MANIPAL INSTITUTE OF TECHNOLOGY
MANIPAL
(A constituent unit of MAHE, Manipal)

3BIGS

Biotech
IE - Biotechnology, Manipal Chapter

Dr TMA Pai
1898 - 1979
Padmashree Awardee
125th BIRTH ANNIVERSARY

INSTITUTION OF EMINENCE

NAAC A++ GRADE ACCREDITED

INSTITUTION'S INNOVATION COUNCIL
(Ministry of Education Initiative)

G20 भारत 2023 INDIA

4 QUALITY EDUCATION
8 DECENT WORK AND ECONOMIC GROWTH
9 INDUSTRY, INNOVATION AND INFRASTRUCTURE
17 PARTNERSHIPS FOR THE GOALS

The Institution of Engineers - Biotechnology, Manipal Chapter (IE-Bt)
&
Department of Biotechnology, MIT Manipal

SymBiot'23

A National Level Biotechnology Conference

PROCEEDINGS

October 31st 2023

PATRONS

Dr. Ramdas M. Pai
Chancellor,
Manipal Academy of Higher Education

Lt. Gen. (Dr.) M. D. Venkatesh
Vice Chancellor,
Manipal Academy of Higher Education.

Dr. P. Giridhar Kini
Registrar,
Manipal Academy of Higher Education.

Cdr (Dr) Anil Rana
Director,
MIT Manipal.

Dr. Somashekara Bhat
Joint Director,
MIT Manipal.

ORGANIZERS

Dr. Balaji S
Dr. Subbalaxmi S
Dr. Fayaz S M

STUDENT ORGANIZERS

Shreya Sivaram
Kashish Dagli
Garv Agarwal
Serin Joby Parekkadan
Divydarshini Valavan
Nava Manickam
Meenakshi Harikumar
Baidurja Biswas

DESIGNED & COMPILED BY

Divyadarshini Valavan
Diya Anantheswaran
Shubha Tiwari
Pooja Agarwal
I Vidhula Jessica

GUEST SPEAKERS

Mr. Jagadish Bennale
Market Development Specialist,
Beckman & Coulter.

Ms. Manvi Singh
Scientist,
Aurigene Pharmaceuticals.

JUDGES

Dr. Balaji S
Dr. V Thivaharan
Dr. Ritu Raval
Dr. Naresh Kumar Mani
Dr. Subbalaxmi S
Dr. Divyashree M S
Dr. Ravindranath B.S.
Mrs. Vrunda Nagaraj Katagi
Dr. Ramananda Bhat
Dr. Narasimhan S
Dr. Mukunthan K. S.
Dr. Vijendra Prabhu
Dr. Praveen Kumar
Dr. N Kannan
Mrs. Archana M. Rao

SymBiot'23
A National Level Biotechnology Conference

MAHE

MAHE has branch campuses in Bangalore, Malaysia, Dubai and Antigua in the Caribbean Island. There is also a campus in Mangalore with a medical college, a dental college and a nursing college with attached teaching hospitals. MAHE has an international academic collaboration for twinning programmes in engineering with universities in the US, UK, Australia and other countries. Manipal Group institutions are located on scenic campuses, which provide a high- quality lifestyle and ideal environment for study. All campuses have excellent infrastructure for academic activities, sports and other extracurricular activities. The infrastructure includes air-conditioned lecture halls, a skills lab, air-conditioned hostels, and a multi-cuisine food court. The state-of-the-art health sciences library is fully air-conditioned, accommodates 1300 learners and has over 62,000 books and over 600 journals. The library facilities include Medline, Proquest medical library of online databases, audio-visual, Cochrane library, e-learning, computer and Internet services. The Skills Lab and Anatomy Museum are considered amongst the best in the world. The latest addition to the facilities, a Simulation Lab with computer-driven mannequins, is an achievement, which the university is proud of. It is of considerable help to students in the field of health care.

MAHE believes in providing the finest in infrastructure and facilities to its students when it comes to learning and research. In fact, some of the facilities, like the Innovation Centre, have served as a valuable ‘incubation centre’ for industry and research. The state-of-the-art innovation centre bridges the gap between universities and industries for industrial-academic research.
Other facilities on the campus include a gym, swimming pools, and football and cricket grounds.

The new indoor sports complex is perhaps one of its kind in Asia. The complex has five badminton courts, four squash courts, three tennis courts, a basketball court, gymnasiums and a walking track. Besides being an ISO 9001:2008 and ISO 14001: 2004 certified University, it is home to many top 10 ranked institutions of India. MAHE has won the prestigious IMC Ramkrishna Bajaj National Quality Award and International Asia Pacific Quality Award during 2007- 2008. MAHE attained the Institute of Eminence by MHRD in 2018.

MANIPAL INSTITUTE OF TECHNOLOGY

Manipal Institute of Technology (MIT), one of the Premier Engineering Institutes in India, was among the first self – financed engineering colleges in the country. It was started in 1957 by Padmashree late Dr.T.M.A Pai, as Manipal Engineering College with an undergraduate course in Civil Engineering. In 1965, the institute got affiliated to the University of Mysore from Karnataka University. In 1974, it was renamed as Manipal Institute of Technology (MIT). In 1980 it got affiliated to the University of Mangalore. After the creation of the Visveswaraiah Technological University (VTU), MIT along with a number of other engineering colleges in the state got affiliated to the VTU in 1998. As the Manipal Academy of Higher Education (MAHE) had acquired a Deemed University status, MIT became a constitution institution of MAHE in May 2000.

In 2003, MIT obtained full academic autonomy and adopted credit system with 10 point grading. In 2007 MAHE was renamed as Manipal University and MIT retained its status as a constituent institution of Manipal University. With total student strength of over 7500, MIT has emerged as the largest institute of University. MIT currently offers undergraduate programs (B.TECH) in 16 disciplines and postgraduate courses (M.TECH/MCA) in 24 different streams and Doctoral programs (Ph.D) in all streams of engineering, basic sciences, humanities and management. Academic programs offered by institute are approved by AICTE and have been accredited by the National Board of Accreditation (NBA). The institution plays a vital role in producing world – class engineers tuned to the demands of a fast changing global village.

DEPARTMENT OF BIOTECHNOLOGY

The Department of Biotechnology, MIT, Manipal was founded in the year 2005. The department has state-of-the-art infrastructure, well defined and updated curriculum, and wide range of electives to encourage interdisciplinary research. The faculty are highly qualified and experienced with research interests in diverse and emerging areas of biotechnology. The department has received up to 5 crores in research grants from various funding agencies.

The vision of the department: Excellence in the teaching-learning process and research.

The mission of the department: To impart and disseminate knowledge, develop competencies and to produce industry-ready and academically enriched engineers for the emerging areas of applied biotechnology.

IE-BT

The Institution of Engineers-Biotechnology, Manipal Chapter (IE-Bt) is a premier society of Indian engineers from MIT, Manipal, which organizes technical and non-technical activities relating to different aspects of biotechnology. SymBiot is a national level symposium conducted annually by IE-Bt, Manipal with the aim of providing participants an exposure to a real-life work environment. Every year more than 200 students from all over the country take part in the event.

MANIPAL INSTITUTE OF TECHNOLOGY
MANIPAL
(A constituent unit of MAHE, Manipal)

Bio tech
IE - Biotechnology, Manipal Chapter

Dr TMA Pai 1898 - 1979 Padmashree Awardee
125th BIRTH ANNIVERSARY
INSTITUTION OF EMINENCE
NAAC A++ GRADE ACCREDITED
INSTITUTION'S INNOVATION COUNCIL (Ministry of Education Initiative)
G20 भारत 2023 INDIA

The Institution of Engineers - Biotechnology, Manipal Chapter (IE-Bt)
&
Department of Biotechnology, MIT Manipal

SymBiot'23

A National Level Biotechnology Conference

ABSTRACTS

October 31st 2023

TABLE OF CONTENTS

Characterisation of Recombinant Antimicrobia Protein from *Streptomyces roseolus*

Gouri Illanad, Shreya S Shettar
Dr. Zabin K. Bagewadi

Department of Biotechnology, KLE Technological University, Hubballi, Karnataka 580031

Recombinant antimicrobial protein produced from *Streptomyces roseolus* was cloned and expressed in *E.coli* host system. The protein demonstrated potential antimicrobial activity against tested pathogens like *Bacillus subtilis* and *Bacillus cereus* and the minimum inhibitory concentrations were determined. The protein also revealed significant stability under the influence of various biochemical parameters such as, temperature, pH, hydrolytic enzymes, surfactants, metal ions, organic solvents etc. The protein capped silver (Ag-NPs) and zinc (Zn-NPs) nanoparticles were synthesis that showed pronounced anti-microbial effect at different tested concentrations. The recombinant protein was also screened for various enzymatic potential such as, Glutaminase, Protease, Chitinase, Amylase, Lipase and Gelatinase. The protein possessed attributes of anti-oxidant property based on DPPH assay. The anticancer efficiency of the enzyme showed significant toxic activity toward MCF7 cell line (IC50 40.68 µg/ml). Secondary metabolites of the organism extracted by genome mining and docking studies demonstrate that the compounds have good dock scores and binding affinities with various therapeutic targets in cancer cell proliferation. The protein was characterized by NMR and CD spectroscopy and the mass was deduced by SDS-PAGE analysis. The purity of the desired protein was analyzed by HPLC that revealed a single peak at 2.978 retention time and was compared with standard antimicrobial protein. The biosynthesized Ag-NPs and Zn-NPs were characterised using UV-Vis spectroscopy where the peak for UV-Vis spectrum was observed at 515 nm for Ag-NPs and 365 nm for Zn-NPs. The FTIR analysis confirmed the presence of different bioactive functional groups, such as C-O, O-H, C=C, N-H. TGA showed the thermal stability of the protein and NPs. The AFM further revealed the surface morphology. The present research signifies the potential application of recombinant protein for applications in healthcare with strong antimicrobial potential.

