

ABSTRACT BOOK

**INTERNATIONAL WORKSHOP ON
BIOLOGY AND APPLICATIONS OF
ACTINOMYCETES**

On 31st October-1st November 2019

Organized by

University of Mysore, Mysore, India

In association with

Helmholtz Centre for Infection Research

&

Technical University of Braunschweig, Germany

**Vijnana Bhawan, University of Mysore
Manasagangotri, Mysore**

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Edited by

Dr. Ravishankar Rai. V

University of Mysore, Mysore

WELCOME MESSAGE



It gives me immense pleasure to invite you all to the International Workshop on Biology and Applications of Actinomycetes-2019. It is jointly organized by the University of Mysore, Mysore, Helmholtz Centre for Infection Research, Braunschweig and Technical University of Braunschweig, Germany. This workshop is aimed to bring both young and experienced scientists from all regions of the world to learn advanced techniques on the Diversity, Chemical biology and Ecology of Actinomycetes and the application of modern genomic platforms for the discovery of antibiotics, anti-infectives and anticancer drugs from Actinomycetes.

Actinomycetes are one of the extremely diverse groups of filamentous bacteria capable of surviving in a number of ecological niches due to their bioactive potential. After more than half a century of exploitation, it has become increasingly challenging to find novel natural products with useful properties as the same known compounds are often repeatedly re-discovered when using traditional approaches. Modern genome mining approaches have led to the discovery of new biosynthetic gene clusters, thus indicating that Actinomycetes still harbor a huge unexploited potential to produce novel natural products. In recent years, innovative synthetic biology and metabolic engineering tools have greatly accelerated the discovery of new natural products and the engineering of actinomycetes. The two days Workshop on Biology and Application of Actinomycetes will include deliberations by both internationally recognized and well-established scientists as well as promising young researchers and students who are keen to unfold the natural product repository hidden in the Actinomycetes class. We anticipate active exchanges of ideas, advancements of new knowledges, approaches, techniques, and applications, encompassing nearly all disciplines of modern biology and biotechnology for discovery of natural products from Actinomycetes. The workshop is open to all scientists interested in the biology, ecology, taxonomy and natural product chemistry of Actinomycetes. The workshop is expected to bring forth and foster much new collaboration among the Actinomycetes biologists. I take this opportunity to thank Prof Prof Joachim Wink, Helmholtz Centre for Infection Research, Germany for his support for organising this workshop

I wish this International workshop on Biology and Applications of Actinomycetes a great success and the participating delegates a pleasant stay in Mysore

Prof. Ravishankar Rai. V
Convener

WELCOME MESSAGE



Dear colleagues working with Actinomycetes, it's a pleasure for me to also welcome you to our joint workshop in Mysore.

Microorganisms have been used since nearly 4000 years by humans for many purposes and influenced the development of the different civilizations. For healing wound infections microorganisms have been used by the Egypt, Persian and Greek people, but it took until the 1882 when it was Robert Koch who identified the first bacterium *Mycobacterium tuberculosis* being responsible for tuberculosis one of the mayor infection problems of this time. In the centuries bevor a number of microbial caused diseases like the Black Death caused by *Yersinia pestis*, leprosis in warm tropical countries, caused by *Mycobacterium leprae* und Cholera caused by *Vibrio cholera*, only to give some examples, were responsible for the death of millions of people. After Koch's findings it only took a short time before the first Antibacterial compound Salvasan came on the market, followed by the Sulfonamides. In 1929 Alexander Fleming observed the production of an antibacterial activity produced by a fungus and nearly 10 years later Florey and Chain where able to isolate and characterize the responsible compound Penicillin. Since this time in the so called golden time of antibiotics many novel compound were isolated, most of them from Actinomycetes.

Today there are macrolides, glycopeptides, ansamycines, tetracyclines, carbapenemes and a number of other compound classes as antibiotics on the market that are produced by Actinomycetes and many people believe that we do not need additional antibiotics. Today we know that this is wrong, we have the resistance development in many bacteria, especially the nosocomial ones, increase if resistance of *S. pneumoniae* against Penicillin and Erythromycin, of *S. aureus* against methicillin, of multidrug resistance at *P. aeruginosa* and of vancomycin resistance of Enterococci. We have every year new infection diseases like EHEC, MERS and Zika and the neglected diseases like tuberculosis and malaria are responsible for two million death cases every year. Novel natural products and here the secondary metabolites from Actinomycetes still harbor a huge potential for new antibiotics. So I'm expecting very interesting results around our research field of Actinomycetes in this two days in Mysore. At this I want also to thank Prof. Ravishankar Rai V. for organizing this workshop at this wonderful city Mysore and wish you also a nice time with fruitful discussions.

Prof Joachim Wink

Helmholtz Centre for Infection Research, Germany

PROGRAM AT A GLANCE

INTERNATIONAL WORKSHOP ON BIOLOGY AND APPLICATIONS OF ACTINOMYCETES 31st October to 1st November, 2019 University of Mysore, India	
October 31, 2019	
9:00 AM – 10:00AM	Registration
10.00 AM – 11:00 AM	Inaugural Ceremony
11:00 AM – 11:15 AM	Tea Break
11:15AM – 12:00 AM	Keynote Address: Prof. Joachim Wink. Helmholtz Centre for Infection Research, Braunschweig, Germany Title: Bacterial Biodiversity as Resource for Novel Antibiotics
Plenary Address: Session Chair: Prof.A.K.Dubey and Prof. K.Kannabiran :	
12:00 AM - 12:45 PM	Prof Michael Steinert Institut für Mikrobiologie, Technische Universität Braunschweig, Germany Title: New drug targets in <i>Legionella pneumophila</i>
12:45 PM – 01:30 PM	Prof. Saisamorn Lumyong Center of Excellence in Microbial Diversity and Sustainable Utilization, Chiang Mai University, Thailand Title: Control of Basal Stem Rot Disease in Oil Palm Seedlings by <i>Streptomyces palmae</i> CMU-AB204 ^T
01:00 PM – 02:30 PM	Lunch Break and Poster Session

Session Chair: Prof. Saisamorn Lumyong and Prof. Tohru Dairi

02:30 PM – 03:00 PM	Prof.A.K.Dubey Department of Biological Sciences and Engineering Netaji Subhas University of Technology, New Delhi Title: Anti- <i>Staphylococcus aureus</i> and anti-oxidant activities of the metabolites produced by endophytic actinobacteria <i>Streptomyces californicus</i> strain ADR1
03:00 PM – 03:30 PM	Prof. K.Kannabiran Department of Biomedical Sciences, School of Biosciences and Technology, VIT University, Vellore, India Title: Isolation, characterisation and identification of antiviral compound from <i>Streptomyces</i> species against fish White Spot Syndrome Virus (WSSV)
03:30 PM – 04:00 PM	Dr. Syed G Dastager National Collection of Industrial Microorganisms National Chemical Laboratory, Pune Title: Multifaceted actinobacteria: A polyphasic approach
04:00 PM – 04:15 PM	Tea Break
Oral Presentation	
Session Chair: Prof. Chenghang Sun and Prof V. Ravishankar Rai V	
04:15 PM – 5:30PM	1) Dr. Bijaya Kumar Nayak Kanchi Mamunivar Centre for P.G. Studies, Puducherry. Title: Isolation, Characterisation and antimicrobial activity of Actinobacteria from Ant Hill soil. 2) Dr. Jerrine Joseph Sathyabama Institute of Science and Technology, Chennai. Title: Multifunctional <i>Streptomyces maritimus</i> SACC-152 isolated from Andaman and Nicobar Islands, India

3) Dr. Krishna Naragani

Siddhartha College of Arts & Science, Vijayawada- A.P.

Title: Phylogenetic Characterization of Potential bioactive metabolite producing Actinomycetes from Mangrove sediments

4) Dr. Shiburaj Sugathan

Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Trivandrum

Title: A Calcium Independent and Thermo-stable α -Amylase from *Streptomyces griseus* TBG19NRA1 isolated from forest soil of Western Ghats, Kerala, India

5) Dr. Pramod B. Shinde

Natural Products and Green Chemistry Division, CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar.

Title: Bioactive Macrolide from the Actinomycete *Dactylosporangium aurantiacum*

6) Dr. N.Manoharan

Department of Marine Science, Bharathidasan University, Tiruchirapalli

Title: Marine Endophytic Actinomycetes Act as A Effective Anti-Bacterial Agent Against Multi Drug Resistant Uropathogens

7) Dr.V.Mohanasrinivasan

Department of Biomedical sciences, VIT, Vellore

Title: Fermentative production of extracellular pigment from *Streptomyces coelicolor* MSIS1

8) Dr. Aruna Sharmili S

Department of Biotechnology, Stella Maris College, Chennai

Title: Isolation and characterization of methanol utilizing streptomyces species isolated from jack fruit (*Atrocarpus Heterophyllus*) Rhizosphere

November 1, 2019

Plenary Address:

Session Chair: Prof. Joachim Wink. Helmholtz

10:00 AM – 10:30 AM	Prof. Tohru Dairi Graduate School of Engineering, Hokkaido University, Japan Title: Unique enzymes involved in biosynthesis of natural products produced by Actinomycetes
10:30AM – 11:00 AM	Prof. Chenghang Sun Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijing, China. Title: Discovery of New Antibiotics from microorganism inhabited in special environment by dereplication strategy
11:00 AM – 11:15 AM	Tea Break
Session Chair: Prof Michael Steinert	
11:15AM - 11:45 PM	Dr. Wasu Pathomree Chiang Mai University, Thailand Department of Biology, Faculty of Science Title: Plant Growth Promoting Actinobacteria From Mycorrhizal Spores: Taxonomic Characterization and Their Beneficial Traits to Plants under Drought
11.45:00 PM – 12:15 PM	Dr. C. Subathra Devi, Department of Biotechnology, School of Biosciences and Technology, VIT University, Vellore, India Title: Clot busters from Actinobacteria
12:15 PM – 12: :45 PM	Prof Dayanand Agsar Department of Microbiology Gulbarga University Title: Diversity and Bioprospecting potentials of Actinobacteria from Harsh Habitat of Limestone quarries
Oral Presentation	
Sessional Chair: Prof Dayanand Agsar and Dr. Syed G Dastager	
12.45PM-1.30 PM	1)Dr. V. Gopikrishnan Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai Title: Bioprospecting of insect nest associated actinobacteria with special reference to antimicrobial and anti-HIV activity

