HipB<sup>Xn3</sup> antitoxin proteins were also predicted by COACH program. Moreover, interaction between both proteins was also confirmed by STRING analysis. 150 bp upstream region from *hipB<sup>Xn3</sup>* antitoxin gene was also characterized as promoter for *hipBA<sup>Xn3</sup>* TA module and binding sites for transcription factors *arcA*, *crp* and *rpoS17* were also explored. This novel TA system was further analysed for functional activity via molecular functions and biological processes by gene ontology terms and an evolutionary relationship was also inferred. This study allows initial inferences about the unexplored 3-D structure of the *hipBA<sup>Xn3</sup>* TA module and may be further used in rational design of molecules for structure-function studies.

**Keywords:** *Xenorhabdus nematophila*; Toxin-antitoxin; Persistence; Entomopathogenic; *hipBA*

MM-07

### Drug Repurposing Strategy: A new approach against various diseases

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#### Abstract

In the growing era, the demands for drug development increased due to multidrug resistant pathogen (MDR) and emerging viruses. Similarly, the emerging outbreaks of infection from Zika and Ebola virus becomes a potential hazard to healthful living. Therefore, a new approach Drug repurposing comes in light that defines the utilization of drug in a different way from pre-existing drug. Drug repurposing reduces the time and cost of developing new drug. Combination of two or more compounds increases the potency of drug as well as reduces its toxic level as it required in less amount. Screening of earlier drugs may provide a better drug against the emerging disease. Similarly, a change in structure, change in mode of application of drug, combination of adjuvant and bacterial toxins with drug enhance the activity of drug. So we are focus on the different ways to utilize the existing drug for emerging diseases. This would help us to discover broad spectrum antibiotics against pathogens.

**Keywords:** MDR (multiple drug resistant); Drug Repurposing; Ebola; Zika; Adjuvant

MM-08

### Interaction of glycolytic enzymes with influenza a virus structural protein and its effect on Glut-1 mediated glucose uptake

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### Abstract

Influenza A virus is a respiratory pathogen which belongs to Orthomyxoviridae family. It is an RNA virus which encodes for 17 proteins. During the course of its infection viral proteins interact with an array of host proteins modulating various cellular metabolic processes to establish successful infection in the host. Our earlier studies had demonstrated differential expression of glycolytic enzymes; alpha enolase and pyruvate kinase in response to recombinant influenza A viral nucleoprotein (NP) and their interaction with this protein in virus infected cells. Interestingly, these two glycolytic proteins were reported to be packaged into mature influenza A virion by a proteomic approach. In the current study we sought to investigate the role of viral NP and matrix protein (M1); another abundant structural protein in the regulation of host cell glycolysis. We hypothesize that the interaction of these two viral proteins with alpha enolase and pyruvate kinase might affect the glucose uptake, subsequently host cell glycolysis. Further, its effect on glucose transporters in cells infected with influenza A virus was also evaluated. The interaction between viral M1 with above glycolytic enzymes was confirmed using recombinant GST tagged M1 generated in prokaryotic expression vector pGEX4T1. *In vitro* binding assay demonstrated interaction of alpha enolase and pyruvate kinase with M1 protein alone and in association with viral NP. Interaction of viral and host proteins was further confirmed in cells infected with influenza A virus by immunoprecipitation assay. Our experiments had shown increased glucose uptake in infected cells as compared to mock infected cells during early hours of infection, with no change in Glut1 expression levels between mock and virus infected cells. However, Glut 1 expression was significantly decreased after 3 rd day post infection as compared to mock infected cells. Studies are ongoing to understand the correlation between glucose uptake, glycolysis and glucose transporters in association with viral NP and M1 proteins.

**Keywords:** Orthomyxoviridae family, Enolase, Pyruvate kinase, Glycolysis

MM-09

### Identification and biochemical characterization of *E. coli* *icia* homolog *rv1985c* in *Mycobacterium tuberculosis* as novel nucleoid-associated protein

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### Abstract

*Mycobacterium tuberculosis* (Mtb.), the etiological agent of tuberculosis (TB) has been one of the most successful pathogen in the history of the mankind. The molecular mechanisms associated with the pathogenesis and persistence of Mtb. are scarcely understood. Recent work related with Mycobacterial Nucleoid Associated Proteins (NAPs), links the functionality of NAPs in various cellular, physiological, and virulence mechanisms. In this study we have focused on the identification and biochemical characterization of novel NAP in Mtb. and also elucidated its role in growth of non-pathogenic bacterium *E. coli*. Till date, only seven NAPs have been identified in Mtb., viz., Lsr2, EspR, mIHF, HupB, MDP2, NapM and NapA, which are very less as compared to the number of NAPs identified in other bacteria. Sequence homology studies revealed the presence of *E. coli* *iciA* homolog *rv1985c* in Mtb. Earlier studies established the role of IciA as NAP in *E. coli*. That's why we suspected the role of Rv1985c as novel NAP in Mtb. *rv1985c* gene was PCR amplified from Mtb. genome and further cloned in pETPhos vector. Protein purification was performed by IMAC chromatography technique using Ni<sup>2+</sup> - NTA resin as immobile phase. *In-vitro* DNA binding activity of this protein was checked through EMSA (Electrophoretic mobility shift assay) and this showed that Rv1985c is a non-specific DNA binding protein. Over-expression of this protein in non-pathogenic bacteria *E. coli* showed extension of lag phase in over-expression strain in comparison to vector control strain. This study highlights the importance identification of novel NAPs in Mtb., which

have a significant contribution in survival and pathogenesis of the pathogen and can also act as new drug targets or vaccine candidates.