Keywords: Biochemical characterization, antimicrobial, *in silico*, analytical characterization

Development and Analysis of Probiotic Mixed Fruit and Non-dairy Milk-based Smoothie

Roji Waghmare

Assistant Professor, Department of Food Engineering and Technology, Institute of Chemical Technology, Matunga, Mumbai -400017

This study was undertaken to increase the nutrition value and antioxidant level using probiotics with the use of non-dairy milk. Almond milk and peanut milk were prepared by soaking, grinding and filtering respective raw material. Smoothies were prepared from non-dairy milk like almond milk and peanut milk with the combination of fruits like strawberry and banana, fig and banana with sugar for each non-dairy milk respectively. Non-dairy milk smoothies were inoculated with *Lactobacillus casei* a probiotic bacterium. A comparative study was done on the non-dairy milk smoothies and probiotic non-dairy milk smoothies on the basis of physiochemical parameters like pH, acidity, bacterial enumeration, protein assay, carbohydrate assay and antioxidant activity, along with the evaluation of organoleptic properties after 24hrs of fermentation. Here, we have examined that fermentation with probiotic bacterium leads to increase in the antioxidants content, protein content, acidity, ph, bacterial count in all the smoothies prepared. It was found there was decrease in carbohydrate content and phosphorus content in all probiotic non-dairy milk smoothie than non-dairy milk smoothies.

KEYWORDS: Almond Milk, Peanut Milk, *Lactobacillus casei*, Probiotic smoothie.

Production of Recombinant Carbapenemase of *Acinetobacter baumannii*: An Important Target for Therapeutics

Abhishek and Vaishali Verma

Department of Biotechnology, Bennett University (The Times Group), Greater Noida, Uttar Pradesh, India

Multi-drug resistant (MDR) strains of *Acinetobacter baumannii* (*A. baumannii*) are known for causing nosocomial infections globally, resulting in significant mortality rates. Carbapenems are considered the last-resort treatment of choice for MDR *A. baumannii*. However, due to the emergence of carbapenem-resistant *A. baumannii* (CRAB), WHO has listed it as the 'priority 1' pathogen for research and development to develop new antibiotics. Strategies to counter the emergence of CRAB should not only involve the development of newer antibiotics but should also include the development of reliable, rapid, and easy-to-use diagnostic reagents for timely treatment of the patients for improved clinical outcomes and to ensure controlled use of carbapenem drugs and effective management of infection spread. At present, the detection of CRAB is performed using antibiotic susceptibility testing, which is time-consuming. Biochemical tests such as a CarbAcineto NP or β-CARBA are also available. However, these proprietary tests require proper storage of reagents that have short shelf life and consequently may not be cost-effective for low-income countries. MALDI-TOF MS-based detection platforms or nucleic acid-based methods can also be used. However, these methods require expensive equipment and trained manpower and may not be useful in resource-limited settings. Unfortunately, no single platform allows rapid, efficient, sensitive, specific, and cost-effective detection of CRAB. In this work, we have produced purified tagless recombinant carbapenemase protein of *A. baumannii*. The protein has been characterized using ELISA, Dot Blot and its activity has been qualitatively assessed by antibiotic susceptibility test. This protein will be used to develop reagents for diagnostics and therapeutics.

Keywords: *Acinetobacter baumannii*, anti-microbial resistance, therapeutics
Funding: The work is funded by the Department of Science and Technology–SERB (Start - up Research Grant to Vaishali Verma).

Strain Improvemt for Enhanced Erythritol Production by *Moniliella pollinis* Mutant-58 using Jaggery as a Cost - Effective Substrate

Anil B. Khatape

Vidhya Rangaswamy, Syed G. Dastager (Corresponding author)

From:

1) NCIM-Resource Center, Biochemical Sciences Division, CSIR National Chemical Laboratory, Pune-411008, India

2) Academy of Scientific and Innovative Research (AcSIR), Ghaziabad-01002, India

3) High Value Chemicals group, Reliance Industries Limited, Ghansoli, Navi Mumbai, India, 400701

Various microorganisms, including *Yarrowia, Moniliella, Aureobasidium, and Candida* strains, can produce erythritol. Despite its advantages, erythritol sweetener is used in a smaller amount than other polyols due to its comparatively high cost. This research focused on improving erythritol synthesis by exposing *Moniliella pollinis* strain to N-methyl N-nitro N-nitroso guanidine (NTG), Ethyl methyl sulphonate (EMS), and UV mutagenesis. The mutant cultures were then assessed for improved production of erythritol using different carbon substrates at the shake flask level. Among 150 selected mutants, mutant-58 strain was selected for further assessment which was generated by EMS mutagenesis. The mutant cells were found to have an oval shape, while the wild-type has long elongated rod-like cells. Furthermore, mutant cells produce yellow pigment at end of 72h incubation time which was not observed in the parent strain. In batch culture supplemented with 20% glucose, *M. pollinis* Mutant-58 strain was able to produce up to 90.2 ± 2.3 g/L erythritol with 0.39 ± 0.11 g/g yield and productivity of 1.18 ± 0.02 g/L.h. Interestingly, compared with the wild-type strains, the results of shake-flask culture showed that Mutant-58 increased erythritol production by 30% while decreasing ethanol production by 22%. The optimal conditions for high erythritol production are Temperature- 28°C, pH- 6.0, and Yeast extract- 4 g/L. The economical substrate, jaggery, was assessed for erythritol production, revealing its potential as a cost-effective alternative to glucose, given the comparable erythritol production values. Therefore, current research deals with strain improvement by EMS mutagenesis followed by media optimization leading to a 30% increase in erythritol production and a 25% reduction in ethanol production when cultivated in glucose. This is also the first instance where jaggery has been used as a cost-effective carbon source alternative to glucose for erythritol production.

KEYWORDS: Erythritol, *Moniliella pollinis*, Mutation, Fermentation, Renewable resource, Optimization.

Green Synthesis of Zinc Oxide (ZnO) Nanoparticles using Aqueous Extract of *Lagerstroemia indica*: Its Characterization and Biological Environmental Applications

Keerti Naik

Shifhali Morabad, Keerti Kamat, Uday Muddapur (Corresponding author)

From: Department of Biotechnology, KLE Technological University, Hubballi, Karnataka- 580031

Nanoparticles (NPs) are a wide class of materials that include particulate substances, which have one dimension less than 100 nm. Zinc oxide nanoparticle is among the most researched studies conducted due to their ability to apply in varied downstream applications. The second-most abundant metal oxide after iron is zinc oxide nanoparticle, which is also cheap, safe, and simple to synthesize. Many processes have been developed to synthesize nanoparticles for different applications. Among them, green synthesis by biological systems especially plant extracts have become an emerging field in nanotechnology and is gaining more importance because of their vast applications in physics, chemistry, biology, and medicine. In this study, aqueous extract of *Lagerstroemia indica* was prepared. The zinc oxide nanoparticles were synthesized from the flower extract using zinc acetate as the precursor with continuous stirring in the magnetic stirrer. The formation of ZnONPs was confirmed by UV-Vis spectrophotometric analysis. The absorbance peak was observed at 354 nm. The synthesized ZnONPs were further characterized by Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM) with Energy Dispersive Spectroscopy (EDS). From the XRD data, the average particle size was 22.58 nm. The antimicrobial activity of the ZnONPs was studied against some gram-positive and gram-negative organisms and was found to be effective.

KEYWORDS: ZnO nanoparticles, Green synthesis, flower extracts, antibacterial, phytochemical.

Computational Studies of Dietary Bioactive Compouds Against Apoptotic Inhibitors in Breast Cancer

Thripthi Nagesh Shenoy

Abdul Ajees Abdul Salam (Corresponding author)
From: Department of Atomic and Molecular Physics, Manipal Academy of Higher Education, Manipal – 576104, Karnataka, India.

Breast cancer (BC) is a leading cause of female mortality worldwide, often marked by apoptosis resistance, a critical cell death mechanism. Overexpressed anti-apoptotic proteins like Bcl-2 enable cancer cells to evade apoptosis, promote breast cancer, and develop resistance to treatment, making them essential treatment targets. Dietary bioactives can modulate breast cancer risk, either by worsening or alleviating the disease progression. Given the challenges of tumor relapse, side effects, and drug resistance with chemotherapy, there is a growing interest in natural compounds for breast cancer prevention and treatment. Various dietary bioactive compounds have demonstrated the ability to inhibit breast cancer cell growth and induce apoptosis. Therefore, we investigated the potential of dietary bioactive compounds available in the FooDB and PubChem database against Bcl-2 proteins of breast cancer using molecular docking analysis. Molecular docking was performed using AutoDock Vina for the analysis of binding efficiency. Of the 160 dietary bioactives screened, 19 demonstrated better binding energies than the standard drug obatoclax (-6.9 kcal/mol) and the bioactives mentioned in the literature – cucurbitacin (-6.7 kcal/mol), kaempferol (-6.0 kcal/mol) and mangiferin (-5.7 kcal/mol). Bioactives with better binding energies were analysed for their molecular interactions with the active site amino acid residues of the target. Top three scoring bioactives, currayanine (-8.0 kcal/mol), mahanimbine (-7.6 kcal/mol) and murrayazolinine (-7.6 kcal/mol) were studied to be present in different parts of *Murraya koenigii* (curry leaf plant). Mahanimbine was also found to be present in other medicinal plants like *Clausena anisata* (Horsewood) and *Murraya paniculata* (Kaamini). This suggests that the dietary bioactive compounds have the potential to interact with anti-apoptotic targets in breast cancer and warrant further investigation for their role in breast cancer prevention and treatment.

KEYWORDS: Breast cancer, apoptotic inhibitors, Bcl-2, molecular docking, dietary bioactives

Decolorisation of Synthetic Food Dyes using Indigenous Plant

Harshika. M

Abdul Ajees Abdul Salam (Corresponding author)
From: Department of Atomic and Molecular Physics, Manipal Academy of Higher Education, Manipal 576104, Karnataka, India.

Synthetic food dyes are widely used for their attractiveness and taste in various processed food items such as candies, ice creams, baked goods, canned fruits, gelatin sweets, soda drinks, etc. They cause allergic reactions and hyperactivity in children (ADHD). Consumption of dyes above the permissible limits is also known to cause serious effects in adults including depression of immune system and bladder cancer. These dyes after ingestion are broken down in our body into aromatic amines, benzidines and benzene sulphonic acids, which are reported as potent carcinogens, mutagens and hence can induce deadly effects in cells. The presence of these dye molecules in wastewater channels is also an emerging environmental issue. In this study, we identified a novel indigenous plant which could decolorize the food dyes completely from water. The “seed” and “fruit” of the plant was tested for their decolorization abilities against four of the commonly used industrial food dyes namely: Indigo carmine, Erythrosine B, Tartrazine, and Amaranth. The plant part showed efficient decolorization capabilities in all of the dyes within a minimum period of 24 hours. The same was confirmed by subjecting the mixture to UV-Visible spectroscopy. The use of natural resources such as plants possess various advantages as they are easy to grow, eco-friendly, non-toxic and possess nutritional values. Thus, it can serve as the potential alternative for food dyes degradation.