	<p>2) Dr.I.Manideepa Department of Microbiology, Maries Stella College, Vijayawada, A.P., India</p> <p>3) Dr. T.Ganesan Kanchi Mamunivar Centre for P.G. Studies, Puducherry.</p> <p>Title Isolation, screening and characterization of Actinomycetes from mangrove soil, Puducherry</p> <p>4) Dr. M. Radhakrishnan Institute of Science and Technology, Chennai</p> <p>Title: Bioprospecting of actinobacteria from Indian rare ecosystems with special reference to anti-tuberculosis activity</p> <p>5) Dr. A.H. Sneharani Mangalore University, Kodagu</p> <p>Title: Gut bacterial diversity in worker adults of <i>Apis florea</i> and <i>Apis cerena indica</i> assessed by MALDI-TOF, 16S rRNA and NGS</p> <p>6) Dr. Jayanthi Vellore Institute of Technology, Vellore</p> <p>Title: Polyketides from actinomycetes as potential anti-tumor compounds.</p>
<p>01:20 PM – 02:30 PM Lunch Break and Poster Session</p>	
<p>02:30 PM – 04:30 PM Workshop</p> <p>The Polyphasic Approach In Actinomycetes Taxonomy In The Time Of Full Genome Sequencing</p>	



Bacterial Biodiversity as Resource for Novel Antibiotics

JOACHIM WINK

Helmholtz Centre for Infection Research

Braunschweig, German

Email: Joachim.wink@helmholtz-hzi.de

Since the discovery of the bactericidal effect of the penicillin by Alexander Flemming microorganisms play an important role as antibiotic producers. During the 40th to the 60th of the last century these were particularly the Actinomycetes isolated from soil samples which have dominated the „golden age of the antibiotic research“. Caused by the false assumption that with these active substances the problem of the infection illnesses are solved, most pharmaceutical companies dropped their antibiotic research. The development of resistance of many germs, particularly in the hospitals as well as the return of presumed to be dead illnesses like the tuberculosis has moved the antibiotic research, however, just during the last years again in a new light.

Often the question is raised whether microorganisms may continue to provide modern medicine with new antibiotics, a question closely connected to the remaining potential of these organisms after decades of exploitation. How can we judge whether this resource is exhausted or still promising for the identification of novel scaffolds for development? In this talk I will argue for microorganisms as the most promising source for the future from two perspectives: First, from the amazingly low percentage of biodiversity among microorganisms which has been harvested and second from current genomic knowledge showing enormous potential for the production of an almost inexhaustible number of new secondary metabolites

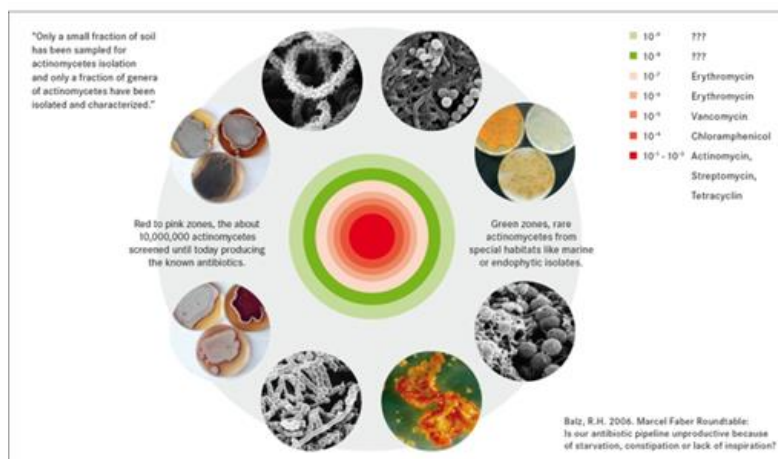
Since the 1940's the history of antibiotic discovery and -development is inseparably connected to microorganisms. Today we know that bacteria exhibiting large genomes (often more than 8 MB) also show highest potential for rich secondary metabolism. Actinobacteria of diverse genera, such as the *Streptomyces*, *Saccharopolyspora*, *Amycolatopsis*, *Micromonospora* and *Actinoplanes*, are the producers of clinically used antibiotics belonging to different chemical classes (for instance, the carbapenemes, anthracyclines, macrolides, glycopeptides, ansamycins, lipopeptides and aminoglycosides (1)). Actinobacteria are a group of gram-positive bacteria characterized by DNA with high GC content. Many of them are soil bacteria, but pathogenic or saprophytic organisms also belong to this group. Many show a characteristic differentiation by forming endospores which are arranged in spore chains, sporangia or are found as single spores on sporophores. Approximately 2500 species in 238 genera, 37 families and 9 suborders are known (Approved List of Bacterial Names) and nearly every week a new species are described.

Already in 1946 Oxford and coworkers described the first bacteriolytic effect based on an antibiotic produced by a myxobacterium (2). The myxobacteria are gram negative bacteria which are also ubiquitously found in soil; most of them degrade biomacromolecules such as cellulose or prey on other microorganisms. Similar to most actinobacteria, myxobacteria are characterized by a differentiation process culminating in the formation of fruiting bodies and more than 50 species in

20 genera, 6 families and 3 suborders are known. In the 1980's sorangicin was isolated from a Sorangium strain as the first myxobacterial antibiotic with high potential for market development (3). Since then many new compounds have been described from this fascinating group of bacteria that have considerably increased the structural diversity found in natural product libraries. Intriguingly, and in contrast to the actinobacterial secondary metabolites, many compounds from myxobacteria are highly active against fungi (e.g. soraphen is an example of a potent antifungal with a novel mode of action(4). Today we know that myxobacteria harbor a large potential for the production of novel secondary metabolites, which holds true for antibacterials and antifungals(5,6), particularly if we look for unexploited isolates or for the still almost unexplored genetic potential of their large genomes. In conclusion actinobacteria and myxobacteria are believed to be potent resources for novel anti-infectives, a probable consequence of their habitat where they live in competition with other bacteria and fungi, making the production of bioactive compounds an obvious strategy for survival. Although the isolation of novel species and families significantly increases the chances of the discovery of novel chemical entities, most of the already known and well described species also harbor a huge and so far almost untapped "hidden" biosynthetic potential in their genome. An understanding of the biosynthesis of the antibiotics is the basis for taking a more direct approach. With the identification of many different biosynthetic pathways and the organization of the corresponding genes in gene clusters, new possibilities for enhancing the production of known metabolites or their modification, as well as the induction of the so called "silent" genes for the production of novel metabolites have opened up. Interestingly, in the actinomycetes and myxobacteria, only a limited number of potential secondary metabolite gene clusters can be correlated to compound production after analysis under standard laboratory conditions (7). The genome sequencing of the first microorganisms known as producers of secondary metabolites (e.g. *S. coelicolor*A3(2), *S. avermitilis* and *Sorangiumcellulosum*) provided an opportunity to analyze complete genomes for their potential to encode novel secondary metabolite biosynthesis gene clusters (8-12). Starting with the sequence information these can be quickly identified using bioinformatics tools (e.g. <http://antismash.secondarymetabolites.org> (13)). However, the corresponding products frequently cannot be detected in fermentation extracts of the respective strains. This will be considered in more detail using selected examples of how to harvest this genomic potential using microbiology, induction of genes and genome mining.

Helmholtz Centre for Infection Research in Braunschweig has dealt during the last years intensely with the search for new antibiotics and, besides, has laid his main focus on two groups of ground-living bacteria. These are on the one hand furthermore the Actinobacteria, the biggest class in the empire of the bacteria with still high potential, and on the other hand the Myxobacterien, a group of the gliding bacteria whose cultivation owns a long tradition in Braunschweig. The biology and active substance production of these both groups as well as the approach in the HZI with the search for new active substances is introduced.

What should we screen in Actinobacteria?



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New Drug Targets in *Legionella pneumophila*

MICHAEL STEINERT

Institut für Mikrobiologie, Technische Universität Braunschweig, Germany

Email: m.steinert@tu-bs.de

Legionella pneumophila, an environmental bacterial pathogen, is able to cause severe pneumonia in humans termed Legionnaires' disease. The Gram-negative bacterium naturally inhabits freshwaters and biofilms, where it parasitizes within protozoan hosts. Upon aerosol formation via man-made water systems, *L. pneumophila* can enter, colonize and damage the human lung. Chest radiographs typically demonstrate patchy, peripheral, non-segmental consolidations. Electron microscopy shows *L. pneumophila* intracellularly within macrophages and neutrophils and it is well documented that the bacteria multiply within a reprogrammed *Legionella*-specific vacuole (LCV). Guinea pigs are highly susceptible to *L. pneumophila* infection and therefore have been the preferred animal model for studies of legionellosis. Guinea pig infections revealed that the *Legionella* virulence factor Mip (macrophage infectivity potentiator) contributes to bacterial dissemination within the lung tissue and the spread of *Legionella* to the spleen. Histopathology of infected animals and human lung tissue explants (HLTE), binding assays with components of the extracellular matrix (ECM), bacterial transmigration experiments across an artificial lung epithelium barrier, and ECM degradation assays were used to elucidate the underlying mechanism of the in vivo observation. The Mip protein, a peptidyl-prolyl-*cis/trans* isomerase (PPIase), which belongs to the enzyme family of FK506-binding proteins (FKBP), was shown to bind to the ECM protein collagen type IV. Transwell assays revealed that Mip enables *L. pneumophila* to transmigrate across a barrier of NCI-H292 lung epithelial cells and ECM. By using peptide arrays, we were able to demonstrate that Mip binds to the NC1-domain of the human collagen IV α 1. A collagen IV derived peptide (P290) was chemically synthesized and analyzed in PPIase- and in vitro transmigration assays. We were able to show that bacterial transmigration is reduced in the presence of P290. NMR studies on the P290-Mip binding revealed a high similarity between the binding region of P290 and the Mip binding pocket for rapamycin. Since drugs which block this process could be an entirely new approach to treat Legionnaires' disease we set out to identify new Mip inhibitors. With the aim of a complementary anti-infective treatment strategy (antibiotics plus Mip inhibitor) we are currently screening new compounds in cellular infection assays, the transwell system and in human lung tissue explants.