**Keywords:** *Mycobacterium tuberculosis*; Nucleoid associated proteins; *rv1985c*; EMSA

MM-10

### Biochemical characterization of a putative serine/threonine phosphatase in *Bacillus anthracis*

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#### Abstract

Post-translational modifications (PTMs) are known to affect the biological properties and functions of proteins and also add a layer of complexity between genome and proteome. In particular, phosphorylation is one of the most studied PTM across eukaryotes that regulate various cellular processes like cell cycle, metabolism, cellular architecture, signal transduction events and response to extracellular signals. As the roles of eukaryotic kinases and phosphatases have been well described in a variety of biological processes, we hypothesize that their counterparts in the *Bacillus anthracis* (BA) might also play an important role in the regulation of key events in the life cycle and pathogenesis of this bacteria. BA is a gram positive, spore-forming bacterial pathogen that causes anthrax. Sporulation and germination are the two major events in the life cycle of BA. To date, only one eukaryotic-like phosphatase named PrpC has been characterized in BA. Here we report a novel putative phosphatase in BA based on sequence homology to the known eukaryotic phosphatases and characterize it using para-nitrophenylphosphate (pNPP) assay. To elucidate the biological function of this novel phosphatase, we made an overexpression strain in BA and studied its effect on the phenotype, growth kinetics, sporulation and germination efficiency. The results obtained with this overexpression strain will be discussed.

**Keywords:** Phosphorylation; *Bacillus anthracis*; Phosphatase; Sporulation; Germination; Overexpression

MM-11

### Thermophilic Xylanase Mining from Hot Spring of Odisha Using Sequence Based Metagenomics Approach

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#### Abstract

Every sphere on earth is inhabited with microbes, be it human body, hot thermal springs, soil, icy glacier or even deep ocean floor. However, only 0.1-1% microorganisms are cultivable rest 99% were non-cultivable due to their unique habitat requirement that we are unaware of. Metagenomics emerged as a method to get into microbial diversity of these non-cultivable microbes and their potential in various industries and diverse ecosystem. Hot springs are springs form due to geothermal activity that heats

ground water and came out from earth's outer layer. Thermophilic enzymes are suitable candidates for application in industrial bioprocesses because they can withstand harsh conditions (High temperature and extreme pH levels) imposed by industrial processes. Xylanases (O- glycoside hydrolases, EC 3.2.1.8) are glycosidases belongs to hydrolase family and known for cleavage of 1, 4-  $\beta$  xylosidic bond present in backbone of xylan. Xylanases having low molecular weight and thermo-stability are recently in demand of pulp and paper industry, feed industry, textile industry and bio-ethanol production. In the present study we have used sequence based metagenomics approach to mine novel xylanases from Atri hot spring of Odisha. Metagenome was isolated using soil as a source of DNA. Enzyme specific degenerate primers were used for amplifying xylanase gene (ODXyn) from Metagenome and cloned in pGEM-T vector and transformed into *E. coli* JM109 highly efficient competent cells. Amplicon of size 500-600 bp was obtained and extracted using gel extraction kit and sequencing was done. ODXyn show 91.12% identity with *Bacillus halodurans* endo-1, 4-beta-xylanohydrolase gene.

**Keywords:** Metagenomics, Xylanase, Metagenome, Thermophilic, Xylan, Degenerate.

MM-12

### Detection and characterization of nitrite reductase gene from anaerobic bacteria causing chronic periodontitis

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#### Abstract

Periodontitis is an inflammatory disease, characterized by the formation of dental plaque or a bio-film of several bacterial species producing various virulence factors. Many bacteria producing such virulence factors have been isolated from the periodontal pockets including species of *Porphyromonas*, *Prevotella*, *Fusobacterium* etc. Analysis of genome sequence of these pathogens suggests that the genome encodes for many enzymes that could be implicated in anaerobic respiration like the anaerobic from patients with clinical symptoms of chronic periodontitis and characterised anaerobic nitrite reductase genes. In the current investigation we have isolated and identified the periodontal bacteria nitrite reduction in these bacteria. Samples were collected from 80 patients in the age range of 30 to 60 years using sterile paper points into TSB broth. The samples were then plated on blood agar plates and incubated in anaerobic chamber for 3-5 days at 37°C. Pure cultures of the periodontal bacteria were isolated and identified using 16SrRNA sequencing and biochemical assays. Two of the important periodontal isolates in our study include *Prevotella* and *Fusobacterium* species. These isolates were further characterized by anaerobic nitrite reduction. Assay and were found to be capable of nitrite reduction as detected by decrease in nitrite concentration from 1mM to undetectable levels over 2-3 days. Further, presence of active nitrite reductase enzyme was confirmed in these bacteria during nitrite enzyme activity assay. Moreover, we were able to detect presence of 501 bps *nrfA* gene fragment in these periodontal strains by degenerate PCR using primers specific for nitrite reductase gene. We are further analysing the degenerate PCR amplified nitrite reductase gene fragments by sequencing and their insilico analysis.