KEYWORDS: Synthetic food dyes, indigenous plant, carcinogen, mutagen

Green Synthesis of Silver Nanoparticles from Selected Plant Extracts: Optimization, Characterization and Antibacterial Potential

Janani Arun

Dr. Laxmikant R. Patil (Corresponding author)
From: Department of Biotechnology, KLE Technological University, Vidyanagar, Hubballi,
Karnataka - 580031.

Nanoparticles are those whose dimensions are in nano scale. The application of Metallic Nanoparticles are in various fields like biology, medicine, pharma etc. This has led to high demand for these nanoparticles and thereby resulting in a significant need for better production of such nanoparticles. Green synthesis of Nanoparticles is an eco-friendly, non-toxic and a cost-effective method. Silver Nanoparticles are majorly used in the field of dental work, wounds and burns treatment and also have properties of Antimicrobial, Antioxidant and Anticancer activities. In this study an attempt was made to synthesize Silver Nanoparticles from the Plant (Leaf) Extracts of *P. pterocarpum*, *M. longifolium* and *A. columnaris*. The process parameters like Temperature (30 o C, 40 o C, 50 o C, 60 o C), pH (6.0, 8.0, 10.0, 11.0, 12.0) and Concentration of Silver Nitrate (1mM, 5mM, 10mM) were optimized using OFAT method of optimization. For all the three Plant Extracts, the optimum Temperature was found to be 60 o C. The optimum pH was found to be Alkaline i.e 12.0 for *P. pterocarpum*, 11.0 for *M. longifolium* and 10.0 for *A. columnaris*. The optimum Concentration of Silver Nitrate for *P. pterocarpum* was 10mM whereas for *M. longifolium* and *A. columnaris* the optimum was 5mM. In the characterization studies, SEM and EDS along with XRD were performed to analyze the structural morphology of the nanoparticles. FTIR characterization was made to analyze the functional groups present in the synthesized nanoparticles. The analysis of Antibacterial Activity of each of the synthesized Nanoparticles was made using different bacterial strains like *cereus, subtilis, mobilis* and *aeruginosa* with standard antibiotic strips as positive control. The results of this study showed that it is an environmentally friendly, inexpensive, and simple approach for successful synthesis of Silver nanoparticles, as well as their potential application as an antibacterial agent.

KEYWORDS: Green Synthesis, Silver nanoparticles, *P. pterocarpum*, *M. longifolium*, *A. columnaris*.

Biochemical, Structural and Functional Characterisation of Serine Protease from *Bacillus velezensis*

Muskan M Naik

Dr. Zabin K. Bagewadi
From: Department of Biotechnology, KLE Technological University,Hubballi, Karnataka-580031

The present study reveals the functional, biochemical, analytical, and *in-silico* characterization of Recombinant collagenase enzymes from the *Bacillus velezensis* organism. It was found that the enzyme had the highest anti-microbial activity of 34mm for *Bacillus subtilis* and the minimum inhibitory concentration was found to be 14mm for 50X dilution. The stability was seen for 10 days and then the plates were discarded. The enzyme showed the highest enzyme activity with the optimum temperature (RT), and pH (10) of 28.07 and 143.63 U/ml. The ions like Cu and Ca acted as inducers for collagenase activity and Mg and mercapto ethanol had an inhibitory effect. It had a quite good solubility effect in DMSO and had a great activity. The surfactants like urea increased the activity whereas SDS, Triton X 100, and Tween 80 acted as inhibitors. The kinetics of the enzyme showed that there was an increasing effect for the substrate up to 2% and it was quite the opposite when the enzyme was immobilized. The immobilization of the enzyme was done by agar and agarose plate method and the activity was found better to be in agar method. The synthesis of nanoparticles by AgNO3 and ZnC4H6O4 was done to know the characterization like SEM-EDS, TGA, FTIR, AFM, HNMR, and CD, the *in-silico* studies followed to find potential drugs against cancer, STD, and Neurological diseases and also those drugs that had inhibitory effects against collagenase enzyme.

KEYWORDS: Collagenase, *Bacillus velezensis*, Nano-particles, Anti-microbial activity.

Green Synthesis of Zinc Oxide Nanoparticles Using *Saussurea obvallata* Root Extract

Shifhali Morabad

Keerti Naik, Keerti Kamat, Uday Muddapur (Corresponding author)
From: Department of Biotechnology, KLE Technological University Hubballi Karnataka-580031

Nanotechnology is a cutting-edge approach to the development, characterization, manufacture, and utilization of materials, devices, and systems by manipulating their shape and size on a nanometer scale, ranging from 1 to 100 nanometers. Zinc Oxide Nanoparticles is a metal oxide nanomaterial with distinct physical and chemical properties, making it a valuable and adaptable inorganic compound. In this study, the *Saussurea obvallata* (Brahmakamala) root extract has been employed for the Green synthesis of zinc oxide nanoparticles. The reaction mixture was found to be present in the resulting precipitate which demonstrated the formation of zinc oxide nanoparticles. Extracted root extract was subject to a comprehensive phytochemical analysis, which revealed the presence of a variety of Phytochemicals such as, alkaloids, saponins, as well as ateroids, resins, diterpenes and coumarins were identified. Optimization of zinc nanoparticles was carried out by synthesizing zinc nanoparticles at different temperature and pH. In addition, ZnO green synthesized nanoparticles were further characterized using UV spectrophotometer, XRD analysis, FTIR, and SEM with EDX. Agar well diffusion method was used to determine the antibacterial activity of zinc nanoparticles against Gram positive and Gram negative bacteria. The maximum and minimum inhibitory zones of zinc nanoparticles were observed against Gram negative bacteria [*Pseudomonas aeruginosa* (10mm)] and Gram positive bacteria [*Zymomonas mobilis* (8mm)] respectively. Antifungal activity was carried out against *Candida albicans*.

KEYWORDS: *Saussurea obvallata*, Phytochemicals, Antibacterial activity, Antifungal activity.

Green Synthesis of Zinc Oxide Nanoparticles Using Aqueous Extract of Papaya peel, Its Characterization, and its Biotechnological Applications

Keerti Kamat

(Corresponding authors - Keerti Naik, Shifhali Morabad, Uday Muddapur)
From: Department of Biotechnology, KLE Technological University Vidyanagar, Hubli, Karnataka-580021

This study aims to indicate the synthesis, characterization, and applications of Zinc Oxide nanoparticles using Papaya peel extract. Green Synthesis of ZnO NPs is extensively used as it is an eco-friendly and cost-effective method. ZnO- NPs were characterized using analytical methods. Peaks of UV-Visible spectra were obtained at 364 nm therefore, the formation of ZnO-NPs is confirmed. SEM Analysis confirmed the generation of nanoparticles was spherical aggregated in shape. EDS verified the use of metallic zinc acetate in the manufacturing of ZnO-NPs. XRD confirmed the generated nanoparticles with a particle size of 26.581 nm. To identify the precise functional groups responsible for reduction, stabilization, and capping agents found in the nanoparticles, FTIR spectroscopy was employed. In Phytochemical analysis Flavonoids, Saponins, Steroids, Triterpenoids, and Resins showed positive results. The parameters such as the effect of pH and temperature were studied. The synthesized nanoparticles of Papaya peel were tested for antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, and gram-negative bacteria *Escherichia coli*, *Zymomonas mobilis* and *Pseudomonas aeruginosa* and *Candida*. Using a 2,2-Diphenyl-1-Picryl-Hydrazyl (DPPH) free-radical scavenging experiment, the antioxidant activity of ZnO nanoparticles was determined. ZnO-NPs produced from Papaya peel have the potential to be used in biomedical and biotechnological applications due to their eco-friendly synthesis, on-toxicity, and biocompatibility.

KEYWORDS: Green Synthesis, ZnO nanoparticles, Papaya Peel Extract, Antibacterial activity.

Isolation, Characterization and Phytochemical Analysis of Bioactive and Nanocompounds from *Premna integrifolia* (Leaf & Root) and their Biomedical Application

Poorvi B Kuppasagoudar

(Corresponding author - Dr. Uday M. Muddapur)

From: Department of Biotechnology, Kle Technological university, Hubli ,Karnataka

Zinc oxide nanoparticles (ZnO-NPs) are one of the metal oxide nanomaterials and a valuable and versatile inorganic compound due to its unique physical and chemical characteristics. ZnO-NPs have been widely manufactured and utilized in various commercial and additive products, including ceramics, cement, plastics, glass, ointments, lubricants, adhesives, sealants, pigments. This study aims to determine the classes of phytochemicals, biosynthesize and characterize the Zn oxide nanoparticles, using *Premna integrifolia* root and leaf extracts. ZnO-NPs have been widely manufactured and utilized in various commercial and additive products, including ceramics, cement, plastics, glass, ointments, lubricants, adhesives, sealants, pigments.. In this study, Zinc oxide NPs were synthesized from the root and leaf extracts of *Premna integrifolia* leaves through a non-toxic, cost-effective and eco-friendly method. The Zinc oxide nanoparticles were characterized using UV-Visible spectroscopy, where the peak between 360 to 365 nm indicates the formation of Zinc oxide nanoparticles. A peak at 362 nm was obtained. Optimization studies on the bulk synthesis of zinc nanoparticles were carried out for different parameters. The applicational study was done on the antibacterial activity and antifungal activity of zinc oxide nanoparticles from the root and leaf extracts of *Premna integrifolia*. The inhibition zone diameters root and leaf extracts are 34-31mm, 27-16mm, 8-7 mm 31-32 mm and 28-27 mm for *Zygomonas mobilis*, *S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida sp*. respectively. 96-well plate method against different strains were carried out. The root and leaf extract showed MIC of 20 and 40 microgram per ml. . The free radical scavenging activity of *Premna integrefolia* was also examined using the DPPH assay . Phytochemical studies have shown the presence of many valuable bioactive compounds such as alkaloids, flavonoids, glycosides, tannins, phenolic compounds and diterpenoids. The extracts and the compounds isolated from leaf, root show a wide spectrum of pharmacological activities including antibacterial, antifungal, antioxidant.. Flavonoid and alkaloid compounds present in the extract were characterized by mass spectrophotometry, UV-visible spectrophotometry, FTIR, XRD, SEM-EDS.

KEYWORDS: *Premna integrifolia*, Zinc oxide nanoparticles, Root leaf extract, FTIR, XRD, SEM-EDS

Microbial Diversity and Dynamics as Affected by Fermentation in *Nuchhu Ambli* - A Traditional Fermented Food of North Karnataka.