Unique Enzymes Involved in Biosynthesis of Natural Products Produced by Actinomycetes

TOHRU DAIRI

Graduate School of Engineering, Hokkaido University, Sapporo 060-8628, Japan

Email: dairi@eng.hokudai.ac.jp

We have been studying unique enzymes involved in biosynthesis of natural products produced by actinomycetes. Recently, the enzymes participating in peptide biosynthesis were extensively studied. In this workshop, the following three topics will be presented.

ATP-grasp ligases using peptides as nucleophiles.

Pheganomycin (PGM) consists of the nonproteinogenic amino acid (*S*)-2-(3,5-dihydroxy-4-methoxyphenyl)-2-guanidinoacetic acid (**1**) at the *N*-terminus and a proteinogenic core peptide derived from NVKDGPT or NVKDR. Recombinant enzyme encoded by *pgm1*, which existed in PGM biosynthetic gene cluster, catalyzed the amide bond formation between **1** and the peptides for the first example of a ATP-grasp ligase utilizing peptides as nucleophiles ¹). Orthologs of *pgm1* was found in genome databases and constitute similar gene clusters. By heterologous expression of the cluster, we confirmed that the cluster was responsible for biosynthesis of a novel pseudo-tripeptide, ketomemicin (KM), with carbonylmethylene structure. Recombinant PGM1 ortholog was revealed to be a dipeptide ligase catalyzing the amide bond formation between amidino-arginine and (pseudo)dipeptides to yield KM ²). We also showed that the carbonylmethylene structure (pseudo-Phe-Phe) was introduced by four unique enzymes ^{3,4}).

Glycopeptidyl-glutamate epimerase for bacterial peptidoglycan biosynthesis ^{5,6}.

D-Glutamate (Glu) supplied by Glu racemases is usually utilized for peptidoglycan biosynthesis in bacteria. By comparative genomics, some bacteria including rare actinomycetes have no orthologs of the genes. We performed shotgun cloning experiments with a D-Glu auxotrophic *Escherichia coli* mutant as host and genomic DNAs of such the bacterium as donor. We obtained complementary two genes and their products were shown to catalyze ligation of L-Glu to UDP-MurNAc-L-Ala and epimerization of the terminal L-Glu of the product.

A novel epimerase responsible for biosynthesis lasso peptide, MS-271 ⁷.

MS-271, produced by *Streptomyces* sp. M-271, is a lasso peptide natural product, which consists of 21 amino acid residues with a D-tryptophan (D-Trp) at its *C*-terminus. Since lasso peptides are ribosomal peptides, the biosynthetic mechanism to introduce the D-Trp residue, is of great interest. The precursor peptide identified by draft genome sequencing contained all 21 amino acid residues including the *C*-terminal tryptophan, suggesting that the D-Trp residue is introduced *via* an epimerization. By heterologous expression of putative MS-271 cluster in *Streptomyces lividans*, a novel enzyme was suggested to catalyze the epimerization.

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Control of Basal Stem Rot Disease in Oil Palm Seedlings by *Streptomyces palmae* CMU-AB204T

KANAPORN SUJARIT¹ AND SAISAMORN LUMYONG^{1,2}

¹Center of Excellence in Microbial Diversity and Sustainable Utilization, Chiang Mai University, 239 Suthep, Muang, Chiang Mai 50200, Thailand

²Academy of Science, The Royal Society of Thailand, Bangkok 100300, Thailand

Oil palm is a primary source of vegetable oil and biofuel production which have economic value in Thailand. However, the plant is usually damaged by fungal infection which results in the yield loss and death of oil palm tree. Basal stem rot (BSR) caused by a fungus, *Ganoderma boninense*, is a pathological problem in oil palm plantation. The present study aimed to selection, development and evaluation of effective actinomycetes to use as inoculants for controlling oil palm disease. A total of 226 actinomycete strains were isolated from rhizosphere soil of healthy oil palms (*Elaeis guineensis* Jacq.) which collected from Chiang Mai University, Chiang Mai Province, Thailand. The visual morphological characteristics exhibited that approximately 90% of all strains belonged to members of streptomyces. Strain CMU-AB21, CMU-AB83 and CMU-AB204 showed the highest inhibitory activity against *G. boninense*, they were chosen as potential biocontrol agents. The efficiency of the three actinomycetes *in vivo* suppression of basal stem rot disease caused by *G. boninense* were evaluated in oil palm seedlings using spore suspension. The most effective inoculant, strain CMU-AB204, reduced percentage of disease severity by 81.6%. Based on phenotypic and genotypic data, strains CMU-AB204 classified as a novel species, for which the name of *Streptomyces palmae* sp. nov. was proposed. *S. palmae* CMU-AB204^T produced various antimicrobials which assigned as actinopyrone A (1), anguinomycin A (7), leptomycin A (8) and four new phenyl alkenoic acids (2, 3, 4 and 5) with a mixture (6) of two new related structures. The best antifungal in this study was observed in leptomycin A, it had a potential to be an antifungal agent for suppressive the growth of *G. boninense* causing basal stem rot disease in oil palm.

Discovery of New Antibiotics from Microorganism Inhabited in Special Environment by Dereplication Strategy

CHENGHANG SUN, SHAOWEI LIU, QINPEI LU

Department of Microbial Chemistry

Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, P. R. China

Email: chenghangsun@hotmail.com

After more than 70 years' antibiotic screening all over the world, rediscovery of known antibiotics has become a bottle-neck issue for discovery of new antibiotic^[1-2]. The process of identifying known compounds responsible for the activity of an extract prior to bioassay-guided isolation is referred to as dereplication^[3]. Combination of modern analytic technique with big data, researchers not only can dereplicate known antibiotics, but also can select new antibiotic producing strains from immense biodiversity of microorganism to overcome the issue. By combination of small scale of fermentation, bioassay and TLC-MS dereplication, new amicoumacins producing strains from Taklimakan desert in China was captured in our group, then, a series of new amicoumacins group antibiotics, Hetiamacins, with new antibacterial target against MRSA, were discovered. After screening by a unique double fluorescent protein reporter system and traditional bioautography against bacteria, Quinomycin producing strains were selected from mangrove-originated *streptomyces*, combination of dereplication strategy with GNPS molecular networking, analogues of quinoxaline group antibiotics were discovered from the culture broth. Our research showed "micro-, rapid-, precise-" identification of known molecules can increase efficiency in discovery of antibiotics.

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Plant Growth Promoting Actinobacteria From Mycorrhizal Spores: Taxonomic Characterization and Their Beneficial Traits to Plants under Drought

WASU PATHOM-AREE

Department of Biology, Faculty of Science, Chiang Mai University,
Muang, Chiang Mai 50200, Thailand
Email: wasu.p@cmu.ac.th

Actinobacteria are large group of Gram-positive bacteria with high %G+C content in their genomes. They are prolific producers of useful bioactive metabolites in particular antibiotics. In the search of beneficial microbes for biotechnological applications, actinobacteria are of priority choice due to their unbeaten track records. They are widely distributed in soils but can be found in many other environments such as marine sediments or associated with other organisms. It is also well accepted that some actinobacteria are potential plant growth promoting bacteria. Our previous work showed that some actinobacteria especially members of the genus *Streptomyces* are potential plant growth promoting and biocontrol microorganisms. Gram positive bacteria associated with AM fungal spores have been reported including several actinobacterial species such as *Streptomyces*. These mycorrhizal associated actinobacteria showed interesting properties on biocontrol and plant growth promoting activities. Currently, we are interested in these mycorrhizal associated actinobacteria which may be a good source of novel taxa for bioprospecting.

In this study, we report on the isolation of actinobacteria from spores of *Funneliformis mosseae* and provide evidence of their potential in agriculture as plant growth promoter in mung bean and rice. Five actinobacteria strains were isolated from spores of *F. mosseae* using selective media. Phylogenetic analyses based on a 16S rRNA gene sequences showed that the isolates belonged to the genera *Pseudonocardia* and *Streptomyces*. These isolates were able to produce siderophores, indole-3-acetic acid (IAA) and solubilized phosphate *in vitro* at varying level. *Streptomyces* sp. isolate S3 produced the highest IAA and high activity of phosphate solubilization and siderophore production. DDH result identified it as *S. thermocarboxydus*. The results provide evidence that actinobacteria were associated with arbuscular mycorrhizal spores of *F. mosseae*. The inoculation of mung beans (*Vigna radiata*) with this strain resulted in a significant increase in fresh weight, root length and total length as an effect of IAA production. In an experiment with rice (*Oryza sativa*), *S. thermocarboxydus* isolate S3 promoted the growth of rice plants grown in low nutritional soil under induced drought stress. The ability of this organism to promote plant growth confirms the potential of using actinobacteria for agricultural purposes.

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Isolation, Characterization and Identification of Antiviral Compound from *Streptomyces* Species Against Fish White Spot Syndrome Virus (WSSV)

KRISHNAN KANNABIRAN

Department of Biomedical Sciences, School of Biosciences and Technology,
Vellore Institute of Technology (Deemed to be University), Vellore -632014 Tamil Nadu, India.
Email: kkb@vit.ac.in

The aim of the study was to evaluate the antagonistic activity of actinomycetes against fish white spot syndrome virus (WSSV) of shrimp. Marine soil samples collected from different locations of South India yielded 42 actinomycetes, among the isolates VITMK2 exhibited strong antiviral activity against WSSV. The isolate was identified by morphological, biochemical and molecular taxonomic characterisation and identified to be belonged to the genus *Streptomyces* and designated as *Streptomyces* sp. VITMK2. The antiviral activity of ethyl acetate (EA) extracts of VITMK2 against WSSV was studied in *Litopenaeus vannamei* at a concentration of 500 µg per shrimp. *Streptomyces* sp. VITMK2 exhibited better inactivation of WSSV resulting in 92.2% mean survival of shrimp. Purification of EA extract of *Streptomyces* sp. VITMK2 by silica gel column chromatography yielded two compounds, C1 and C2. The C1 compound (500 µg, 250 µg, and 125 µg) treated shrimp infected with WSSV showed survival rates of 88.89%, 83.33% and 55.56% respectively when compared to C2 compound. The chemical nature of the C1 compound was identified by FT-IR, HRMS, ¹H and ¹³C NMR analysis. Based on the spectroscopic data, the C1 compound was identified as 9(10H)-Acridanone (lead compound) with a molecular formula of C₁₃H₉NO and a molecular mass of 195.1048 Da. Docking of the lead compound with WSSV target proteins VP26 and VP28 showed the least binding energy of -5.71 Kcal/mol and -5.21 Kcal/mol respectively predicting the strong interaction of the compound with WSSV target proteins. Molecular dynamic simulation studies further confirmed the stability of the interaction of the lead compound with the WSSV target proteins.