**Keywords:** nitrite reduction; anaerobic respiration; virulence; *Prevotella*; *Fusobacterium*

## Role of CRP and Adenylate cyclase in Virulence of *Aeromonas hydrophila*

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### Abstract

*Aeromonas hydrophila* is an important gram-negative pathogenic bacterium known to cause red fin disease in fishes and several intestinal and extra-intestinal diseases in humans. It has several known virulence factors such as hemolysin, cytotoxins, enterotoxin and aerolysins. Additionally, the genome sequence of *A. hydrophila* also shows presence of cyclic adenosine monophosphate receptor protein (CRP) (AHA\_0951) and adenylate cyclase (*cya*) genes (AHA\_0470, AHA\_2412, AHA\_3770). The role of these genes and encoded proteins in motility, biofilm formation and virulence of *A. hydrophila* is not known. In our current study we are investigating the role of these regulatory proteins in virulence of *A. hydrophila*. Insertional mutants of CRP and adenylate cyclase genes were generated using plasmid Per21 having R6k origin of replication. For insertional inactivation of the target gene the internal approximate 500 bps of the target gene is amplified by PCR and cloned into PER21 vector. The plasmid with the cloned fragment is transformed into *A. hydrophila* strain. Recombination leads to insertional inactivation of the target gene as indicated by gain of antibiotic resistance marker. The biofilm formation was significantly reduced in the CRP mutant (AHA\_0951) and two of *cya* mutant (AHA\_2412 and AHA\_3770) as compared to the wildtype strains. These results indicate the involvement of the regulatory proteins in regulation of pilin biosynthesis and other virulence mechanism. For complementation of the CRP and adenylate cyclase mutants, full length wildtype gene will be amplified and cloned in to broad host range vector PJB3Cm6 and transformed into the respective mutant strains. Further studies will involve cell culture studies, fish infection assays and attachment assay with the wildtype, mutant and their complemented strains. We are also performing mutagenesis studies to identify the functional pili in biosynthesis genes. Further we will analyze the role of these regulatory proteins in controlling the expression of the identified pilin biosynthesis genes.

**Keywords:** *Aeromonas hydrophila*; Virulence; Regulation; Biofilm; Pili

MM-14

## Development of real-time loop mediated isothermal amplification assay for the detection of *Yersinia pestis*, the causative agent of plague

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### Abstract

*Yersinia pestis*, the causative agent of plague is a Gram-negative, rod shaped coccobacilli, which mainly infects rodents, while humans are only accidental host. Plague can be transmitted through flea bite, direct handling of infected animal tissues, or inhalation of aerosolized bacteria. Depending on the route of infection, there are three major clinical manifestations of plague: Bubonic (by flea bite), Pneumonic (by respiratory droplets) and septicemic plague. *Y. pestis* is classified as a category 'A' agent by NIAID, USA due to its high mortality and easy person to person dissemination. The conventional diagnostic methods available for *Y. pestis* show cross-reactivity with other enteropathogenic bacteria which make its

detection difficult. Rapid and reliable point-of-care detection of *Y. pestis* is essential for timely initiation of medical treatment. Therefore in the present study, a sensitive and specific loop mediated isothermal amplification assay has been developed for rapid detection of *Y. pestis*. LAMP is a simple, rapid and sensitive assay suitable for application in the field areas. A set of LAMP primers were specifically designed targeting the *cafI* gene located on pFra, a prototypical plasmid of *Y. pestis*. Isothermal amplification was accomplished at a constant temperature of 65 °C for 40 min. Primer concentrations and assay conditions were optimized and the results were monitored by real-time turbidimeter. The amplified products were also visualized using SYBR green, DNA intercalating dye under visible and UV light. The analytical sensitivity of the assay was found to be 500 fg genomic DNA of *Y. pestis* and 100 copies of target DNA harboured in a recombinant plasmid. The assay was found to be highly specific as it did not cross-react with other closely related species. The assay however needs to be further validated clinical and environmental samples for detection of *Y. pestis*.

**Keywords:** Plague; *Yersinia pestis*; LAMP; SYBR green dye.

MM-17

### Characterization of nucleoid associated proteins (NAPs) from *Staphylococcus aureus*

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#### Abstract

Bacteria possess certain DNA architectural and modulating proteins called the Nucleoid Associated Proteins (NAPs) or Histone-Like Proteins (HLPs). NAPs are low molecular weight basic peptides, functionally similar to the eukaryotic histones and play a key role in the bacterial nucleoid organization via DNA bending, bridging or forming aggregates. NAPs also play crucial roles in gene regulation, transcription, recombinatorial repair, replication, virulence, stress resistance, etcetera. The most extensively studied NAPs are from the model bacterium *Escherichia coli* (Gram-negative), however the identification and characterization of NAPs in Gram-positive bacteria remains a less-explored territory. H-NS, HU, FIS and IHF are some highly characterized NAPs found throughout the Gram-negative bacteria. H-NS homologues are present in a wide variety of bacterium and play crucial roles. From literature, we know that the C-terminal domain of H-NS aids in DNA-binding and the N-terminal domain is the dimerization domain. One such H-NS homologue is the focus of study in our work presented here. DNA compaction is one of the pre-requisites for any DNA-binding protein to be characterized as a NAP. Therefore, here we show the effect of an H-NS homolog found in *Staphylococcus aureus* NCTC 8325 strain on the chromatin condensation. We did a heterologous expression of this NAP from *S. aureus* into *E. coli* cells. Upon over expression, nucleoid compaction is observed, as opposed to uninduced cells, wherein, the chromatin is dispersed throughout the cell-length. A full-length construct versus a truncated version of the same protein are overexpressed in the cell to contrast the effect of different domains of the protein on nucleoid compaction. The truncated version used here lacks the N-terminal domain, which is supposedly essential for dimerization. NAPs are not only important to be studied just to assess nucleoid compaction but also to identify their roles in other biochemical pathways critical to the cell growth.

**Keywords:** Nucleoid Associated Proteins; H-NS; *Staphylococcus aureus*; DNA compaction

## Cloning and expression of Pyrroloquinoline quinone (pqq) from a commercially important phosphorous solubilizing *Burkholderia cenocepacia* PS27

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### Abstract

*Burkholderia cenocepacia* strain PS27 (previously known as *Pseudomonas striata*) is a Gram-negative bacterium, possessing the ability of solubilising mineral phosphate, potassium from compounds and zinc from oxides, carbonates and sulphate. Previously, sequencing of its genome revealed the presence of gene clusters and operons for mineral phosphate solubilization (MPS) and uptake (Pst system and Pit system), K-solubilisation and uptake (kdg system) and Zinc solubilisation and uptake (*zntB*, *znuC*, *zntR* and *zur*). *B. cenocepacia* PS27 has a strong ability to solubilise insoluble phosphate, for which it secretes gluconic acid. The gluconic acid is produced by direct extracellular oxidation of glucose by a glucose dehydrogenase, using pyrroloquinoline quinone (PQQ) as a cofactor. In present study, pqq gene cluster (- 3000 bp) was cloned, using pet28α bacterial plasmid expression vector (5369 bp). The final construction (- 8000bp) was transformed and expressed in *Escherichia coli* BL-21 strain. Further screening of recombinant clones was done by performing blue white screening technique. Clones containing recombinant plasmids produced higher clearing halozones on Pikovskaya's agar plates with insoluble tricalcium phosphate as the P source, in comparison with those of without plasmids, demonstrating the successful cloning. This genetic manipulation allowed the increase in MPS ability of the strain, enhancing the potentialities as growth promoters of agricultural crops.