Jyeshta. G. P

(Corresponding authors - Nidhi Kadadi, Shreya. Pujar, Neelu. Nargund, S.V. Desai, V.S.)
Hombalimath, Neelu. Nargund
Department of Biotechnology, KLE Technological University, Hubballi,Karnataka- 580031

Traditional fermented foods are known to play a crucial role in human nutrition and health by harnessing the metabolic activities of beneficial microorganisms to transform raw ingredients into products that are safe and beneficial to humans. *Nuchhu Ambli* is one such Sorghum-based traditional food of Karnataka which is prepared by fermenting broken jowar with curds and thus, is a classical example of symbiotic food. The present study deals with the dynamics and diversity of different classes of microorganisms (total mesophilic count, aerobic count, lactic acid bacteria, coliforms and yeasts & molds) in control and treated food samples at 0 and 12 hours of fermentation. Appropriate plating methods (pour and spread plate) and microbiological media were employed for the isolation and enumeration. In regard to fermented food with a time period of 12 hours, there was an increase in total mesophilic count (3.9 to 4.4 log 10 cfu/g), aerobic count (2.4 to 3.8 log 10 cfu/g), lactic acid bacteria (2.6 to 3.2 log 10 cfu/g) and a decrease in yeast & molds (2.1 to 1.2 log 10 cfu/g). A remarkable decrease in coliforms was observed (2.5 to 1.1 log 10 cfu/g) indicating the potential of lactic acid bacteria (LAB) in controlling the coliforms growth. The isolates were characterized morphologically and tested for their motility. Rod-shaped, gram positive and gram negative organisms with motility were observed. Lactic Acid bacteria were found to be gram positive and non-motile. The results indicate the dynamics of the microbial population as a result of fermentation in *Nuchhu Ambli*. Further characterization of the isolates would give insights into the type of microorganisms involved and their succession in the process.

KEYWORDS: *Nuchhu Ambli*, Fermentation, LAB, Coliforms

Green Synthesis of Silver Nanoparticles using Different Leaf Extract, Optimization of Process Parameter using CCD and Its Biotechnological Application

Shrawani Ganesh Dasa

(Corresponding author-Dr.VS Homballimath)
From: Department of Biotechnology, KLE technological university, Hubli.

Green synthesis of nanoparticles has acquired a lot of importance because of their cost effectiveness, environmentally friendly nature and even their small size. In this study, we have focused on the synthesis of silver nanoparticles using different plant extracts and the plants used were *Ricinus communis* (castor plant), *Artocarpus heterophyllus* (jackfruit tree), and *Syzygium cumini* (jamun plant). Optimizing the process parameter for the synthesis of silver nanoparticles using leaf extract of *Ricinus communis* (castor plant), *Artocarpus heterophyllus* (jackfruit tree), and *Syzygium cumini* (jamun plant). Ultraviolet-visible (UV-Vis) spectrophotometer was used for the confirmation of AgNP synthesis; castor leaf extract showed a peak at 433 nm, jackfruit leaf extract showed a peak at 426 nm and jamun leaf extract showed a peak at 452 nm. The Silver nanoparticles were further characterized by NMR spectroscopy, SEM microscopy, TEM and FTIR analysis. The effect of process parameters like volume of leaf extract, volume of AgNO3, and pH were studied using central composite design (CCD) for all three plant sources. The response is measured in terms of wavelength and absorption. The volume of AgNO3 solution and volume of plant extract solution showed a significant effect on *Syzygium cumini* plant source and pH, and the volume of AgNO3 and the interaction of pH with pH showed a significant effect in terms of absorption. Phytochemical screening of leaf extract of castor, jackfruit and jamun leaf extract was conducted for the qualitative detection of various phytochemicals like Carbohydrates Terpenoids, Proteins, Steroids, Tannins, Alkaloids, Saponins, Acidic compounds, Flavonoids, Coumarins, Phenols, Quinones, Glycosides and Phlobatannins. The synthesized AgNPs were examined for antimicrobial properties against *Z. mobilis*, *B. cereus*, *P. aeruginosa* and *B. subtilis*, two gram-positive bacteria and two gram-negative bacteria. The synthesized AgNPs were examined for antidiabetic assay using α- amylase assay and castor leaf extract showed the highest antidiabetic property at 0.1mg/ml concentration.

Bioprocess optimization of *Penicillium funiculosum* NCIM 1228 aims to enhance the production and hydrolytic efficiency of cellulases on Lignocellulosic biomass

Sambhaji Chavan

Mahesh Dharne (Corresponding author)

From: CSIR-National Chemical Laboratory, Pune-411008, Academy of Scientific and Innovative Research (AcSIR), Ghaziabad - 201002, India

The cellulase enzyme currently ranks as the world's third-largest commercial enzyme due to its increasing demand across various industries such as textiles, food, waste management, pharmaceuticals, agriculture, pulp paper, biofuels, and more. In this study, the response surface methodology (RSM) was employed to optimize both media components and process parameters, leading to a successful increase in cellulase production from *Penicillium funiculosum* NCIM 1228. Statistical optimization for hyperproducing cellulases was achieved through the application of RSM. The critical factors affecting cellulase production medium were investigated using the Plackett-Burman design (PBD) approach. Subsequently, the Box-Behnken design (BBD) method was utilized to statistically determine the optimum values and conditions that significantly influenced cellulase production. The estimated optimal parameter combinations for cellulase production included urea (0.2%), CaCl 2 (0.2%), MgSO 4(0.05%), peptone (1.5%), microcrystalline cellulose (5.0%), wheat bran (2.5%), Corn steep liquor (CSL) (2.5%), KH 2 PO 4 (0.15%), inoculum (10.65%), agitation (157 rpm), pH (5.88), and temperature (29.84°C). In conclusion, experimental validation under optimal conditions revealed a substantial increase in the production of both filter paper assay (FPase) and β-glucosidase by 3.82 and 3.61-fold, respectively. Moreover, there was a notable 1.66 and 1.57-fold improvement in the specific activity of FPase and β-glucosidase, while xylanase activity was enhanced by 3.29-fold. Furthermore, the enzyme exhibited an impressive 51.30 percent hydrolysis efficiency on Sugarcane bagasse lignocellulosic biomass (LCB) when dosed at 7 FPase units per gram of cellulose. P. *funiculosum* NCIM 1228 offers the advantage of producing cellulase, along with a complete cellulolytic system of enzymes that can be synthesized extracellularly, making it a promising biocatalyst for the biofuel industry.

KEYWORDS: Cellulase; *Penicillium funiculosum*; Response Surface Methodology; Submerged Fermentation; Xylanase; Lignocellulosic biomass.

Botyrococcus braunii Microalgae-derived peptides as promising therapeutics against drug-resistant *Pseudomonas aeruginosa*

Sachin Sudhakar Pujeri

Bhavan Ganesh K, Jayaram L Narayana, Akshatha Prasanna
From: Department of Biotechnology, Dayananda Sagar College of Engineering (An Autonomous Institute Affiliated to Visvesvaraya Technological University, Belagavi), Kumaraswamy Layout, SM Hills, Bengaluru 560 111, India.

Antimicrobial resistance (AMR) is one of the significant concerns towards global public health. *Pseudomonas aeruginosa* has been declared as one of the critical pathogens by the World Health Organization due to its resistance to currently available antibiotics. One of the alternative to overcome the AMR are Antimicrobial peptides (AMP). The present study explores the microalga *Botryococcus braunii* as a potential source of antimicrobial peptides (AMPs) against drug-resistant Pseudomonas aeruginosa. The workflow includes the identification of AMPs from *B. braunii* based on physiochemical properties using various prediction and activity tools. The PBIT tool was used to identify non-homologous protein targets within *P. aeruginosa* PA01. Subsequently, two potential AMPs were subjected to docking studies, followed by validation through molecular dynamics (MD) simulations. These molecular predictions provide that two identified AMPs AMPs exhibit high docking scores of -29.6 and -26 kcal/mol respectively for the protein responsible for prenylated Flavin Mononucleotide (FMN) synthesis, likely to have a central role in bacterial electron transport and metabolic reactions. The molecular dynamic simulation determined the interaction stabilities between the AMPs and the protein at the binding site. Thus, the high binding affinity and insights from the molecular interaction signify that the identified AMPs from *B. braunii* can serve as potential alternative treatments against drug-resistant *P. aeruginosa*.

KEYWORDS: Antimicrobial Peptides, Antimicrobial resistance, Gram Negative pathogens, *Botryococcus braunii*, *Pseudomonas aeruginosa*

In Silico approach to identify candidates against MDR Enterococcus faecalis clinical isolates

Indupriya M

Bhavyashree V, Hamsaveni S M, Sahana M, Prashantha Karunakar, Akshatha Prasanna

From: Department of Biotechnology, Dayananda Sagar College of Engineering (An Autonomous Institute Affiliated to Visvesvaraya Technological University, Belagavi), Kumaraswamy Layout, SM Hills, Bengaluru 560 111, India.

Enterococcus faecalis is a prevalent opportunistic pathogen and causes nosocomial infections. Over the decades *E. faecalis* have emerged resistant to antibiotics such as the β–lactam class of antibiotics and vancomycin. Due to the rise of MDR, there is a need to identify new targets for the discovery of antibacterial medicines to combat *E. faecalis*. In this context, phytochemicals have been found as promising alternative therapeutic agents. The present study aims to identify potential antibacterial phytoligands against *E. faecalis* Indian isolates.

The methodology involves retrieval of publicly available genome data of *E. faecalis* Indian clinical isolates (n=4). Antimicrobial resistant (AMR) genes of the assembled fasta files were identified by ResFinder and the genome was annotated using Prokka. The variations in Indian strains were identified by comparing with the *Enterococcus faecalis* V583 reference genome. PBIT pipeline builder was used to identify non-homologous proteins in *E. faecalis* analysed against human proteome and gut microbiota. The sequences of non-homologous proteins identified as potential drug targets involved in the DNA replication pathway were retrieved from the Prokka results. Homology modelling was done on these selected drug targets using Swiss Model and validated models were docked using PyRx against selected 60 phytoligands.

The result of molecular docking revealed that lowest vina score was observed in Carpaine (-8.1 Kcal/mol) of Papaya Carica against the proteins DnaB and DnaD, respectively to inhibit the replication initiation activity. Stigmasterol of *Ricinus communis* showed the lowest Vina score of -7.2 kcal/mol to inhibit unwinding and separation of double stranded DNA in DNA replication protein. Based on the Vina score and binding interaction the two compounds Carpaine and Stigmasterol can be considered as potential antibacterial agents against *E. faecalis* infections.