Anti-*Staphylococcus aureus* and Anti-Oxidant Activities of The Metabolites Produced by Endophytic Actinobacteria *Streptomyces Californicus* Strain Adr1

RADHA SINGH, ASHOK K. DUBEY*

Department of Biological Sciences and Engineering, Netaji Subhas University of Technology, New Delhi 110078

*Email: akdubey@nsut.ac.in

S. aureus is one of the major causes of hospital and community-acquired infections, resulting in serious health consequences. It can affect the bloodstream, skin and soft tissues, and lower respiratory tract and can cause infections related to medical instrumentation, such as central-line associated bloodstream infection (CLABSI) etc. Thus, there is an urgent need to develop new antibiotics or antibiotic formulations to effectively fight such infections.

In this context, various endophytic actinobacteria from different local plants around NSUT, New Delhi were isolated and screened for their antimicrobial properties. One of the isolates from the plant *Datura* sp., identified as *Streptomyces californicus* strain ADR1, was chosen for further studies based on its strong potential to produce bio-active metabolites against a panel of Gram-positive bacteria including *S. aureus* and MRSA strains. The secondary metabolites produced by the strain ADR1 were recovered and characterized for their anti-bacterial properties and other therapeutic potential such as anti-oxidant activity. The ethyl acetate extract prepared from the cell-free broth of ADR1 culture, was evaluated for its bactericidal activities on various Gram-positive pathogens in terms of the MIC₉₀ values. The anti-oxidant activity was also determined in terms of IC₅₀ values by using different assays. The probable compounds in the ADR1 metabolite mixture were putatively assigned by employing GCMS analysis. Bioactivity guided purification of the anti-infective compounds had been undertaken by using column chromatography. The isolated compounds were characterized for their anti-*S. aureus* activities. Further, they have been analyzed by HPLC, LCMS, NMR and FTIR for an insight into their structure.

Keywords: Endophyte, actinobacteria, anti-bacterial metabolites, anti-oxidant, *Staphylococcus aureus*

Multifaceted Actinobacteria: A Polyphasic Approach

SYED G DASTAGER

Senior Scientist

National Collection of Industrial Microorganisms, CSIR-National Chemical Laboratory, Pashan Road, Pune-411008, Maharashtra, India.

Email: sg.dastager@ncl.res.in; syedmicro@gmail.com

Microbial diversity encompasses the spectrum of variability among all types of microorganisms (bacteria, fungi, viruses and many more) in the natural world and as altered by human intervention. Current evidence suggests there exists perhaps 300,000 to 1 million species of prokaryotes on earth yet only 8000-9000 bacteria are described in Bergey's Manual. New technologies are being developed that are based on diverse organisms, from diagnostics to biosensors and to biocatalysts. Much more needs to be done, however, on how to understand better the microorganisms, inventory their diversity, maintain reference cultures of them, and find ways to exploit them beneficially. The untapped diversity of microorganisms is a resource for new genes and organisms of value to biotechnology. The importance and myriad applications of microorganisms are well known and human beings have been making microorganisms work for them for a very long time, even before knowing the basic facts about microorganisms. The microorganisms are providing a vast array of products for the welfare of the human kind and the success of microbial biotechnology relies on the diversity of microorganisms. Microbial systematics is the science of identification, classification and naming of living organisms. Taxonomic work involves study of morphological characteristics and phylogenetic relationship of organisms, which is essential for applied biological sciences, such as medicine, agriculture, forestry and fisheries. Development of biotechnologies and their industrial applications depend heavily on taxonomy and their application in biotechnology process, depending on the place within the phylogenetic framework, a taxonomically unassigned strains needs to be characterized by a wide range of approaches to obtain a broad range of informative data from the genetic and epigenetic level, including morphology, physiology, chemistry, DNA patterns, gene sequences and whole genome hybridization. We have contributed new knowledge by isolating and identifying a large number of bacterial species and reported first time from Indian soil. Many new species and their potential application have been reported. These microorganisms have diverse feature, which could make effective tools for biotechnological applications by using polyphasic taxonomical approach we have described more than 65 novel species of actinobacteria/actinomycetes from different ecological niches of the country.

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Diversity and Bioprospective Potentials of Actinobacteria from Harsh Habitat of Limestone Quarries

DAYANAND AGSAR*, SYED G. DASTAGER, SYED R. QUADRI, MADHUSUDAN D.N., BI BI ZAINAB, SHIVAVEER KUMAR, RAGHAVENDRA NAIK, SEELAM SIVA DEEPTHI, UMESH M.K., SUDARSHAN A, SHILANJALI B, RESHMA S

A-DBT Research laboratory, Department of Microbiology,
Gulbarga University, Kalaburgi-585106
Email: dayanandagsar@gmail.com

Habitat and ecosystem are two interlinked important microenvironments for microorganisms according to microbial ecology, because they dwell and interact with other biotic and abiotic factors. Deccan trap (Deccan: Dakshin; Trap: a sedimentary basaltic rock), is a large igneous province in India which harbours many trap rocks including limestone quarries or lime powder deposits. These limestone quarries are primarily comprised of calcite, siderite, pyrite and other minerals, which probably constitutes a harsh habitat for the existence of microorganisms. Among various groups of microorganisms, actinobacteria are unique and owing to their high metabolic diversity, genome complexity and greater adaptability, they are expected to thrive in by their distinct survival strategies.

The research was intended to unveil the diversity of actinobacteria from lime stone quarries/ powder deposits and explore their potential bioactive molecules. The basic-, molecular- and chemo- systematic studies have led to the discovery of one novel genus *Actinorectispora indica*, and six novel species of actinobacteria namely, *Allostreptomyces indica*, *Nonomurea indica*, *Saccharomonospora saliphila*, *Streptomyces deccanensis*, *Streptomyces tritolerans* and *Streptomyces gulbargensis*. A few novel isolates of actinobacteria were screened for tyrosinase and melanin. Enhanced production of tyrosinase (369.41IU) and melanin (5.29g/L) were achieved employing Response Surface Methodology with Central composite Design. A biosensor for detection of phenolic constituents in industrial effluents, was developed by conjugation of tyrosinase with gold nanoparticles. Droplets of soluble melanin on the slant culture of *Streptomyces sp.* was a phenomenal observation. Actinobacterial melanin was explored as sunscreen and surface protective agent. Its application as textile/hair dye is under progress.

Clot Busters from Actinobacteria

C.SUBATHRA DEVI.

Department of Biotechnology, School of Biosciences and Technology,
Vellore Institute of Technology, Vellore- 632 014, Tamil Nadu, India

Natural products have been successfully used for the treatment of various diseases through many centuries. Clot-busting enzyme provides a new hope for cancer, stroke and heart patients. Now-a-days, the thromolytics at minimal expenditure and easy to obtain is from marine sources. Marine actinomycetes are one of the most efficient groups of bioactive metabolite producers. Screening for new bioactive metabolites from marine actinobacteria is gaining interest in recent years. Actinokinase (AK) remains the most well-known thrombolytic operators. Serine protease from *Streptomyces* sp. assimilated a lead toward thrombolytic treatments. The marine environment is attractive as it holds promise as a source of entirely a new enzymatic therapy. Marine is the unique environment which is recognized widely for the most valuable resources with immense diversity of bioactive metabolites from actinomycetes and is attractive as it holds promise as a source of entirely novel compounds. The South Eastern coast of India is extensively known and recognized as a diverse source of potential actinomycetes for bioactive secondary metabolites. Although exclusive number of bioactive compounds has been derived, there is an urge for new bioactive metabolites to treat cardiovascular diseases. The focus of our current research is to explore the fibrinolytic potential of marine actinomycetes. In our study, actinobacteria was isolated from marine sediments & marine sponges. All the actinobacterial strains were screened for fibrinolytic activity. Potent strains were selected for mass production of fibrinolytic protease. An isolate screened from marine sediments of South East coast of Chennai was identified as *Streptomyces violaceus* VITGYM. The blood clot lysis activity of the actinoprotease was compared with the standard streptokinase. It showed 97.43 % of clot lysis activity. In another study, a potent strain, *Streptomyces radiopungnans* VIT SD8 was isolated from marine brown tube sponge, *Aeglas conifer*. Based on the results it was found that the enzyme could lyse both natural clots as well as synthetic clots of fibrinogen, plasmin, and thrombin. The potential contribution of marine actinomycetes to the discovery of new thrombolytic agent is increasingly challenging. Natural thrombolytic drugs are safer and less costly. The existing thrombolytic agents that include tissue type plasminogen activator, urokinase and streptokinase, nattokinase and lumbrokinase are popular in the treatment of intravascular thrombosis. They induce haemorrhagic adverse effects, have short half-life in the body, and expensive. Therefore, it is necessary to search for novel thrombolytic agents and there is still scope to search new agents which overcome these drawbacks. The current research will ensure justice to the thrombolytic therapy from marine natural products, from *marine actinomycetes* which can deleterious effects and gain optimal therapeutic benefit with minimal risk.