**Keywords:** *Burkholderia cenocepacia*; pyrroloquinoline; recombinant; K-solubilisation

## Morphological and genomic characterization of mycobacteriophages isolated from Delhi-NCR

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### Abstract

In the prevailing 'post-antibiotic era', an urgent need for non-antibiotic strategies are required for the treatment of drug-resistant infections. Phage therapy (and phage-derived enzyme therapy) is emerging as a promising alternative. Mycobacteriophages are virus specific to *Mycobacterium* spp., having the potential for treating tubercular and non-tubercular (NTM) infections. Besides, Mycobacteriophages can be recruited in diagnosis, phage typing and as tools for genetic manipulation of mycobacteria. Given their various applications, we are building a repository of local Mycobacteriophages (phages for short) from soil samples collected from hospitals/garbage dumping locations, with *M. smegmatis* mc<sup>2</sup>155 as the bacterial host. A total of 125 soil samples have been screened and phages were found at a frequency of about one in three. So far, 30 lytic phages with high titre (- 1\*10<sup>12</sup> pfu/ml) have been purified by double-agar overlay method; the plaques obtained are largely clear with a halo, are 0.35 to 0.5 cm in size and their TEM analysis have revealed siphoviral morphotypes. On testing the stability, phages endure a pH range of 6-12 and up to 55°C temperature. Further, based on the similarities in the nucleotide sequences, phages



are grouped into clusters and sub-clusters. On cluster analysis by PCR amplification of a conserved region, we found 33% phages to belong to of B1 cluster and the remaining belonging to Y, E and F1, indicating genomic diversity in the isolated phages. On testing the methylation status, interestingly all the 30 phages showed non-methylated status. Currently, WGS and functional annotation is underway for complete genomic characterization of the repository. The growing emergence of drug-resistant TB and NTM infections has stimulated research into discovery of mycobacteriophages. We believe developing libraries of well-characterized novel phages will be a useful national asset, to be tested against the MDR clinical isolates and as a promising source of therapeutic enzymes.

**Keywords:** MDR; Mycobacteriophages; Phage therapy

MM-22

### **Bio-engineered probiotics as biotherapeutic for the management of Type 2 diabetes**

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#### **Abstract**

Type 2 diabetes (T2DM) characterized by hyperglycaemia is a common metabolic disorder which can be due to improper utilization of Insulin or inadequate production of Insulin. From the past few decades, the number of T2DM patients is increasing rapidly. Nearly one million people die every year due to diabetes. According to Indian Heart Association, 105 million people are already suffering with diabetes. Glucagon-like peptide-1 (GLP-1) is a metabolic hormone, belongs to class of incretin hormones, secreted by epithelial cells of intestine which stimulate islets of pancreas to secrete Insulin similar to that of glucose dependent mechanism. It is the most potent insulinotropic hormone which is released right after eating. Potent food-grade probiotics such as *Lactobacillus* species can be used to corroborate their compatibility for oral therapeutic administration. Therapeutic peptides or proteins expression on the microbial cellular surface, especially in lactobacilli is very appealing for an ideal vaccine/therapeutic design. This allows to explore probiotic microorganism by expressing GLP-1 on their surface to manage T2DM in both in-vitro and in-vivo model.

**Keywords:** Probiotics; Diabetes; T2DM; GLP-1; Therapeutic

MM-23

### **An active surveillance on circulating serotype of Dengue virus in the patients visiting a tertiary care hospital of Uttarakhand**

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#### **Abstract**

Dengue fever is one of the most serious public health concerns worldwide. It is a viral infection caused by a single stranded RNA virus which is spread by *Aedes* species of mosquito. This Flavivirus with its four serotypes (DEN-1 to DEN-4) spread epidemics annually. According to WHO, 3.9 billion people are living with the risk of dengue virus infection in 128 countries including India. As per the WHO and NVBDCP report on dengue in India, 220 deaths were reported in 2015 followed by 172 deaths reported in 2018. This

data shows almost 300 percent hike in dengue cases from the year 2009. In light of the above information, a surveillance study of Dengue viral infections was done in a tertiary care hospital of Uttarakhand from Jan 2018 to Dec 2018. Patients visiting AIIMS Rishikesh, with complaints of acute febrile illness followed by headache, myalgia, arthralgia were primarily screened using Rapid Test followed by Enzyme Linked Immunosorbent Assay (ELISA) for Dengue NS1 antigen. Confirmed samples were further processed for RNA isolation (Qiagen), followed by Sanger Sequencing for identification of the genotypes. 2737 dengue suspected cases from Jan 2018 to Dec 2018 visiting the hospital were screened. All these cases were primarily screened by rapid test, out of which, 239 were NS1 positive. These NS1 positive were confirmed by ELISA that revealed 205 NS1 positive for Dengue. Further, the RNA isolation and cDNA preparation were done to proceed for sequencing which further asserted the DEN2 as the circulating serotype of DV in patients coming from different regions of Uttarakhand.