KEYWORDS: Multidrug resistance, AutoDock Vina, PyRX, Phytoligands.

The Ectomycorrhizal Shield: P*axillus involutus* Fortifies Poplar Seedlings Against Aluminum Stress via Asada Halliwell Pathway

Dhiraj Naik

From: Botany Department, Bajaj College of Science, Civil Lines, Wardha Maharashtra-442001

Aluminum (Al) toxicity is a significant threat to forest trees, especially *Populus*, known for its resilience in acidic and metal-enriched soils. Ectomycorrhizal (EM) fungi are crucial partners, mitigating Al's harmful effects in the root zone. While EM-induced stress tolerance in trees is well-established, the underlying molecular mechanisms, known as priming, have been elusive. In our study, we conducted a comprehensive transcriptome analysis of EM-associated poplar seedlings to unravel Al toxicity mitigation and enhanced tolerance in *Populus*. We identified 1,143 Al-responsive genes in mycorrhizal roots, surpassing non-mycorrhizal roots. EM roots also exhibited higher expression of Al-induced transcripts under prolonged Al stress, highlighting the key role of EM symbiosis in enhancing *Populus'* Al stress response. EM-associated Al-stressed plants displayed significant upregulation of genes related to oxidative stress and early signaling pathways. This heightened activation of stress-related genes indicates that EM symbiosis primes the plant's response to Al stress, equipping it to combat oxidative damage and trigger rapid stress responses. Our analysis also revealed osmoprotectant genes' involvement, further enhancing their capacity to endure Al-induced stress. Interestingly, auxin-related pathways were suppressed, suggesting a trade-off between growth and stress responses in the presence of Al stress. In conclusion, our study underscores the power of genome-wide transcriptome profiling in understanding *Populus'* response to Al stress. EM symbiosis activates stress-related genes and signaling pathways, priming the plant for enhanced abiotic stress tolerance. This newfound understanding of the interplay between EM fungi and *Populus'* responses to Al stress offers innovative strategies to enhance tree growth and sustainability in challenging environments. It holds promise for improving biofuels production and carbon sequestration, even in acidic and metal-enriched soils.

KEYWORDS: *Populus*, model forest tree, ectomycorrhizal fungi, Microarray, functional genomic, Aluminum stress

Evolutionary Divergence of Toll-Like Receptor 9 (TLR9)

Manisha Ghosh

Surajit Basak (Corresponding author)
From: Division of Bioinformatics, ICMR-National Institute of Cholera and Enteric Diseases, Kolkata-700010.

Background: Toll-Like Receptors (TLRs) are considered as the primary sensors of invading microbial pathogen in the innate immune system because they detect pathogen-associated molecular patterns (PAMPs). The transmembrane pattern recognition receptor TLRs are best known for their roles in innate immunity via recognition of pathogen and initiation of signalling response. Mammalian TLRs recognize molecular patterns associated with pathogens and initiate innate immune response and most mammalian species share a similar repertoire of TLR homologs with few exceptions. Therefore, study of the evolutionary diversity of mammalian TLR genes is important for understanding the differences in immunological response.

Methods: In this study, the sequences of mammalian TLRs were retrieved from the sequence repositories. Then multivariate analysis was performed to study the amino acid usage pattern of the TLRs. Phylogenetic tree construction and estimation of synonymous and non- synonymous substitution rates of TLRs were done to understand the evolutionary perspective. Ancestral sequence reconstruction was done based on the phylogenetic tree. Subsequent analyses were performed to explain the gradual changes in the evolution of TLR9.

Result: The comprehensive analysis of mammalian TLRs revealed a distinct pattern of evolution of TLR9. Various sequence-based features such as amino acid usage, hydrophobicity, GC content etc. and evolutionary constraints are found to influence the divergence of TLR9 from other TLRs. Ancestral sequence reconstruction analysis also revealed that gradual evolution of TLR genes in several ancestral lineages lead to the distinct pattern of TLR9.

Conclusion: This study demonstrated evolutionary divergence with the progressive accumulation of mutations contributing in the distinct pattern of TLR9. Reconstruction of ancestral sequences is a key aspect of the molecular evolution of TLR to track changes across the TLR genes. It will elucidate the biological significance of TLR9 and provide evidence for their distinct contributions in response to host defence.

KEYWORDS: TLR, Evolution, Phylogenetic tree, Divergence, Ancestral sequence

Biodegradation of Plastic Using Microbes Isolated from Landfill Soil

Sahana F Goudar

(Corresponding author-Dr. V.S.Hombalimath)
From: Department of Biotechnology, Kle Technological University, Hubli, Karnataka

Accumulation of Plastics, especially polyethylene and other plastics, has increased rapidly which causes threats and is harmful to the environment. Given that the current study is to degrade the LDPE (Low-Density Polyethylene) using the microorganisms isolated from soil. In this study, the soil bacteria was isolated from plastic contaminated soil sample. The bacterial isolates were identified by morphological and biochemical characterization. The biodegradability test for LDPE was analyzed by considering the weight reduction of LDPE after 30-35 days of incubation. Optimization of process parameters using the Central Composite Design (CCD) approach of Response Surface Methodology was done. The LDPE plastic films were characterized by FT-IR & SEM analysis.

KEYWORDS: LDPE (Low-Density Polyethylene), Optimization, CCD, FT-IR, SEM.

Exploring the Efficacy of Cutting-Edge Nano-probiotic Formulation for Optimized Restoration of Gut Dysbiosis and Holistic Health Improvement

Manish Singh Sansi

(Corresponding authors-Daraksha Iram , Sunita Meena)
From: Biofunctional Peptidomics & Metabolic Syndrome Laboratory, Animal Biochemistry Division, ICAR-National Dairy Research Institute, Karnal, India
Antimicrobial Peptides, Biofunctional Probiotics & Peptidomics Laboratory, Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal, India

Introduction: This study presents a comprehensive evaluation of the restoration of gut dysbiosis through the application of a novel nano-composite formulation comprising milk peptides, probiotics and nanocellulose.
Objectives: Dysbiosis has emerged as a global health concern, necessitating innovative approaches for its management. The synergistic potential of combining milk bioactive. peptides, probiotics, and nanocellulose in a nanocomposite has garnered significant interest due to their individual health benefits and potential for enhanced therapeutic outcomes.
Methods: In this study, we comprehensively assess the restoration of gut dysbiosis using a novel nano-composite formulation of milk peptides, probiotics, and nanocellulose. Dysbiosis is a global health concern, necessitating innovative approaches. The synergistic potential of this combination has attracted significant attention due to its individual health benefits and potential for improved therapy.
Results: In this research, we investigate restoring obesity-induced gut dysbiosis using a nano-composite formulation through both experiments and computational methods. We integrated milk peptides to regulate metabolism and appetite, probiotics to improve gut health, and nanocellulose as a stabilizer. We carefully examined parameters like lipid metabolism, gut microbiota, and body weight regulation. Our preliminary metagenomics data indicates the potential of this nanocomposite for treating gut dysbiosis. This study contributes to the field of nanotechnology-based dysbiosis interventions, highlighting the promise of synergistic nanocomposites in addressing this global health concern.
Discussion: Our research utilized a nanocomposite to address obesity-related gut dysbiosis, incorporating milk peptides for appetite and metabolism control, probiotics for gut health, and nanocellulose as a stabilizer. Evaluated parameters like lipid metabolism and gut microbiota. Preliminary metagenomics data shows promise for treating gut dysbiosis, contributing to the field of nanotechnology-based interventions for this urgent global health concern.
Conclusion and Key Message: In summary, our research utilizing a nanocomposite to combat obesity-related gut dysbiosis, with the inclusion of milk peptides, probiotics, and nanocellulose, shows promise. The assessment of lipid metabolism and gut microbiota composition, along with preliminary metagenomics data, highlights the potential of this innovative approach. It advances the field of nanotechnology-based interventions for the urgent global health concern of gut dysbiosis.

Innovative Nanohydrogel Featuring Potent Antimicrobial Peptides and Nanoparticles for Exceptional Efficacy against Resilient Pathogens

Daraksha Iram

Manish Singh Sansi B and Shilpa Vij

From: A Antimicrobial Peptides, Biofunctional Probiotics & Peptidomics Laboratory, Dairy

Microbiology Division, ICAR-National Dairy Research Institute, Karnal, India

Biofunctional Peptidomics & Metabolic Syndrome Laboratory, Animal Biochemistry Division, ICAR-National Dairy Research Institute, Karnal, India

Introduction: The rise of antibiotic-resistant pathogens is a major global health threat. This study seeks to understand them using various methods and explores a new antimicrobial peptide from cow colostrum whey, combined with copper nanoparticles, to combat these pathogens.

Objectives: The study analyzed antibiotic resistance mechanisms, pathogenicity genes, and metabolic changes. A novel antimicrobial peptide combined with CuNPs demonstrated efficacy against *E. coli*, *S. aureus*, *and A. baumannii*.

Methods: Cow colostrum whey fermentation produces valuable antimicrobial peptides. This study identified and synthesized these peptides, then combined them with nanoparticles to combat resistant pathogens. The conjugate showed enhanced antimicrobial effectiveness with low MIC values, and it was safe for cells. Additionally, a nanohydrogel was prepared and tested for wound healing on rats' skin.

Results: In this study, identifying 240 bioactive peptides from a pool of 1234 peptide sequences. Among these, 126 peptides were found to have antimicrobial properties, and 56 were anti-biofilm peptides. Antimicrobial peptides are substances that can kill or inhibit the growth of harmful microorganisms, such as bacteria. The researchers then synthesized these antimicrobial peptides and combined them with copper nanoparticles (CuNPs). Furthermore, novel antimicrobial peptide were isolated from colostrum whey and conjugated with CuNPs to enhance its antimicrobial efficacy and zone of inhibition were found against sensitive bacteria *E. coli, S. aureus and A. baumannii* (27.0±0.1, 25.6±0.1 and 26.6±0.1 in mm), in case of resistant bacteria ESBL, MRSA and *A. baumannii* 1379 the inhibition zone were found (23.0±0.1, 21.6±0.1 and 22.6±0.1), The conjugation process was optimized to ensure stability. The efficacy of the conjugate was assessed against a panel of characterized antibiotic-resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), *E. coli* (ESBL), and *Acinetobacter baumannii* 1379. The antimicrobial activity of the conjugate against characterized antibiotic-resistant pathogens was evaluated. Minimum inhibitory concentration (MIC) assays demonstrated the enhanced antimicrobial efficacy of the conjugate, with significantly lower MIC values compared to both alone peptide and CuNPs. The conjugated antimicrobial peptide showed effective crystal violet uptake at 2xMIC concentration against resistant bacteria. Mechanistic studies revealed multiple synergistic actions, including membrane disruption, membrane permeability, and cellular imaging. Combining peptides with copper nanoparticles resulted in a promising antimicrobial agent effective against both resistant and non-resistant bacteria. Detailed characterization of these conjugates is essential for optimizing their antimicrobial properties. Furthermore, the study explored their potential for wound healing using a nanohydrogel, showing promise in tissue regeneration and reducing inflammation. This research is significant for combating antibiotic resistance and has broader therapeutic implications, demonstrating the potential of combining peptides and nanoparticles for dual applications in healthcare.