Unexplored Cyclic Dipeptide from Marine Actinomycetes Targeting Cell Cycle in Triple Negative Breast Cancer Cells

KGK DEEPAK AND RAMA RAO MALLA*

Cancer Biology Lab, Dept of Biochemistry and Bioinformatics,
GIS, GITAM (Deemed to be University), Visakhapatnam – 530045
Email: dr.rrmalla@gmail.com

Introduction: Triple-negative breast cancers (TNBC), characterized by high aggressive nature and poor prognosis due to lack of specific targets and targeted therapeutics. Recently, our group and others proved CD151, a member of tetraspanin as potential target of TNBC. Cyclo (L-Leucyl-L-Prolyl) is a natural cyclic dipeptide reported in marine actinobacteria isolated from Kakinada coast, Bay of Bengal, Andhra Pradesh. This study is aimed to target metastasis in TNBC cells.

Methods: The effect of CLP on MDA-MB 231 and MDA-MB 468 cell death evaluated by MTT, BrdU, TUNEL, γ H2AX, Dead green. The efficacy of CLP on cell cycle was evaluated by flow cytometry and possible mechanism was studied by Western blotting.

Results: In study of mechanism of action, CLP reduced the viability of TNBC cells at $IC_{50} < 73.4 \mu M$, while human healthy breast epithelial cell line, MCF-12A at $IC_{50} > 100 \mu M$. At $73.4 \mu M$, CLP inhibited 70% proliferation of TNBC cells. It induced DNA strand breaks, G₂M cell cycle arrest, DNA damage, cell death. It also inhibited the expression of cell cycle regulatory proteins cyclin D, CDK4, PAK, RAC1, and P27kiP1.

Conclusion: This study concludes that CLP suppresses the cell cycle of TNBC cells by inducing DNA damage and reducing the expression of cyclin D and CDK4. Thus, CLP can be explored as novel therapeutic for targeted therapy of TNBC.

Keywords: *Actinomycetes, cell cycle, proliferation, TNBC and viability*

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**ORAL
PRESENTATION**

1. Isolation, Characterization and Antimicrobial Activity of Actinobacteria from Ant Hill Soil

B. K. NAYAK, UMADEVI D. and A. NANDA*

Department of Botany, K.M. Centre for Post Graduate Studies (Autonomous),
Airport Road, Lawspet, Puducherry-605008, India

*Department of Biomedical Engineering, Sathyabama Deemed University, Chennai-600119

*Corresponding author email: bijuknaya@gmail.com

During the day today life, multi-drug resistant pathogens are remaining as the major problem and became challengeable for every microbiologist in order to find novel antibiotics to prevent and put a full stop from their reign. Henceforth, our current research work is aimed to find out better compounds from the actinobacteria from a new source i.e., ant hill. During the period of research work, a total of 43 different isolates of Actinobacteria were isolated from the ant hill samples collected from our college campus. Among the 43 isolates, 25 isolates produced pigments on the PDA media. All the 25 isolates were subjected for antibacterial and anticandidal activity. The pathogenic bacterial and *Candida* test organisms were obtained from MTCC, Chandigarh, India. The treated MTCC cultures were viz., *Vibrio cholerae* (MTCC-3906), *S. Epidermis* (MTCC-435), *Bacillus substilis*(MTCC-1755), *Pseudomonas aeruginosa* (MTCC-424), *Candida albicans* (MTCC-439). Among the actinobacterial isolates; A4,A5,A6,A8,A10,A11,A13,A14 showed very good antimicrobial properties. These isolates were selected for future investigation on their Morphological, Biochemical, Molecular and chemical characterization.

Keywords: Antimicrobial activity, actinobacteria, multi-drug resistant pathogens, PDA, MTCC, ant hill soil

2. Multifunctional *Streptomyces maritimus* Sacc-152 Isolated from Andaman and Nicobar Islands, India

JERRINE JOSEPH^{1*}, M. RADHAKRISHNAN, K. MANIGUNDAN M¹, A. SIVARAJAN², R. BALAGURUNATHAN²,

¹Actinobacteriology Laboratory, Centre for Drug Discovery and Development, Col. Dr. Jeppiaar Research Park, Sathyabama Institute of Science and Technology, Chennai – 600 119. Tamil Nadu. INDIA

²Actinobacterial Research Lab, Department of Microbiology, Periyar University, Salem – 636 011. Tamil Nadu. INDIA

*Corresponding author: jerrine.jj@gmail.com

Streptomyces is the most versatile and metabolically talented genus under the phylum actinobacteria. A member of this phylum from under studied sources remains the largest producer of new multifunctional secondary metabolites. A mangrove derived actinobacterial strain SACC-152, from Andaman and Nicobar Islands, was investigated for their multiple bioactive and biosynthetic properties. Morphology, cultural, physiological and molecular studies showed that the strain SACC-152 belongs to the genus *Streptomyces maritimus*. Bioactive metabolites from the *Streptomyces maritimus* SACC-152 was produced by submerged fermentation and extracted using ethyl acetate. Further, the ethyl acetate extract was tested for their antimicrobial, anti-tubercular, anticancer, antioxidant and larvicidal activities. The ethyl acetate extract of *Streptomyces maritimus* SACC-152 exhibited a broad-spectrum antimicrobial activity against gram-positive bacteria *Staphylococcus aureus*, *Mycobacterium smegmatis* and *Bacillus subtilis*; gram-negative bacteria, *Escherichia coli*, *Salmonella paratyphi*, *Aeromonas hydrophila*, *Klebsiella pneumonia* and *Providenciavermicola* and fungal pathogens *Candida albicans* and *Cryptococcus neoformans*. In Luciferase Reporter Phage (LRP) assay, strain SACC-152 showed more than 80% inhibition against standard laboratory strain *Mycobacterium tuberculosis* H37Rv, drug sensitive and MDR *M. tuberculosis* strains. In MTT assay, the extract showed promising activity against HT-29 colon cancer with the IC₅₀ value of 9.68 µg/ml. In the antioxidant assay, the extract showed maximum of 61.35±2.46% free radical scavenging activity at 500 µg/ml concentrations. The extract of SACC-152 caused highest mortality against the mosquito larvae of *Aedes aegypti* (LC₅₀ 19.6 µg/mL, r₂ = 0.61). Further, the genomic DNA of strain SACC-152 was found to harbor secondary metabolite biosynthetic genes such as polyketide synthases (PKS type I), the adenylation domains of non-ribosomal peptide synthase (NRPS) and halogenase (Halo), which depict its diverse secondary metabolic potential.

3. Isolation, Screening and Characterization of Actinomycetes from Mangrove Soil, Puducherry

JANAKI.T, GANESAN T., *B. K. NAYAK, ANIMA NANDA

Department of Botany, K.M. Centre for Post Graduate Studies (Autonomous),
Airport Road, Lawspet, Puducherry-605008, India

*Department of Biomedical Engineering, Sathyabama Deemed University, Chennai-600119

*Corresponding author email: bijuknayak@gmail.com

Aim:

To find out the novel compounds from mangrove actinomycetes for drug resistance MDRs

Materials methods:

There were 167 mangrove actinomycetes isolated from four soil samples from the rhizosphere region of *Avicennia marina* in the back water of Ariyankuppam, Puducherry. Soil samples were analysed for physiochemical properties. All the isolates were subjected for primary and secondary antimicrobial activity against ten bacterial and five fungal pathogens. Among the isolates tested against antimicrobial activity, the isolates M20 showed broad spectra towards the tested pathogens. Then, the isolate M20 was subjected for morphological, biochemical, molecular and chemical characterization.

Results:

The M20 isolate was identified as *Streptomyces cacaoi* sub sp. *cacaoi* and showed positivity towards many enzymes. Active antimicrobial, anti-larvicidal and anti-candidal properties were identified. GCMS results showed that the *Streptomyces cacaoi* sub sp. *cacaoi* had antimicrobial properties.

Conclusion:

From our present study, it was found that the mangrove actinomycetes have the ability to produce novel compound so that, these isolate, particularly, *Streptomyces cacaoi* sub sp. *cacaoi* could be used in the field of medicine, agriculture and industry.

Key words: Mangrove actinomycetes, characterization, antimicrobial activity.

4. Bioprospecting of Insect Nest Associated Actinobacteria With Special Reference to Antimicrobial and Anti-Hiv Activity

GOPIKRISHNAN. V*, POOJA.B, YASASWINI. KG, MANIGUNDAN.K, VIGNESH. A,
RADHAKRISHNAN M, AND JERRINE JOSEPH

Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology (Deemed to be University),
Jeppiaar Nagar, Chennai 600 119, Tamil Nadu, India.

*Corresponding Mail: gopivtk@gmail.com

It is becoming increasingly apparent that the vast majority of multicellular animals as well as many plants and fungi engage in mutualistic interactions with microorganisms that are often essential for successful growth and reproduction of the host. Because of their abundance in terrestrial and marine habitats and their metabolic versatility, bacteria are the most common symbiotic partners of eukaryotes. Insects have occupied almost every environmental niche while in the meantime, symbiotic and/or pathogenic microorganisms have adapted specifically to insects as host systems. As an immediate response, insects were colonized by symbiotic microorganisms that are often required by the insect host to provide necessary nutritional and immunological effectors (obligate symbiont). The micro biota may account for 1–10% of the insect biomass, implying that the insect, as well as any other higher organism, can be regarded as a multi-organismal entity. Actinobacteria, filamentous bacteria found in numerous ecosystems around the globe, produce a wide range of clinically useful natural products (NP). In natural environments, actinobacteria live in dynamic communities where environmental cues and ecological interactions likely influence NP biosynthesis. The present study attempted to explore actinobacteria from different insect nest samples for antimicrobial activity. Totally 43 actinobacterial colonies were recovered from ant nest, termite nest, wasp nest and blanket worm nest samples by adopting standard spread plate method. Antimicrobial activity of actinobacterial strains was determined by agar plug method. Two actinobacterial strains AN1 and AN5 showed promising activity (14-18 mm inhibition) against *S. aureus*, *E. coli*, *P. aeruginosa* and carbapenem resistant *K. pneumoniae* and *Mycobacterium smegmatis*. Both the strains produced antimicrobial compound earlier on ISP agar when compared to ISP2 broth. Crude antimicrobial from the strains AN1 and AN5 was produced by adopting agar surface fermentation and extracted using ethyl acetate. Three well separated spots observed on AN1 and AN5 ethyl acetate extract using thin layer chromatography. Based on the studied phenotypic characteristics, actinobacterial strains AN1 and AN5 isolated from ant nest was identified as *Streptomyces sp.* In addition to antimicrobial activity, extracts also showed Anti-HIV activity. Findings of the present study concluded that insect nest is the promising source for bioactive actinobacteria. Two potential *Streptomyces sp.* AN1 and AN5 isolated from ant nest will be a promising source for antimicrobial metabolites against drug resistant bacteria, retrovirus and mycobacterial pathogens.