**Keywords:** Dengue Virus (DV); Aedes mosquito; serotype; Uttarakhand.

MM-26

### A Green Approach towards Sustainability of Sea Food Waste through Novel Marine Chitinolytic enzymes

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#### Abstract

The advent of increase in human population has also led to an increase in the sea food industry. This has led to increased dumping of the by-product waste primarily chitin in the sea beds. Chitin is a  $\beta$ -1,4 linked homopolymer of N-Acetylglucosamine (GlcNAc) arranged either in antiparallel ( $\alpha$ -chitin) or in the parallel arrangement ( $\beta$ -chitin), with  $\alpha$ -chitin found more abundant as compared to the other isomeric forms (Rathore and Gupta, 2015). In spite of its annual production of around  $10^{11}$  metric ton globally, the accumulation of chitin is highly negligible (Paulsen et al., 2016). This recycling of chitin can be largely attributed to the chitinase, chitosanase and chitin deacetylase secreted by the marine organisms. This motivated us to isolate these chitin modifying enzymes and study their mechanism of action. The chitin modifying organisms from the Arabian Sea were isolated. The sample was collected from 12°48' N and 74°40' E at a depth of 12 m. The initial screening resulted in nine morphological different colonies which were screened for their chitinase activity. This yielded in an isolate; MS7 which upon molecular characterization was identified as *Bacillus aryabhatai*. Homology based modelling of the chitinase (BaChiA) showed the presence of two N-terminal LysM domains which has been earlier reported in plants, ferns, and algae (Kitaoku et al. 2017; Onaga and Taira 2008) of molecular mass 42 kDa, was purified from the leaves of a fern (*P. ryukyuensis*). The BaChiA was able to work on the highly crystalline  $\alpha$ -chitin flakes. This might be due to the presence of the two LysM domains present in the enzyme structure. The chitin deacetylase (BaCDA) was able to deacetylate the products after working in succession of BaChiA. This is the first report of a microbial chitinase with LysM domain. This synergistic action of BaChiA and BaCDA on chitin can be exploited to obtain patterned products for various biomedical and industrial applications and add value addition to the sea food waste.

**Keywords:** *Bacillus aryabhatai*; Chitinase; LysM domains; Chitin deacetylase; Chitinolytic enzymes

## Bacteriocin coated silver nanoparticles as potent antimicrobials against multi-drug resistant *Acinetobacter baumannii*

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### Abstract

Multidrug resistant *Acinetobacter baumannii* (MDR-Ab) has emerged as a significant hospital pathogen, quickly becoming resistant to commonly prescribed antimicrobials and thus has gathered worldwide attention. Peptide conjugated metallic nanoparticle is one of the promising approaches in combating multidrug resistant pathogen. In the current study we have used bacteriocin capped silver nanoparticle in combating multidrug resistant *Acinetobacter baumannii*. Two bacteriocins from lactic acid bacteria were used to synthesize peptide coated silver nano particles. The synthesized nanoparticles were characterized by using biophysical techniques such as UV-Vis excitation spectra, TEM, FTIR, Zeta potential. The MIC of bacteriocin coated silver nanoparticles were determined by broth micro dilution method. The time-kill kinetics assays were used to determine bactericidal activity of the nanoparticles. Live- dead staining, Scanning Electron Microscopy, Biofilm inhibition assays was done to investigate the potential of synthesized bacteriocin capped silver nano particles. Resistance development assay was carried out by passaging *A. baumannii* AYE to repeated exposure of subinhibitory concentration of bacteriocin capped silver nano particles. The experimental findings demonstrated that peptide capped silver nanoparticles are effective against carbapenem resistance strains of *Acinetobacter baumannii* without detectable resistance.

**Keywords:** Multidrug resistant; Silver nanoparticles; Bacteriocins; Biofilm inhibition

## Uncovering the mechanism of action of L6-Ag<sup>+</sup> complex as antibacterial compound

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### Abstract

The rising problem of antimicrobial resistance (AMR) has posed a serious threat to the healthcare system globally. Pathogens are evolving with time to gain resistance against antibacterial agents including final resort antibiotics. It is very important for the scientists to discover new antibiotics against which bacteria cannot develop resistance. The scientific community is investigating alongside healthcare systems intensively to search for novel drug candidates that can help in combating this issue. In the present study, we have utilized forward chemical genetics approach in an attempt to find a novel antibacterial molecule. A compound L6-Ag<sup>+</sup> was synthesized and was tested against various Gram-positive and Gram-negative strains as well as clinical strains. The compound depicted broad-spectrum antibacterial activity. We then attempted to decipher the mode of action of the compound. Based on the DCFH DA assay, L6-Ag<sup>+</sup> was found to be a reactive oxygen species (ROS) generating compound. Further, we analysed DNA damaging property of the compound using a cell-based biosensor. Membrane damaging activity of L6-Ag<sup>+</sup> was also investigated with the help of fluorescence microscopy and spectro-fluorometry. ROS was

established as the major cause of cell death with the help of antioxidant, vitamin C. Overall, this study represents a compound L6-Ag<sup>+</sup>, which exhibits a broad spectrum and better antibacterial potential than silver nitrate.