Discussion: In summary, this study's novel combination of bioactive peptides from cow colostrum whey with copper nanoparticles shows promise in fighting antibiotic-resistant bacteria and supporting wound healing. However, further research is required to uncover the mechanisms, ensure safety, and assess scalability for real- world medical use.

Conclusion and Key Message: This study shows promise in combating antibiotic-resistant pathogens with peptides and copper nanoparticles, along with potential wound healing benefits. However, it's in the early stages, requiring further research for safety and scalability. If successful, it could have a significant impact on healthcare.

In-depth Genome Analysis of Antibiotic Resistant Halotolerant Probiotic Bacterium *Paenibacillus sp.* S-12

Rajnish Prakash Singh

From: Department of Biotechnology, Jaypee Institute of Information Technology (JIIT) Noida

Members of *Paenibacillus* genus have been reported from a wide variety of environments and attracted great attention due to their multifarious properties. However, till now, very little information is available for this genus. In the present study, we characterized the genome of a halotolerant bacterium *Paenibacillus* sp. S-12. Molecular identification by PCR approach and 16S rRNA gene sequencing showed its closest match to other *Paenibacillus* species. The complete genome size of S-12 was 5.69 Mb, with a GC-content 46.5%. The genome analysis unraveled the presence of an open reading frame (ORF) encoding the functions related to environmental stress tolerance, adhesion processes, multidrug efflux systems, and heavy metal resistance. Additionally, it showed the presence of CAZymes, probiotic, and stress protected genes that equipped the strain for industrial and agricultural purposes. The antiSMASH analysis identified genes for antimicrobial peptides (AMPs), secondary metabolite production, NRPSs, and PK synthesis. Various NRPs regions related to paenibacterin, guadinomine, polymyxin B, chejuenolide A/ chejuenolide B, fusaricidin, pelgipeptin, and octapeptin-C4 were identified. The pangenome analysis indicates the S-12 strain harbor many unique genes which are not shared by other strains and thereby gene pool size would increase further increased number of genomes incorporated in the analysis. The current findings provide the in-depth investigation of a probiotic Paenibacillus bacterium that possessed various unique genome features and also shows the strong ability for probiotic application purposes.

KEYWORDS: antiSMASH, CAZymes, Genome, Paenibacillus, PCR

Isolation, Screening and Characterization of Lactic Acid Bacteria and the Optimization of their Media Components and Process Parameters for Production of Lipase through Submerged Fermentation.

Uday G

Manasa Gouda, Keerti G Naik, Shreenivas, Dr.V.S.Hombalimath. (Corresponding author)

From: Department of Biotechnology, KLE Tech University, Hubballi, Karnataka-580031

Locally fermented vegetable pickles are tested for the presence of Lactic Acid bacteria strains, and their probiotic properties are assessed. Four *Lactobacillus* strains are isolated from selected fermented pickles which are grown under anaerobic conditions for 24-48 hours and enumerated. All the isolates are screened for lactic acid production using agar-well diffusion method and the isolates are able to tolerate the pathogenic activity against *B. cereus, B. subtilis* and *P. aeruginosa*. The isolates are found to be Gram positive, non-spore forming and non-motile organisms and negative for catalase and MR test. The results showed that the local traditional fermented vegetable pickles are good source of potentially beneficial *Lactobacillus* probiotic strains. Lactic acid bacteria isolated from the pickles were screened on various oil containing media to check for lipase production. The culture was tested against olive oil, tributyrin and tween 80. LAB of amla pickle sample showed the highest zone of clearance against the media containing olive oil as substrate. It was stored in nutrient agar slants until further analysis. The culture was then added into the production media and incubated for 48 hours. Then the statistical optimization was carried out for different media components and process parameters. The significant factors of the production media were screened using Plackett-Burman (PB) design at two levels- high and low. The culture was centrifuged at 6000rpm for 15 minutes. Enzyme activity and protein content was estimated using titrimetric analysis and Lowry's method respectively. The results were analyzed based on the pareto chart. Pareto chart showed pH, (NH4)2SO4 and RPM to be significant factors. These factors were subjected to optimization. The optimization studies were done using Central Composite Design (CCD) at three levels: low, mid and high. The results were analyzed based on the contour plots. Enzyme activity was found to be 157.98 µg/ml/min and 1.288 µg/ml of protein. An optimized culture media was prepared and was subjected to partial purification by ammonium sulphate precipitation and dialysis and stored at - 20°C.

KEYWORDS: Lactic Acid Bacteria, Lipase, Optimization, Plackett-Burman design, CCD.

Screening and Validation of Reference Genes for Expression Studies in Black Tiger Shrimp, *Penaeus monodon*

Preety Sweta Hembrom

Mottakunja Deepthi , Tony Grace
From: Department of Genomic Science, School of Biological Sciences, Central University of Kerala, Kasaragod, Kerala- 671316, India

Gene expression profiling through RT-qPCR is a sensitive and effective method for understanding the complex molecular mechanisms governing growth and development. However, in the context of *Penaeus monodon*, a widely farmed marine crustacean, there has been a lack of comprehensive evaluation of suitable reference genes for such studies. This research aimed to assess the stability of seven potential reference genes (ACTB, 18S, EF-1α, AK, PK, cox1, and CLTC) in adult tissues (hepatopancreas, gills, and stomach) from both small and large *P. monodon* populations. The stability of these genes was assessed using NormFinder, BestKeeper, and geNorm. The comprehensive ranking was carried out through the web-based tool RefFinder. Overall, 18S and CLTC emerged as the most stable genes in the hepatopancreas and stomach, while CLTC and AK exhibited high statistical reliability in the gills of adult *P. monodon*. These findings were validated using a growth-associated gene, insr-1, which confirmed 18S and CLTC as reliable reference genes for qPCR analyses focused on *P. monodon* growth. This study provides the first comprehensive assessment of reference genes for RT-qPCR in adult *P. monodon*, offering crucial guidance for future research involving similar crustaceans.

KEYWORDS: *Penaeus monodon*, Gene expression profiling, Reference genes, Normalization

Metagenomic Exploration Unveils Divergent Microbiome Composition in Native Cattle Kasaragod Dwarf and Holstein Crossbred Cattle

Mottakunja Deepthi and Tony Grace

From: Department of Genomic Science, School of Biological Sciences, Central University of Kerala, Kasaragod-671320, Kerala, India.

Kasaragod Dwarf cattle, a non-descriptive native cattle variety that is noted for its high feed efficiency and greater resistance to diseases, asserts its importance over exotic cattle. This study about the fecal bacterial community of native Indian cattle, Kasaragod Dwarf, and the crossbred variety Holstein attempts to determine the degree to which the cattle breed impacts the compositional changes in the microbiota and its interaction with host physiology. To compare and contrast the unique microbiota composition and their relationship with the host, we performed a marker gene-based taxonomic analysis of fecal metagenome of the two genetically distinct cattle, Kasaragod Dwarf, and Holstein crossbred. Alpha diversity analysis showed a higher bacterial richness in Kasaragod Dwarf cattle compared to Holstein cattle (Mann-Whitney U test, P < 0.0001). The PCoA plot on the weighted and unweighted UniFrac distance metrics clearly showed a substantial difference between the microbiota composition of the two different cattle types (ANOSIM, P < 0.001). The dissimilarities observed between the two cattle types were further confirmed by the signature taxa identified in each cattle type following Random Forest analysis. Furthermore, the study observed a positive correlation between bacterial genera associated with feed efficiency, such as *Anaerovibrio*, *Succinivibrio*, *Roseburia*, *Coprococcus*, *Anaerostipes*, *Paludibacter*, *Elusimicrobium*, *Sutterella*, *Oribacterium*, *Coprobacillus*, *and Ruminobacter*, and the Kasaragod Dwarf cattle. To the best of our knowledge, this marks the first microbiome profiling study conducted on Kasaragod Dwarf cattle. Further molecular characterization is solicited to better understand the microbial role in the conversion of low-quality feeds into more efficient animal products, a well-defined characteristic of indigenous cattle.

KEYWORDS: Kasaragod Dwarf cattle, Microbiota, Holstein cattle, Fecal sample

Electrode Configuration Influence on the Performance of Bioelectrogenesis in Microbial Fuel Cells (MFCS)

Anagha R Nadiger

Naik P. Sneha , Devu Pengadeth , Gunda Mohanakrishna
Centre for Energy and Environment, School of Advanced Sciences, KLE Technological University, Hubli, Karnataka
2 Department of Biotechnology, KLE Technological University, Hubli, Karnataka

The major bottle necks when it comes to the development of sustainable energy are high cost of generation and imbalance of extensive energy consumption during its production. Microbial fuel cell (MFC) that produces bioelectricity while oxidizing the organic substances in wastewater is a comprehensive approach to waste management, energy recovery and valuable product generation. Scaling up of this process is the primary challenge hindering the widespread adoption of this technology. To address this concern, it is valuable to study innovative configurations of MFCs. With this in mind, in this study a novel multiple electrode MFC is developed. A single chamber of working volume 1700 mL MFC is constructed with grooved graphite electrodes to increase the surface area of electrodes for improved electrical contact. Three anodes and cathodes are used with each anode and cathode having a surface area of 121 cm^2 and 111 cm^2, respectively. Synthetic wastewater containing glucose, sodium acetate and mineral solution along with activated microbial consortium is introduced. The electrodes generated stable power outputs with concomitant removal of organics in wastewater. The first cycle showed the highest potential, generating an open circuit voltage (OCV) of 458 mV and closed-circuit voltage (CCV) of 101 mV at 120 h of operation. The degradation of carbohydrates and proteins was analyzed using standard procedures, which registered around 90% and 50% reduction respectively. Maximum chemical oxygen demand (COD) removal obtained was 43%. The maximum volatile fatty acids concentration was observed halfway through the cycle, the highest being 1145 mg/L. This research delves into the design, creation, and performance evaluation of MFCs, specifically those with multiple electrodes. This study further aims towards the enhancement of energy generation efficiency as well as improvement of wastewater treatment through bio electrochemical processes.