5. Bioprospecting of Actinobacteria from Indian Rare Ecosystems with Special Reference to Anti-Tuberculosis Activity

RADHAKRISHNAN, M^{1*}, R. BALAGURUNATHAN², K. SIVAKUMAR³ AND VANAJA KUMAR⁴

¹Actinobacteriology Laboratory, Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai – 600 119. Tamil Nadu. INDIA

²Actinobacterial Research Lab, Department of Microbiology, Periyar University, Salem – 636 011. Tamil Nadu. INDIA

³CAS in Marine Biology, Annamalai University, Parangipettai – 608 502. Tamil Nadu. INDIA

⁴Department of Bacteriology, National Institute for Research in Tuberculosis (ICMR), Chennai – 600 031. Tamil Nadu. INDIA

*Corresponding author: radhakrishnan.cddd@sathyabama.ac.in ; mrkactinos@yahoo.com

Actinobacteria are one among the largest bacterial phylum comprised of group of Gram positive bacteria with tremendous ecological and industrial significance. Bioprospecting of members of this group yielded numerous novel bioactive metabolites including anti-TB antibiotics over the past 8 decades. However, the discovery of new bioactive natural products from this promising bacterial group has declined over the years due to the redundant investigations on routine terrestrial habitats. Rare ecosystems like desert, forest, mountain, caves and marine ecosystems are the potential store houses for novel actinobacteria which produce unique bioactive metabolites. For the past 17 years we are investigating rare/ understudied terrestrial and marine ecosystems in India with special reference to anti-tuberculosis activity. Extracts from actinobacterial strains were produced by agar surface fermentation and extracted using methanol. Anti TB activity of actinobacterial extracts was tested by adopting rapid Luciferase Reporter Phage (LRP) assay against standard strain *M. tuberculosis* H37Rv as well as against clinical strains of drug sensitive and MDR *M. tuberculosis*. Present work focused on bioprospecting of actinobacteria from rare ecosystems with special reference to the isolation of antituberculous compounds. The results showed that around 70% of actinobacterial strains isolated from under studied terrestrial and marine ecosystems were showed inhibition against one or more number of *M. tuberculosis* strains tested with or without showing activity against routine bacterial pathogens. We have isolated two chromopeptide compounds from *Streptomyces* sp D25 and *Streptomyces* sp. BCA1 isolated from Thar Desert Rajasthan and Borra caves, Andhra Pradesh, respectively. Both the compounds showed promising activity against standard and drug sensitive and MDR *M. tuberculosis* strains. In addition, we are also in the process of isolating anti TB compounds from actinobacteria isolated from mangrove sediments and sponges collected from Andaman and Nichobar Islands. Findings of our research revealed that Indian rare ecosystems are the richest source for bioactive actinobacteria which deserves the potential for the isolation of novel anti TB compounds to fight against drug resistant tuberculosis.

6. Phylogenetic Characterization of Potential Bioactive Metabolite Producing Actinomycetes from Mangrove Sediments

KRISHNA NARAGANI¹ MANI DEEPA INDUPALLI AND VIJAYALAKSHMI MUVVA³

¹Department of Botany, PB Siddhartha College of Arts & Science, Vijayawada-10, Andhra Pradesh, India

²Department of Microbiology, Maris Stella College, Vijayawada-520008 Andhra Pradesh, India

³Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur -522510, Andhra Pradesh, India

The main objective of the present study was to isolate and identify the actinomycetes which produce potential antibacterial and antifungal metabolites and to analyze the phylogenetic relationship. The soil samples were collected from mangrove sediments of South coast of Andhra Pradesh. The potent secondary metabolite producing strains were isolated and designated as VLK1-VLK-25. The antimicrobial activity of the strains was evaluated by using agar well diffusion assay. The crude ethyl acetate extracts of the strains were tested against Gram positive and Gram negative bacteria as well as fungi. Among the strains, one of the strains designated as VLK-24 possessed good activity against fungi and bacteria tested. Identification of the strain was carried out by employing the morphological, cultural, physiological, biochemical and molecular characteristics. Phylogenetic analysis of 16S rRNA gene sequence revealed the identity of the strain VLK-24 as *Streptomyces rubrus* VLK-24. Ninety six hours of incubation was found to be the optimum for bioactive metabolite production by the strain. Among the ten media tested, ISP-2 media was found to support the high production of bioactive compounds. The optimal pH and temperature for secondary metabolite production were recorded at 7.0 and 30°C respectively. Studies on nutritional factors revealed that highest antimicrobial metabolite production was obtained when glucose (0.5%) and peptone (0.5%) were used as carbon and nitrogen sources respectively.

Key words: *Streptomyces rubrus*, Mangrove ecosystem, Bioactive compounds

7. Fermentative Production of Extracellular Pigment from *Streptomyces Coelicolor* MSIS1

V. MOHANASRINIVASAN

Department of Biomedical sciences, School of Biosciences and Technology,
Vellore Institute of Technology, Vellore- 632 014, Tamil Nadu, India

In the present research work, the pigment producing actinomycetes was isolated from a rhizosphere soil of ornamental plants and identified as *Streptomyces coelicolor* MSIS1 (FR856603). The pigment was produced in shake flask as well as in bioreactor. The results were evident that there was threefold increase in the pigment production in bioreactor compared with shake flask. The extracted pigment was characterized based on TLC, HPLC and FT-IR. The HPLC data showed that the compound may be one of actinorhodinic acid but on the other side FT-IR data infers that there were no presence of aromatic ring but prominent aliphatic stretch has been found.

8. Structural Elucidation of The Bioactive Compound Produced by *Streptomyces cellulosa* Isolated from Mangrove Habitats of Andhra Pradesh, India

MANIDEEPA INDUPALLI ¹ KRISHNA NARAGANI ² AND VIJAYALAKSHMI MUVVA *

¹ Department of Microbiology, Maries Stella College, Vijayawada, A.P., India

² Department of Botany, P.B. Siddhartha Arts & Science College, Vijayawada, A.P., India

* Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, A.P., India.

The microorganisms especially actinomycetes are virtually unlimited sources of novel compounds due to their diversity and ability to produce bioactive metabolites of high therapeutic value. Attempts were made to isolate the potent strain, to optimize the cultural parameters for enhanced production of bioactive metabolites as well as characterization of active biomolecule. An actinobacterial strain designated as VJDS-1 possessing high antimicrobial activity was isolated from mangrove habitats of Nizampatnam, Andhra Pradesh (India) and identified as *Streptomyces cellulosa* VJDS-1. Modified yeast extract-malt extract-dextrose broth supported the production of bioactive metabolites by the strain as compared to other media tested. Glucose (1%) and tryptone (0.5%) were found to be the suitable carbon and nitrogen sources respectively for the optimum production of bioactive metabolite. Maximum production of metabolite was detected when the strain was grown in culture medium with an initial pH 7.0 incubated for five days at 30°C under shaking conditions. Secondary metabolites obtained from the strain lead to the isolation of a compound active against Gram positive and Gram negative bacteria as well as fungi. The structure of the active fraction was elucidated as Catachin using FT-IR, electrospray ionization, mass spectrometry, ¹H and ¹³C NMR.

9. Gut bacterial diversity in worker adults of *Apis florea* and *Apis cerena indica* assessed by MALDI-TOF, 16S rRNA and NGS

GANESH PRASAD D N¹, YOGESH S SHOUCHE² AND A.H. SNEHARANI ¹

Jnana Kaveri P.G. Centre, Mangalore University, Chikka Aluvara, Somwarpet Tq, Kodagu Dist - 571232
National Centre for Microbial Resource and National Centre for Cell Science, Pune

Gut microbiota research is an explicating field that improves our understanding of the biological and beneficial elements of the gut. Honey bees are eusocial organisms has a very critical role in stabilizing natural and agricultural ecosystems. Honey bees are super-organisms due to their complex social systems, dynamic and tight-knit interactions with one another and their environment.

The present study was performed to identify and characterize the bacteria harboring in the gut of honey bees - *Apis florea* and *Apis cerena indica*, indigenous to south Indian sub-continent. The gut microbiome of *Apis florea* (dwarf bee) was identified and characterized by by culture-based and culture-independent methods. The alimentary canal from worker honey bees of *A. florea* were used to analyze the microflora. Bacterial colonies were cultivated using different media. For culture based identification and characterization, matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) and 16S rRNA sequencing and for culture-independent identification next generation sequencing (NGS) technique, were used respectively.

Bacterial species belonging to 15 different phyla were identified by both culture based and culture independent methods. Bacteria from phyla Proteobacteria and Firmicutes were identified by culture based methods and from culture independent method, in addition to above phylum, Bacteroidetes were identified along with bacterial species belonging to 12 other phyla. From NGS, the prevalence of majority of bacteria from different phyla are as follows; Bacteroidetes (52 %), Proteobacteria (45%), < 1 % each from Euryarchaeota, Actinobacteria and Firmicutes and distribution of major bacterial genera were Prevotella (59.3%) and Escherichia-Shigella (33.3%).

The bacterial communities of *Apis cerena indica* was analyzed by culture-independent method. 16S rRNA gene amplicon sequencing yielded 14 bacterial phyla in the gut of *A. cerena indica*. Bacterial phyla distributions were as follows; Proteobacteria (81.5%), Bacteroidetes (16.4%), Firmicutes (2%) and the rest (0.01%) constituted the minor phyla Euryarchaeota, Actinobacteria, Acidobacteria, Chloroflexi, Verrucomicrobia, Cyanobacteria, Planctomycetes, Gemmatimonadetes, Thaumarchaeota, FBP and Armatimonadetes. Bacterial phylum was found to constitute Proteobacteria and Bacteroidetes as major phyla. Distribution of bacterial genera in the gut of *A. cerena indica* were Escherichia-Shigella (52.8%), Prevotella (21%), Orbus (15.1%), Steroidobacter (5.4%), Methylobacterium (2.7%), Lactobacillus (2.35%), Klebsiella (0.2%) and Lactococcus (0.2%).