**Keywords:** Antimicrobial resistance; Antibacterial; Reactive oxygen species

MM-31

### Occurrence of mobile colistin resistance gene *mcr-1* among bacterial isolates from aquatic environment in Delhi, India

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#### Abstract

The rapid increase in the prevalence of multidrug-resistant pathogenic bacteria is a significant global health concern. Antibiotic-resistant bacteria are one of the major causes of mortality worldwide. Apart from fundamental applications in clinical settings, antibiotics are extensively used in agriculture, food industry and aquaculture. Presence of antibiotics in the ecosystem, serves a potent stimulus to elicit a bacterial adaptation response to develop antibiotic resistance. Increased use of colistin, a last resort drug due to failure of Carbapenems has possibly contributed in development and spread of resistance to colistin among *Enterobacteriaceae*. The colistin belongs to the family of Polymyxins, cationic lipopeptides, with broad-spectrum activity against Gram negative bacteria. In majority, colistin resistance is chromosomally mediated involving modulation of two component regulatory systems e.g. *pmrAB*, *phoPQ* and its negative regulator *mgrB* leading to modification of lipid A or total loss of the lipopolysaccharide(LPS). Some new reports indicate horizontal transfer of colistin resistance *mcr-1* gene with multiple variants known worldwide. In this study we obtained 253 non-duplicate bacterial isolates from sewage water and River Yamuna in Delhi and phenotypically screened for colistin resistance. Of the 47 positive isolates colistin resistance gene *mcr-1* was detected among 10 isolates. Based on 16s rRNA based identification bacterial isolates were found to be *E.coli*, *Aeromonas veronii*, *Providencia rettgeri*, and *Aeromonas dhakensis*. ESBL resistant determinants CTX-M and TEM were detected in all 10 *mcr-1* positive isolates. On the basis of literature survey, this is first report of *mcr-1* gene from *Aeromonas veronii* and *Aeromonas dhakensis*. Furthermore, *mcr-1* gene has not been reported earlier from sewage water in India. Antibiotic susceptibility test of all 10 isolates against 9 different classes of drugs revealed multidrug resistant phenotype with high MIC values. *In vitro* trans-conjugation studies showed successful transfer of *mcr-1* and other ESBLresistant determinants. Results of our conjugation studies further highlight the risk for dissemination of *mcr-1* gene to other bacteria including clinically important pathogens.

**Keywords:** Colistin resistance; *mcr-1*; ESBL; LPS; Aquatic environment.

MM-32

### Understanding the antibiotic resistome of the soil sample by culture dependent approach

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### Abstract

Antibiotic resistome is a term used to describe the collective antibiotic resistance gene and their precursors present in an environment. One of the major threats to human health and welfare is the presence of antibiotic resistance in microbial communities. Recent studies have shown that antibiotic resistance genes are widespread throughout the globe. Various studies have suggested that soil is a major reservoir of antibiotic resistance genes and use of antibiotics in clinical as well as non-clinical settings has not only created selective pressure for antibiotic resistant microbes but also created ideal environment for the proliferation of antibiotic resistance microbes. Distribution of antibiotic resistome across soil microbiomes of clinical importance is not well understood. In the present study we propose to investigate a composite soil sample collected from various sites from a hospital ground located at Kakryal region of Jammu and Kashmir by culture dependent approach to get a better idea about the natural distribution of antibiotic resistome in clinical setting in relation to the present scenario. So far we have isolated 44 antibiotic resistant bacterial strains. The strains are resistant to different antibiotics like Amoxycillin, Ampicillin, Cefotaxime, Chloroamphenicol, Kanamycin, Naladixic acid, Rifampicin, Streptomycin and Tetracyclin. The antibiotic resistant bacterial strains have been isolated as pure cultures and have been maintained using aseptic techniques. Detailed results will be presented in the conference. This study will help us to understand the natural composition of antibiotic resistome of the soil found in clinical settings and help us to determine the strategies for tackling the threat of antibiotic resistance.

**Keywords:** Antibiotic resistance; Antibiotic resistome; Culture -dependent; Selective pressure

MM-33

### Novel strategies to investigate integration and excision mechanisms of pathogenicity islands

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### Abstract

Mobile Genetic Elements (MGEs) such as plasmids, bacteriophages, insertion sequences, transposons, integrons & pathogenicity islands (PAIs) constitute 5-10% of bacterial genomes and contribute significantly in the evolution of bacteria. Although, the dynamics of plasmids and phages are well studied, little is known about the acquisition and dissemination mechanisms of PAIs. Therefore, in our laboratory, we are working to decode the acquisition and dissemination mechanisms of PAIs using vibrio pathogenicity island-1 (VPI-1) as a model system. For this, we have constructed reporter strains using wild type *V. cholerae* clinical isolate N16961 in which *tcpA* gene has been replaced with *sacB-cat* allele. The *sacB* gene act as a counter-selectable in the presence of sucrose. *V. cholerae* cells containing *sacB* in the genome unable to growth in optimal growth conditions in the presence of sucrose. We have measured the excision frequency of VPI-1 in *V. cholerae* and isolated VPI-1 devoid derivative. We also measured the integration efficiency of VPI-1 integration module (VPI-1<sub>im</sub>) at the *attB*<sub>vpi</sub> site of *V. cholerae* chromosome. We observed that integration of VPI-1 is reversible. We have observed that both the recombinases mediate the integration/excision independently; however integration efficiency in the presence of *int*<sub>vpi</sub> is higher than *vpiT*. Besides this, we also developed a calorimetric assay to observe integration/excision event. For this, the synthetic *attB*<sub>vpi</sub> site was cloned in *lacZ* gene in such a way that *lacZ* gene retains its functionality but in the event of integration, *attB*<sub>vpi</sub> site get interrupted resulting non-functional *lacZ* gene. In the presence of chromogenic substance X-gal the integrated colonies turned white, whereas, in the event of excision, the colonies remain blue.

The genetic tools we developed in our laboratory could also be used for other bacteria to investigate integration and excision mechanisms of different pathogenic islands.