KEYWORDS: Carbon electrodes; Scale-up design; Mixed consortia; Carbohydrate removal.

Vegetable Waste and its Hydrolysates for the Production of Bioelectricity Using Microbial Fuel Cells (MFCs)

Sahana B. Undodi

Naik P. Sneha , Basavaraj S. Hungund , Gunda Mohanakrishna
From: Centre for Energy and Environment (CEE), School of Advanced Sciences, KLE Technological University, Hubli, Karnataka
Department of Biotechnology, KLE Technological University, Hubli, Karnataka

Nowadays, the disposal of waste has increased drastically and it is predicted to increase even more in the near future. Waste disposal has massive environmental impact and it can cause severe problems to the ecosystem. Vegetable waste (VW), a major component of municipal solid waste (MSW) that being generated in large quantities and holds the potential to generate value-added products. Microbial fuel cell (MFC) is a bio-electrochemical device that uses microorganisms to convert organic matter into electricity, which found to have promising option for vegetable waste handling. Present study explores the utilization of VW as a sustainable resource for bioelectricity generation along with simultaneous waste bioremediation with dual chambered MFC that designed with carbon electrodes and cation exchange membrane having working volume of 400 mL. Bioelectricity generation was studied with raw and pretreated VW, followed by supplementation with sewage water in the anodic chamber. Electrochemical studies such as monitoring the open circuit voltage (OCV), closed circuit voltage (CCV), and polarization studies were conducted to monitor the bacterial activity, current and power density respectively to enhance the performance of MFC. Upon analysis it was observed that, 62.5% of VW was treated which representing chemical oxygen demand (COD) removal from 3 g/L to 1.125 g/L during 240 h of cycle operation. The cycle showed a power density and current density of 35.60 mW/m^2 (at OCV 716 mV and CCV 318 mV) and 133.90 mA/m^2 , respectively. MFC operation with VW is suggesting sustainable utilization for bioelectricity generation under ambient operational conditions along with treatment.

KEYWORDS: Vegetable waste, chemical oxygen demand, mixed bacterial consortia, bioanode, waste treatment.

Identification and Characterization of Potential Druggable Targets among Essential Uncharacterized Proteins of *Trichuris trichiura*

Kanchan Rauthan and Sudhir Kumar

From: Department of Biotechnology, H.N.B. Garhwal University, Srinagar (Garhwal), Uttarakhand, India

Trichuris trichiura is a parasitic soil transmitted helminth which causes a disease named as trichuriasis in humans especially children. This parasite invades the intestine of humans after ingestion of contaminated food. Resistance to antibiotics used in treatment of this disease warrants the development and identification of new drug targets. 19% Proteome of *Trichuris trichiura* contains hypothetical proteins, whose function and structure are unknown. Functional and structural annotation of these hypothetical proteins may give an opportunity to identify potential therapeutic drug targets. This study relies on *in-silico* approach in which various web-servers and software's were used for the annotation of these hypothetical proteins. For functional annotation, ExPasy's ProtParam (for physicochemical parameters), FUEL-mLoc server (for localization prediction), and InterProScan, Motif, NCBI-CDART etc (for domain analysis) were used. The performance of various tools used in domain analysis was assessed using receiver operating characteristic curve analysis (ROC). Non-homology searches were conducted for hypothetical proteins having a functional domain in the human protein database. The pharmacological properties of these non-homologous hypothetical proteins were evaluated by identifying similarity to a drug bank database, as well as determining their essentiality. Functional analysis of few hypothetical protein has been done using various server, reflecting the functional aspect of protein which we can used for structural analysis. We were able to assign functions to 184 uncharacterized proteins using programs for predicting physicochemical parameters, motif and domain searches, pattern searches, and localization predictions. A variety of proteins were involved, including enzymes, transporters, membrane proteins, and binding proteins. We observed that 87 proteins are non-homologous to humans and hence could be selected for drug designing targets. Screening of these hypothetical proteins using functional and structural approach gives an insight of pathogenesis and identification of probable therapeutic targets which may reduce the burden of this disease.

KEYWORDS: Soil Transmitted Helminth, Annotation, Druggability, Non-homologous, Domain, ROC.

Exploring the Role of Autophagy Pathway During Saline Stress in Rice (Oryza sativa L.)

Mohd Shahanbaj Khan

From: Department of Biotechnology, H.N.B. Garhwal University, Srinagar (Garhwal), Uttarakhand, India

Autophagy is a pivotal self-regulating process to maintain cellular homeostasis. Autophagy was first reported in yeast and mammals. This mechanism mediates the damaged cellular components to the vacuole via autophagosome formation in plants. The exact mechanism of autophagy in plants is still unknown. However various studies reported that autophagy plays a crucial role and enables the plants to survive under nutrient starvation and biotic and abiotic stresses. Our study highlighted the physiological, biochemical, cellular and molecular response in control and salt-stressed rice plants and role of autophagy and PCD during saline stress. The results of the study showed the inhibitory effect of salt-induced stress on treated plants compared to control. The molecular analysis of key antioxidant genes (CAT1, SOD and GPX,) transcription factors responsible for salinity including MAPK-1, WRKY53, BAX Inhibitor-1 and autophagy-related genes (ATGs) showed the differential expression responses. In conclusion, our study underscores the significance of autophagy and programmed cell death (PCD) in promoting saline stress resilience in rice. The findings serve is a robust basis for identifying autophagy targets, crucial in the development of stress- tolerant rice cultivars for sustainable agricultural practices.

KEYWORDS: ATG, PCD, Saline stress, Transcriptional regulation

Autotrophic Mechanism of Microalgae as Biocathode in Bioelectrochemical Systems for the Treatment of Wastewater

Sunidhi Ganeshkar , Naik P. Sneha

Gunda Mohanakrishna (Corresponding author)
From: Centre for Energy and Environment, School of Advanced Sciences, KLE Technological University, Hubli, Karnataka
Department of Biotechnology, KLE Technological University, Hubli, Karnataka

Bioelectrochemical systems (BES) such as Microbial fuel cells (MFC) that utilize microbial interactions to enable the conversion of organic matter into electricity and valuable products have been extensively studied for bioremediation of wastewater and pollutants along with co-generation of bioelectricity. Autotrophic microorganisms such as microalgae capable of fixing carbon dioxide (CO 2) through photosynthesis, producing organic compounds and oxygen have been studied for their role in BES systems. Integration of microalgae to the cathodic compartment of the MFC can lead to enhanced organic matter removal, nutrient recovery and electricity generation along with biomass formation. In this study, dual-chambered MFC having a total volume of 450 mL with anodic and cathodic compartments separated by a proton exchange membrane (PEM) was used. Grooved carbon electrodes having a surface area of 0.0142 m^2 was used for both compartments. The anodic compartment consisted of vegetable waste hydrolysate supplemented with sewage and the cathodic compartment contained microalgal culture grown in BG11 media. The cathodic compartment was studied and the media for microalgae was changed from distilled water to BG11 media. Electrochemical analysis studies such as open circuit voltage (OCV), closed circuit voltage (CCV) and polarization studies were conducted on the cathodic compartment to monitor microalgal activity in cathodic reduction along with carbon dioxide fixation that generates biomass. Mixed microalgae inoculated in cathode was 2.54×10 5 cells/mL was gradually increases at a rate of 2.74 ×10 4 cells/mL.day in 19 days that resulted to reach algal biomass of 7.76 × 10 5 cells/mL. The process helped for cathodic oxygen reduction reaction and CO2 fixation in terms of algal biomass. The process also evaluated for cathodic half cell potentials to understand the relation between bioelectricity generation and algal biomass growth.

KEYWORDS: Mixed microalgae, CO2 fixation, aerobic cathode, bioelectricity generation.

In silico Assessment of Conventional Drugs for FRD Control in *Areca catechu*

Ajay A Shettigar

(Corresponding authors-Shailashree C Ganiger, Bhuvaneshwari, Dr. Sharada P)
From: Department of Biotechnology, Basaveshwar Engineering College, Vidyagiri,
Bagalkote, Karnataka-587102.

In this study, we propose to use an *in-silico* approach to evaluate the efficacy of conventionally used drugs against Fruit Rot Disease in *Areca catechu*. This approach is cost- effective and allows for the screening of multiple compounds, helping to identify potential drug combinations and novel therapies. Using a combination of computational methods like docking studies and virtual screening techniques, we aim to repurpose existing drugs and identify their potential effectiveness in treating Fruit Rot Disease in *Areca catechu*. The objectives of this research were to determine the efficacy of registered fungicides or fungicides likely to be registered in the near future for control of fruit rot, as well as analyse specificity of these fungicides for the principal pathogens that cause the disease in *Areca catechu*. This research project aims to leverage the power of in silico assessment to evaluate the efficacy of conventional drugs against Fruit Rot Disease in *Areca catechu*. The research project seeks to utilize computational methods and virtual screening techniques to evaluate the efficacy of conventional drugs in controlling Fruit Rot Disease in *Areca catechu*. This approach can help in identifying potential drug candidates and their effectiveness in treating the disease. By using an *in silico* approach, we can potentially save time and resources by identifying promising drug candidates before moving on to costly and time-consuming experimental trials in the field. Additionally, this approach may also provide insights into the specificity of these drugs towards different pathogens causing Fruit Rot Disease.

KEYWORDS: *In-silico* approach, Fruit Rot Disease, Docking studies, Virtual screening.

Nanoparticles and Environmental Pollution

Anurag Jyoti

From: Amity Institute of Biotechnology, Amity University Madhya Pradesh, Maharajpura Dang, Gwalior-474005, India

Nanotechnology has enormous applications in many areas including human health, agriculture, and the environment. Due to their overuse and enormous applications, they are being discharged into the environment. Various nano-based products are available in current market. More and rapid use of Nanoproducts, have become hazardous for both human health and environment. Nanoparticles (NPs) have been increasingly released into environments through various means.