Keywords: Honey bee; *Apis florea*; *Apis cerena indica*; gut microbiota; bacteria, MALDI-TOF MS, 16S rRNA, next generation sequencing

10. A Calcium Independent and Thermo-stable α -Amylase from *Streptomyces griseus* TBG19NRA1 isolated from forest soil of Western Ghats, Kerala, India

SHIBURAJ SUGATHAN*

Division of Microbiology, Jawaharlal Nehru Tropical Botanic Garden and Research Institute,
Palode, Trivandrum, Pin-695562, Kerala, India
Mob: +91 9495826669, E-mail: drshiburaj@gmail.com

A mesophilic actinomycete, *Streptomyces griseus* TBG19NRA1 (MTCC 3756) previously named as *S. setonii* (Synonym) was isolated from soils collected from Neyyar WLS, Western Ghats of Kerala. It showed potential amylase activity and different media were tried for the enzyme production under submerged condition. The M3 medium resulted high activity hence chosen for further optimization studies. The maximum α -amylase production was achieved at the end of 48 h of incubation at pH 7. Sucrose, (1%), starch (2%) as carbon source, Calcium nitrate (0.5%) as nitrogen source, Valine (0.3%), CaCl_2 (0.025%), Triton X-100 (0.05%) and Mannitol (0.5%) enhanced (61.5%) the amylase activity. All the experiments were done in triplicates and results were statistically analysed by one way ANOVA ($p < 0.05$).

On purification studies, the supernatant was subjected to centrifugation, NH_4SO_4 precipitation, dialysis and further purified by gel filtration using sephadex G-100. The purified enzyme showed a molecular weight of 60 kDa and confirmed by zymogram analysis. The purified protein was subjected for LC-MS/MS analysis and the protein was identified against the Uniprot database using the MASCOT search engine which showed similarity to α -amylase of *Streptomyces albulus*. Further the Amy gene also done. The purified amylase exhibited maximal activity at 70 - 80°C and showed decrease in activity above 90°C. The thermo stability of purified enzyme was compared with commercial (Sigma-Aldrich) α -amylase from *Bacillus licheniformis*. The purified enzyme incubated at 80°C had retained (100 % activity) and was stable up to 1h of incubation without the addition of CaCl_2 . Among the different metal ions, Fe^{3+} (122%), Mg^{2+} and NH_4^+ ions resulted enhancement in enzyme activity. . As calculated from Lineweaver-Burk plots, apparent K_m and V_{max} values were 1.6 mg/ml and 28 mg/ml/min at pH 7 which confirmed the efficiency of this enzyme for diverse applications. The thermostability and Ca-independency of α -amylase activity from the *S. griseus* TBG19NRA1 could be observed as an appropriate alternative for industrial application, especially in starch hydrolysis industry

11. Bioactive Macrolide from the Actinomycete *Dactylosporangium aurantiacum*

PRAMOD B. SHINDE*, YEDUKONDALU NALLI, PANKAJ KUMAR, VISHAL GHADGE, SANJU SINGH

Natural Products & Green Chemistry Division (NPGC), CSIR-Central Salt & Marine Chemicals Research Institute (CSIR-CSMCRI), Bhavnagar, Gujarat

*pramodshinde@csmcri.res.in

Actinomycetes are considered to be prolific producers of bioactive secondary metabolites, specially macrolides. The actinomycetes strain *Dactylosporangium aurantiacum* is reported to produce important antibiotics such as SF-2185 and tiacumicin complex. The tiacumicin B3 is also known as fidaxomicin and is clinically used to cure infection caused by the pathogen *Clostridium difficile*. More than 20 fidaxomicin analogues have been generated through genetic modification of fidaxomicin biosynthetic pathway. With the aim to identify other macrolides apart from fidaxomicin congeners, the present research was undertaken. The strain *Dactylosporangium aurantiacum* was obtained from ATCC, revived, and its culture was optimized for bioactivity against a panel of microbial pathogens, both human and plant pathogens. It was cultured on large scale in 1 L flasks and the extract was prepared with ethyl acetate under vacuum. The ethyl acetate extract was fractionated on reversed phase silica column using Combiflash MPLC to obtain 30 fractions. Fraction 27 containing major metabolite was further purified using reversed-phase HPLC with PDA detector. The 1D- & 2D-NMR data of obtained pure compound was acquired and studied carefully to identify its structure. The obtained structure is confirmed with HR-MS and HR-MS/MS.

12. Isolation and Characterization of Methanol Utilizing Streptomyces Species Isolated from Jack Fruit (*Atrocarpus heterophyllus*) Rhizosphere

ARUNA SHARMILI. S* AND S. ANU SHEEPA. A

Department of Biotechnology, Stella Maris College (Autonomous), Chennai-600086

*Corresponding Author: arsharmilis@gmail.com

Two isolates AAG and AAP isolated from the rhizosphere of Jack fruit (*Atrocarpus heterophyllus*) isolated on Ammonia mineral salts media (AMS) supplemented with 0.5% methanol were found to be Gram positive cocci with a smooth surface morphology. The isolates were able to grow on Actinomycetes isolation agar, nutrient agar, Kuster's agar, Czapek dox agar, ISP-1 to ISP-7 agar and Ammonia mineral salts media (AMS). Isolates AAG and AAP showed rapid and good growth on AMS agar medium supplemented with 0.5% methanol. Growth of AAG and AAP was optimum at pH 7 and could tolerate NaCl concentration up to 7%. Both the isolates AAG and AAP was able to utilize formaldehyde, methylamine, maltose, fructose, xylose and lactose as sole carbon sources and ammonium containing compounds such as ammonium nitrate and ammonium chloride as sole nitrogen sources. The isolates were able to hydrolyse casein, starch, lipid and also showed positive result for nitrogen fixation and produced enzymes, cellulose and protease. Isolates AAG and AAP was able to produce Indole acetic acid (IAA) in presence of 0.1% tryptophan. Maximum IAA production was achieved on day 10 by AAG (2.241µg/ml) and AAP (2.211µg/ml). The crude ethyl acetate extract of both the isolates showed antibacterial activity against *E.coli*, *S.aureus*, *E.faecalis*, *K.pneumoniae*, at the concentration of 7mg/ml. GC-MS analysis of the crude ethyl acetate extract of AAG showed the presence of 7 compounds whereas AAP showed 11 compounds.

Key words: Streptomyces Spp., Ammonia mineral salts media, methanol utilization, IAA, Antibacterial activity, GC-MS

13. Polyketides from Actinomycetes as Potential Anti-Tumor Compounds.

S. JAYANTHI,
VELLORE INSTITUTE OF TECHNOLOGY, VELLORE-632014

Actinomycetes are virtually unlimited sources of novel compounds with many therapeutic applications. The search for and discovery of rare and new actinomycetes is of significant interest to drug discovery due to growing need for development of potent inhibitors. Hence, we aim to propose lead compounds for VEGF anti-cancer inhibition by computational approach. Computational studies such as virtual screening and docking studies were performed. Virtual screening was done for the bioactive compounds from actinomycetes. The structure of VEGF protein is used as target and actinomycetes compounds as the ligand dataset. Ligands were docked into active site of VEGF protein based on structure-based drug design and the top leads with high binding affinity are selected. This computational study reports the potential lead compound from actinomycetes bacteria and provides more insights on the inhibition mechanism of VEGF with lead candidate and further suggesting them as potential hits for drug development.

Keywords: Actinomycetes, VEGF, Virtual screening, Molecular docking.

14. Marine Endophytic Actinomycetes Act as A Effective Anti-Bacterial Agent Against Multi Drug Resistant Uropathogens

RAMACHANDRAN GOVINDAN¹, RAJIVGANDHI GOVINDAN^{1,2}, MANOHARAN NATESAN^{1*},

¹Microbiology and Marine Pharmacology Laboratory, Department of Marine Science, Bharathidasan University, Tiruchirappalli-24, Tamil Nadu, India.

²Key Laboratory of Biocontrol and Plant Resource, Department of Microbiology, School of Life Sciences, Sun Yat-Sen University, Guangzhou-510275, China.

*Email id: biomano21@yahoo.com.

Aim: The aim of the present study is to identify potential secondary metabolites from marine endophytic actinomycetes (MEA) for inhibition of multi drug resistant (MDR) gram negative bacteria (GNB).

Methods: The isolation and molecular identification of endophytic actinomycetes (EA) was detected by using bergey's manual and its anti-microbial potential was screened by various bioassay guided fractionation. The purified fractions of the compound was evaluated against MDR uropathogens by minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). Further, the intracellular damage and morphological alteration of the actinomycete compound treated bacteria was detected by confocal laser scanning electron microscope and scanning electron microscope (SEM). Furthermore, the toxicity effect of identified compound was performed against marine shrimp *Artemia franciscana* (*A. franciscana*).

Result: The molecular identification of isolated EA was confirmed as a *Nocardiopsis Sp.* The purified HPLC fractions of the crude compound exhibited excellent anti-bacterial activity against GNB were observed at increasing concentration. The UV-Spectrometer, FT-IR, analytical HPLC, GC-MS and LC-MS results were confirmed the presence of anti-bacterial compound in purified fractions of HPLC. The MIC and MBC of the identified compound was exhibited excellent anti-bacterial activity at 90 µg/mL concentration. The architectural and morphological damages of the tested bacteria was also identified at same MIC by CLSM and SEM. Finally, the *invivo* toxicity effect of the compound treated *Artemia franciscana* (*A. franciscana*) was showed an increased mortality rate at 90 µg/mL.

Conclusion: The result was concluded that the marine sources are a reservoir of bioactive compound for endophytic actinomycetes, which could be ideal bio-resource for extraction of novel anti-bacterial compounds against drug resistant pathogens.