**Keyword:** Mobile Genetic Elements; Pathogenicity Islands; Integration and excision; Reporter strain

MM-34

### Transcriptional responses of mosquito hemocytes against microbial challenge in Indian malarial vector *Anopheles culicifacies*

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#### Abstract

Mosquito's during their whole life deals with diverse nature of pathogens such as aquatic & blood meal associated. Unlike vertebrates the defense system of mosquito leans only on innate immunity mediated by mosquito blood cells (Hemocytes) and their released factors. The hemocyte mediated killing mechanism involves multiple immuno-physiological responses such as phagocytosis, lysis, melanization, degranulation etc, however understanding the nature of the molecular interaction between hemocytes and microbial pathogen remains challenging. *Anopheles culicifacies* is the main vector of human malaria in rural India causing more than 65% malarial cases annually. Due to limited information availability for *A. culicifacies* and to get benefits of the advance technologies towards malaria vector control strategies, cDNA libraries for examining immune tissues specific response of *An. culicifacies* against bacterial challenge were generated by infecting with mixed bacterial culture of *E. coli* and *B. subtilis*. The Illumina based pair end sequenced transcriptomic data of Control and bacterial challenged hemocytes, retrieved raw data of size 2.2GB and 4.0GB respectively. DGE analysis was performed using edgeR software, which shows that both control and treated hemocytes share around 2860 common transcripts along with 91 and 1249 unique transcripts respectively. An experimentally validated up-regulation of several immune transcripts such as Cactus, Trsferrin, interferons & LITAF (LPS induced tumor necrosis factor) against the bacterial infection suggested their potential role to encounter the invaders. An additional finding of specific novel Immune transcripts notably APHAG, REL, PPO, TAK, PGRP, GNBP & GALE etc., provide a valuable source of unique targets which may play crucial role in immune activation with in mosquito host. Future functional analysis of selected transcripts analysis can help to better understanding genetic basis and the role of the hemocyte in the malaria transmission dynamics.

**Keywords:** *Anopheles culicifacies*; Mosquito; Malaria; Hemocytes

MM-35

### Crosstalk between amidases and lytic transglycosylases during cytokinesis in *Caulobacter crescentus*

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### Abstract

Bacterial Peptidoglycan cell wall determines the morphology and provides mechanical strength during various stress conditions. The biosynthesis of this structure is accomplished by a large set of enzymes. The biochemical function of most of these proteins are well investigated but their spatiotemporal regulation as well as interaction with other divisome proteins is still poorly understood. The focus of our research is to study the enzymes involved in cytokinesis of  $\alpha$ -proteobacterium *C. crescentus* has a dimorphic life cycle and is an ideal model organism for study of cell shape as it undergoes series of coordinated morphogenetic changes during its cell cycle. Recent work has shown three different set of divisome components: LytM like endopeptidases, soluble lytic transglycosylases and amidase localized at midcell at distinct stages in *C. crescentus*. LytM domain protein F (LdpF) is a LytM domain containing protein with a signal peptide, two N-terminal CC domains, and a C-terminal LytM domain. SLTs are family of hydrolases, consisting cleavable signal sequence and is required for cell envelope integrity. We have created SLT deletion strain which shows hypersensitivity to beta lactam antibiotics whereas overexpression of both SLT and LdpF leads to morphological defects in *C. crescentus*. With the help of all three enzymatic proteins (LdpF, SLT, amidase) knockout strains and their fluorescent tagging, we have analyzed their role in cytokinetic machinery of *C. crescentus* as well as their functional interactions with each other and other divisome complex proteins. The research further unravels the contributions of SLTs, LdpF and amidases to cell wall biogenesis of *C. crescentus*.

**Keywords:** *C. crescentus*; Peptidoglycan; Lytic transglycosylases; Endopeptidases; Amidases

MM-36

### Molecular characterization of root nodule bacteria associated with *Dichrostachys cinerea* from soils of different Agro-climatic zones of India

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### Abstract

*Dichrostachys cinerea* (sickle bush) is fast growing nodulating shrub of mimosoid clade in subfamily *Caesalpinioideae sensu lato* (family *Leguminosae*). It is naturally distributed in various states of India and is of great ecological significance e.g. used in preventing soil-erosion, land reclamation and improving the soil fertility. To trap the rhizobia, seeds of *D. cinerea* were grown in soils collected from six states [Rajasthan (RJ), Haryana (HR), Punjab (PB), Madhya Pradesh (MP), Pondicherry (PY) and Meghalaya (ML)] of India. Total thirty-six root nodule bacterial (RNB) strains were isolated and genetic grouping {Rapid Amplification of Polymorphic DNA (RAPD)} was done using RPO1 primer. A significant genetic diversity was observed among strains that grouped into twenty-six RAPD genotypes. Twelve selective RAPD genotypes were characterized using house-keeping (*recA*) and symbiotic (*nodA*) genes. Based on protein coding sequences of *recA*, the five strains were identified as species of *Ensifer* and seven as *Bradyrhizobium*. In *recA* phylogeny strains from MP (DC-MP 27, DC-MP29) and HR (DC-HR18) clustered close to newly described species of *Ensifer* (*E. aridi*) from Thar Desert of India. Strain from RJ (DC-RJ16) formed novel lineage close to *E. teranga* and from PB (DC-PB24) clustered close to *E. kostiensis*. The *recA* phylogeny of *Bradyrhizobium* strains showed that strains isolated from alkaline soils of RJ (DC-RJ13), MP (DC-MP30, DC-MP31, DC-MP32) and PY (DC-PY35) were novel and diversified from *B. yuanmingense* whereas those strains (DC-ML38, DC-ML39) isolated from wet-acidic soils of ML are close to *B. embrapense*. The *nodA* phylogeny of DC-*Ensifer* strains showed incongruence from the housekeeping gene phylogeny and were diversified from *E. fredii*. Present study indicates that *D. cinerea* is a promiscuous host nodulated by novel species of *Ensifer* and *Bradyrhizobium*, this ability helps it in spreading to diverse agro-climatic zones of India.