Different NPs may have different levels of toxicity and effects on various organisms. In environment, particularly water bodies get severely deteriorated with discharged nanomaterials. This has resulted into the lethal effects on flora & fauna. This urgently needs to develop a technology that can absorb and reduce the toxic nanoparticles from aquatic body and minimize their hazardous effects. The present paper focuses on the toxicity of nanoparticles. It also focuses on the mechanisms of uptake, translocation and biosorption of NPs by microalgae. This can provide a possible solution for minimizing the toxic effects of nanomaterials.

KEYWORDS: Nanotechnology, Nanoparticles, Toxicity, Biosorption.

Cloning and Functional Characterization of Squalene Synthase Gene from *Brassica juncea*

Rajkumar Bangkim Sana

Priya Panjabi (Corresponding author)
From: Department of Botany, University of Delhi, Delhi-110007, India

Squalene synthase (SQS) enzyme plays a significant role in the biosynthesis of squalene, a key precursor of triterpenoid, phytosterol and cholesterol biosynthesis. Phytosterols have gained significance owing to their cholesterol reducing, anti-cancer, anti-diabetic and anti- inflammatory effects. In the present study, SQS paralog, co-localizing within a QTL identified for phytosterol biosynthesis in *B. juncea* was isolated and functionally characterized. The amplified coding sequence (BjSQS-A08 paralog) is 234 bp long and encodes for 410 amino acid long SQS protein. The BjSQS-A08 sequence exhibits high similarity with SQS orthologs from *Arabidopsis thaliana* (AtSQS) and other *Brassica* species. Both the full length and a 24-amino acids C-terminal deleted version of the BjSQS cDNAs were cloned in the protein expression vector pET-28b. The C-terminal deletion in SQS protein has been previously shown to facilitate isolation of soluble protein. Recombinant BjSQS proteins (44 kDa full length and 43 kDa truncated version), were purified using Ni-NTA affinity chromatography and confirmed on SDS-PAGE. The expressed SQS proteins were able to convert in-vitro the substrate farnesyl diphosphate (FPP) to squalene, the presence of which was identified using GC-MS analysis, confirming the functionality of the BjSQS cDNA isolated. Further the ~1.5 kb and ~1 kb promoter regions of BjSQ-A08 and BjSQ-B07 paralog, respectively were amplified and fused with the GUS reporter gene. The recombinant genes were introduced in *A. thaliana*. Both promoters were found to be active in majority of plant tissues analyzed for GUS expression, including leaves, stems, inflorescence and siliques. However, paralog specific expression was also observed in some regions, the results of which have been presented.

KEYWORDS: *Brassica juncea*, GUS reporter gene, Phytosterol, Squalene synthase

Screening, Characterization, and Studies on Halophilic Bacteria Producing Alginate Lyase

Rohan Dharwad

Dr B.S Hungund (Corresponding author)
From: Department of Biotechnology, KLE Technological University, Vidyanagar, Hubli, Karnataka-580021

Enzymes are specialized proteins produced in an organism, which are capable of catalyzing a specific chemical reaction. Among the different categories of enzymes obtained from microbial sources, Alginate lyase is an acidic linear polysaccharide, a biocatalyst as well as important group of enzymes that degrades alginate to produce oligosaccharides, which have many bioactive functions and could be used as renewable biofuels. The sample of brown marine algae was collected from Karwar beach, Karnataka. Halophilic Bacteria were utilized through Subculturing Techniques. Nine isolates were screened for production of alginate lyase through Minimal-sea Salt Agar Media. Maximum alginate lyase producing isolates were selected based on the highest zone of clearance. Gram staining was conducted to characterize and identify the bacterial species. Endospore staining and motility test were also done inorder understand morphology as well as colony morphology is also done. Biochemical characterization was done with respect to all nine cult ures where in Catalase test was conducted to differentiate catalase positive and catalase negative enzyme and differentiation between genera. Methyl Red is another biochemical characterization test conducted to determine the production of acid. Based on primary screening results top three cultures were chosen for bacterial growth curve studies- G1, G8 and G9 are the three cultures chosen and their doubling time was calculated. For further optimization studies G1,G8 and G9 cultures will be used. Alginate lyase have wide application in food and pharmaceutical industry, they can be used as growth promoters for plants and therapeutic agents such as anticoagulants and tumor. It is used in curing cystic fibrosis. The mild degradation of this enzyme have been the focus for various fields.

KEYWORDS: Halophilic bacteria, Alginate lyase.

NRF2 Regulated Genes as Prognostic Biomarkers in TCGA-Esophageal Squamous Cell Carcinoma (ESCC)

Samanvitha Suresh

Dr Akhileshwar Namani (Corresponding author)
From: Department of Molecular research, Sri Shankara Cancer Hospital and Research Centre, Bengaluru, Karnataka – 560004

Mutations in the KEAP1-NFE2L2-CUL3 axis genes lead to the hyperactivation of the transcription factor-NRF2 signaling pathway in various cancers including Esophageal Squamous Cell Carcinoma (ESCC). 23% of The Cancer Genome Atlas (TCGA)-ESCC patients harbor these mutations that lead to drug and radioresistance. Identification of NRF2 modulated downstream genes, regulated pathways and biomarkers associated with these mutations allows the researchers and clinicians to provide personalized medicine and a quicker diagnosis. In this current study, we carried out a multi-omics analysis of exome and transcriptomic data of KEAP1-NFE2L2-CUL3 axis mutated TCGA-ESCC patients against non-mutated counterparts. As a result, we identified the genes and pathways associated with KEAP1-NFE2L2-CUL3 mutations and identified the prognostic genes which could be used as potential biomarkers in the NRF2 pathway activated ESCC patients. Our findings might be useful in the early diagnosis of ESCC patients.

KEYWORDS: NRF2 pathway, mutations, Esophageal Squamous cell carcinoma, prognostic biomarkers, TCGA.

High Purity Prebiotic Isomalto-Oligosaccharides Production by Cell Associated Transglucosidase of Isolated Strain *Debaryomyces hansenii* SCY204 and Selective Fermentation by *Saccharomyces cerevisiae* SYI065

Saravanan Rengarajan

Rameshthangam Palanivel (Corresponding author)
From: Dept. of Biotechnology (DDE), Alagappa University, Karaikudi, Tamil Nadu, India

An efficient recycling method was used to develop the continuous production of high purity isomalto-oligosaccharides (IMOs) by cell associated transglucosidase of a novel strain, *D. hansenii* from maltose and selective fermentation by S. cerevisiae. The most potent transglucosidase producer was screened, isolated and identified as *Debaryomyces hansenii* using LSU region sequencing. Parameters optimization studies were investigated using whole cells of *D. hansenii* (4023 units L−1 α-glucosidase activity) from 10 L fermenter to increase the enzyme activity through biotransformation. IMOs were continuously synthesized by reusing the cell biomass (6 %) using microfiltration membrane system with 30 % maltose concentration under controlled temperature of 34 °C in an average of 12 h for 5 cycles. The obtained low purity IMOs (67 %) was further incubated with cell pellet of isolated strain *Saccharomyces cerevisiae* (4 %, w/v) in 3 L bioreactor for 1 h to utilize glucose completely without affecting the product to obtain high purity IMOs by recycling method. This novel study using these yeasts was found to utilize more than 98 % maltose with higher conversion efficiency for production of IMOs with > 91 % purity, 79 % yield and highest productivity of 198.79 g L-1.h which was confirmed by HPLC.

KEYWORDS: Biotransformation, *Debaryomyces hansenii*, Isomalto-oligosaccharides, Microfiltration membrane system, *Saccharomyces cerevisiae*, Transglucosidase

CRISPR-Cas Systems in Oral Microorganisms and their Role in Periodontal Therapy

Aditi Chopra

From: Department of Periodontology, Manipal College of Dental Sciences, Manipal, MAHE, Udupi, Karnataka-576104s

Clustered Regularly Interspaced Short Palindromic Repeats technology (CRISPRs) are DNA sequences that can remove, edit, and manipulate a genome sequence by either activating or inhibiting a specific gene sequence. CRISPR technology utilizes the enzyme attached to the CRISPR strand, known as the 'Cas gene' that acts like a pair of molecular scissors, capable of cutting strands of DNA. The oral cavity contains numerous microorganisms that naturally harbour many variants of CRISPR-Cas systems. Oral bacteria use CRISPR sequences to acquire nutrients, inter-species and intra-species communication (quorum sensing), invasion, bacterial virulence, evade the host immune response, horizontal gene transfers, biofilm formation, and antibiotic resistance. CRISPR-Cas technology is also employed to modulate the function of various oral bacteria, affecting the pathogenesis of periodontal diseases. Although 'CRISPR-Cas' is "a revolutionary genomic tool" in various fields, limited papers discuss the role of the CRISPR-Cas system in oral bacteria, and how it can be employed to manage periodontal disease. Hence, this review paper aims to discuss the types, mechanisms, and types of CRISPR genes in oral microbiota and how they can be employed for managing periodontal disease pathogenicity and periodontal therapy.

KEYWORDS: CRISPR; DNA; RNA; Molecular biology; Periodontal disease; Periodontitis; Periodontal therapy; Genetic manipulation; Gene therapy

SYMBIOT'23 WINNERS

Position	Name	Institute	Designation	Location	Mode
Poster Presentation					
1st	Sahana B. Undodi	KLE Technological University	Undergraduate	Vidyanagar	Offline
2nd	Uday G	KLE Technological University	Undergraduate	Vidyanagar	Offline
3rd	Jyeshta GP	KLE Technological University	Undergraduate	Vidyanagar	Offline
Oral Presentation					
1st	Saravanan Rengarajan	Tata Chemicals Ltd.	Research Scholar	Tirupathi	Online
2nd	Sambhaji Balkrushna Chavan	CSIR-National Chemical Laboratory	Research scholar	Pune	Online
3rd	Anil Balasaheb Khatape	CSIR-National Chemical Laboratory	Research scholar	Pune	Online

SymBiot'23

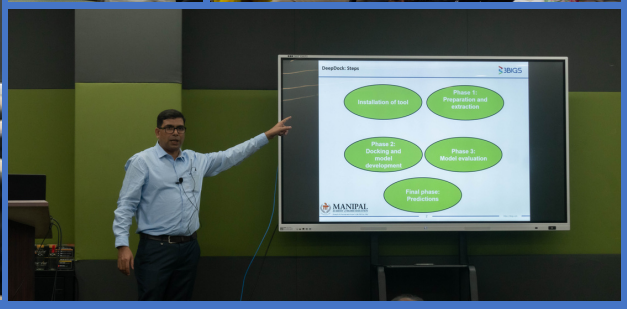
A National Level Biotechnology Conference













Photographs by: Eshita Bathwal, Nithya Sappani & Navya S A