**Keywords:** *Dichrostachys cinerea*; RNB; Phylogeny; *Ensifer*; *Bradyrhizobium*

## Metabolic engineering for production of useful secondary metabolites for health improvement

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### Abstract

Microbiota have an effective health promoting role which are ingested through fermented functional foods, specifically probiotic microorganisms. They are now being administered orally which in turn serves the purpose of potent alternative medication for intestinal disorders by replacing antibiotics. Most frequently used probiotics are *Lactobacilli* and *Bifidobacteria*. For many other therapeutic usage new probiotic strains are being modified to understand their mechanism of action and to strengthen them further through bioengineering. Metabolic engineering shows pathway for modulation of the organism's metabolism so that the maximum amount of desired metabolites can be extracted through genetic manipulations. During the fermentation process, the probiotic strains produce secondary metabolites and they aid in health promoting properties. Some critically effective examples include B-vitamins and bioactive peptides which get liberated from food-derived proteins. Genetically engineered LAB (Lactic acid bacteria) when overproduce vitamins, they improve the nutritional composition of fermented foods. An essential vitamin named Folic acid helps in growth and reproduction of vertebrates including treatment for coronary heart disease, neural tube defects and specific classes of cancer whereas, Bioactive peptides possess the property of inhibition against angiotensin-I-converting enzyme which exhibits anti-hypertensive effect, including antimicrobial and opiate nature, cholesterol-lowering property, possess quality of immunostimulation and mineral-utilization. Reported probiotic yeast *Saccharomyces boulardii*, used for gut health and diarrheal diseases exhibits beneficial phenotypes and is a good host to produce desired biomolecules in gut but the lacking property of auxotrophic strains of definite genetic framework hindered its usage and thereafter, the buildout of clearly defined auxotrophic mutants with the aid of clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9-based genome editing can be cast-off as a host for initiating diverse genetic perturbations that is a wonderful gift of metabolic engineering.

**Keywords:** Lactobacilli; Bifidobacteria; immunostimulation; CRISPR-cas9; *Saccharomyces boulardii*

## To study the expression of TLR5 in vagina and cervix of Balb/C mice against symptomatic and asymptomatic isolates of *Trichomonas vaginalis*.

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### Abstract

Trichomoniasis is a sexually transmitted disease caused by *Trichomonas vaginalis*. It is one of the most common and less studied non-viral STI globally so the study is conducted to have a better understanding of host-parasite interaction. *T. vaginalis* is a facultative anaerobic, flagellated parasitic protozoan of the lower urogenital tract. Infection can occur both in male and female but most of the males remain asymptomatic whereas the females could be symptomatic and asymptomatic. In symptomatic women, clinical manifestation is strawberry cervix, vaginitis, and cervicitis, frothy and foul discharge. The innate immunity



is the first line of defence mechanism against *T. vaginalis*. Innate immune recognition mediated through the expression of the pattern recognition receptors (PRRs), mainly by toll-like receptors (TLRs) and NOD-like (NLRs) receptor, which interacts with pathogen-associated molecular patterns (PAMPs) of the pathogen to release of inflammatory cytokines. In current study TLR5 is discussed which is an exosomal membrane receptor senses flagellin. The current study investigated TLR5 expression in the Balb/C mice infected with symptomatic and asymptomatic isolates of *Trichomonas* by Reverse Transcriptase PCR. Results showed that from second day post infection (dpi) Balb/C mice infected with symptomatic isolates showed increased TLR 5 expression from second day up to eighth day and there after gradual decrease upto 14<sup>th</sup> day. While in the case of asymptomatic isolates continuous increased expression was observed from eighth dpi to 14<sup>th</sup> dpi. The study concluded that there is a role of TLR5 against *T. vaginalis* infection and early immune response in case of symptomatic isolates and late immune response in asymptomatic isolates.

**Keywords:** *Trichomonas vaginalis*; PAMPs; TLRs; Reverse Transcriptase PCR



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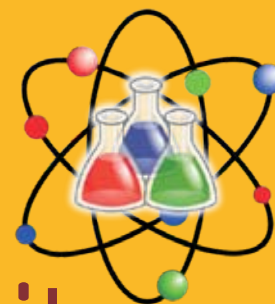
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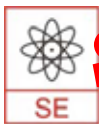
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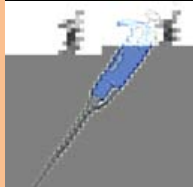
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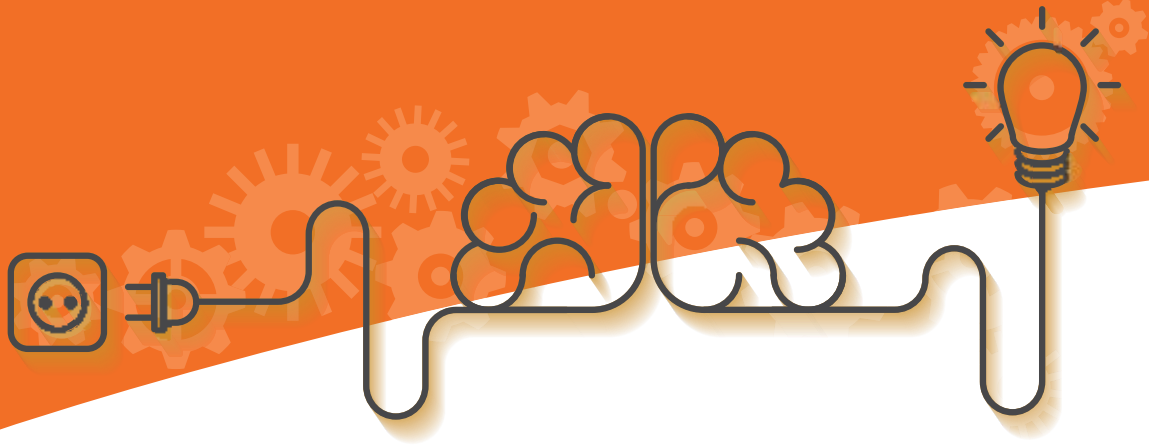
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