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**POSTER
PRESENTATION**

1. Bioprospecting of insect nest associated actinobacteria with special reference to antimicrobial and anti-HIV activity

GOPIKRISHNAN. V*, POOJA.B, YASASWINI. KG, MANIGUNDAN.K, VIGNESH. A,
RADHAKRISHNAN M, AND JERRINE JOSEPH

Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology (Deemed to be University),
Jeppiaar Nagar, Chennai 600 119, Tamil Nadu, India.

*Corresponding Mail: gopivtk@gmail.com

It is becoming increasingly apparent that the vast majority of multicellular animals as well as many plants and fungi engage in mutualistic interactions with microorganisms that are often essential for successful growth and reproduction of the host. Because of their abundance in terrestrial and marine habitats and their metabolic versatility, bacteria are the most common symbiotic partners of eukaryotes. Insects have occupied almost every environmental niche while in the meantime, symbiotic and/or pathogenic microorganisms have adapted specifically to insects as host systems. As an immediate response, insects were colonized by symbiotic microorganisms that are often required by the insect host to provide necessary nutritional and immunological effectors (obligate symbiont). The micro biota may account for 1–10% of the insect biomass, implying that the insect, as well as any other higher organism, can be regarded as a multi-organismal entity. Actinobacteria, filamentous bacteria found in numerous ecosystems around the globe, produce a wide range of clinically useful natural products (NP). In natural environments, actinobacteria live in dynamic communities where environmental cues and ecological interactions likely influence NP biosynthesis. The present study attempted to explore actinobacteria from different insect nest samples for antimicrobial activity. Totally 43 actinobacterial colonies were recovered from ant nest, termite nest, wasp nest and blanket worm nest samples by adopting standard spread plate method. Antimicrobial activity of actinobacterial strains was determined by agar plug method. Two actinobacterial strains AN1 and AN5 showed promising activity (14-18 mm inhibition) against *S. aureus*, *E. coli*, *P. aeruginosa* and carbapenem resistant *K. pneumoniae* and *Mycobacterium smegmatis*. Both the strains produced antimicrobial compound earlier on ISP agar when compared to ISP2 broth. Crude antimicrobial from the strains AN1 and AN5 was produced by adopting agar surface fermentation and extracted using ethyl acetate. Three well separated spots observed on AN1 and AN5 ethyl acetate extract using thin layer chromatography. Based on the studied phenotypic characteristics, actinobacterial strains AN1 and AN5 isolated from ant nest was identified as *Streptomyces sp.* In addition to antimicrobial activity, extracts also showed Anti-HIV activity. Findings of the present study concluded that insect nest is the promising source for bioactive actinobacteria. Two potential *Streptomyces sp* AN1 and AN5 isolated from ant nest will be a promising source for antimicrobial metabolites against drug resistant bacteria, retrovirus and mycobacterial pathogens.

2. Synthesis and characterization of silver nanoparticles using *Streptomyces* sp. KBR3 and its biomedical activities

MANIGUNDAN, K¹., JASWANT SAMUEL², M. MASILAMANISELVAM², A. VIGNESH¹, V. GOPIKRISHNAN¹, JERRINE JOSEPH¹, D. SHANMUGASUNDARAM³ AND M. RADHAKRISHNAN¹*

¹Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai – 600 119. Tamil Nadu.

²Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai – 600 119. Tamil Nadu.

³DRDO- Centre for Life Sciences, Bharathiar University campus, Coimbatore, Tamil Nadu

*Corresponding author: radhakrishnan.cddd@sathyabama.ac.in

Biogenic synthesis of metal nanoparticles has been well proved by using bacteria, fungi, algae, actinobacteria, plants, etc. Among the different microorganisms used for the synthesis of metal nanoparticles, actinobacteria are less known. Although, there are reports, which have shown that actinobacteria are efficient candidates for the production of metal nanoparticles both intracellular and extracellular. The nanoparticles synthesized by the members of actinobacteria present good polydispersity and stability and possess significant biocidal activities against various pathogens. The present review focuses on biological synthesis of metal nanoparticles using *Streptomyces* KBR-3 and their application in medicine. The synthesized AgNps showed the characteristic absorption spectra in UV–vis at 420 nm, which confirmed the presence of nanoparticles. HRTEM, SAED and XRD showed that the crystalline monodispersed nonuniformly spherical nanoparticles were within a size range below 36 nm. Fourier transform infrared analysis suggested the role of water-soluble polyols present in the *Streptomyces* sp. extract in mediating the synthesis of AgNps. The synthesized AgNps showed significant antibacterial activity against *E. coli*, *S. aureus*. AgNps showed an enhanced scavenging activity DPPH radical scavenging assay. AgNps exhibited affect the viability of VERO cell line at dose dependent manner. Anticancer activity against H357 oral cancer cell lines showed reduced viability of 77% inhibition. This is the first report on silver nanoparticles synthesis *Streptomyces* sp. KBR-3 showing anti-oral cancer activity.

3. Isolation, screening and characterization of Actinomycetes from mangrove soil, Puducherry

JANAKI.T, GANESAN T., *B. K. NAYAK, ANIMA NANDA

Department of Botany, K.M. Centre for Post Graduate Studies (Autonomous),
Airport Road, Lawspet, Puducherry-605008, India

*Department of Biomedical Engineering, Sathyabama Deemed University, Chennai-600119

*Corresponding author email: bijuknayak@gmail.com

Aim:

To find out the novel compounds from mangrove actinomycetes for drug resistance MDRs

Materials methods:

There were 167 mangrove actinomycetes isolated from four soil samples from the rhizosphere region of *Avicennia marina* in the back water of Ariyankuppam, Puducherry. Soil samples were analysed for physiochemical properties. All the isolates were subjected for primary and secondary antimicrobial activity against ten bacterial and five fungal pathogens. Among the isolates tested against antimicrobial activity, the isolates M20 showed broad spectra towards the tested pathogens. Then, the isolate M20 was subjected for morphological, biochemical, molecular and chemical characterization.

Results:

The M20 isolate was identified as *Streptomyces cacaoi* sub sp. *cacaoi* and showed positivity towards many enzymes. Active antimicrobial, anti-larvicidal and anti-candidal properties were identified. GCMS results showed that the *Streptomyces cacaoi* sub sp. *cacaoi* had antimicrobial properties.

Conclusion:

From our present study, it was found that the mangrove actinomycetes have the ability to produce novel compound so that, these isolate, particularly, *Streptomyces cacaoi* sub sp. *cacaoi* could be used in the field of medicine, agriculture and industry.

Key words: Mangrove actinomycetes, characterization, antimicrobial activity.

4. Isolation and Screening of Rare Actinobacteria From Mangrove Ecosystem of Andhra Pradesh For Bioactive Compounds

CH. CHIRANJEEVI, M. VIJAYALAKSHMI*, G. ROSAIAH

Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur - 522510, Andhra Pradesh, India.

*Email-id: profmvijayalakshmi@gmail.com

Actinomycetes are widely exploited group of microorganisms for the production of secondary metabolites and enzymes of commercial importance in medical and agricultural applications. Mangroves, unique habitats in tropical and subtropical tidal areas, are known to be highly productive ecosystems. There is an unambiguous evidence that the mangrove ecosystem contains a large diversity of actinomycetes, which have the potential of producing bioactive secondary metabolites. The mangrove sediment samples collected at a depth of 6 to 10 cm were pretreated with calcium carbonate. The pretreated soil samples were serially diluted in sterile distilled water and plated on yeast extract malt extract dextrose agar. The colonies showing the characteristics of actinomycetes such as rough, chalky, powdery appearance with radiating growth and leathery texture were selected. 20 strains were isolated and screened for antimicrobial activity. Among them, 5 isolates exhibited antagonistic activity against test bacteria and fungi. Attempts are in progress to identify the strain VLCH-6 which exhibited strong antimicrobial activity using polyphasic taxonomical approach followed by purification and chemical characterization of its bioactive compounds.

5. Production of Microbial Secondary Metabolites from Marine Sources

ZABIN K. BAGEWADI*, JAYATEERTH S. BHAVIKATTI, UDAY M. MUDDAPUR AND DEEPAK A. YARAGUPPI

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

Email: zabinb@gmail.com

Microbes are known to produce secondary metabolites of significant importance in various fields such as, biomedicine, agriculture, bioremediation, biopharmaceuticals etc. The current research engages in the production and applications of secondary metabolites produced from microbial sources isolated from marine soil and water regions and forest areas. Several bacterial isolates were isolated and screened for the production of bacterial cellulose (BC) and antimicrobial metabolites, using the standard Hestrin-Schramm (HS) medium. Among 43 isolates, some bacterial strains produced either BC or bioactive metabolites while some produced both. The highest BC yield was found to be 3.5 g/l dry BC produced by one of the isolates. Many isolates exhibited antimicrobial activity against one or more of selected 8 pathogens (gram -ve and gram +ve pathogens). Two of the isolates were identified as *Bacillus licheniformis* strain ZBT2 (Accession no. MH729361) and *Bacillus sonorensis* strain ZBT9 (Accession no. MH729362) by 16S rRNA gene sequencing. The secondary metabolites produced by these isolates showed significant antimicrobial activity against different pathogens and also exhibited cytotoxic activity against the lung cancer cell lines (A549). Further, the current research aims to enhance the BC yield by statistical methods, followed by functionalisation, characterisation and applications of BC and BC-composites. The study also aims to enhance the production, purification of the bioactive metabolites for applications in biomedicine and plant pest-control, along with structural elucidation of bioactive molecules and molecular docking to understand the actual pathways responsible for their antimicrobial and cytotoxic activities.

Keywords: Bacterial cellulose, Secondary metabolites, Antimicrobial activity, Cytotoxic activity, Molecular Docking

6. Molecular Diversity and Antibiofilm Activity from *Frankia* spp. of Actinorhizal *Casuarina* spp.

M. NARAYANASAMY, D. DHANASEKARAN* and N. THAJUDDIN

Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamil Nadu 620 024, India, Email ID: samymb2013@gmail.com, dhansdd@gmail.com

Bacteria of the genus *Frankia* are mycelium-forming actinomycetes that are found as nitrogen-fixing facultative symbionts of actinorhizal plants. Although soil-dwelling actinomycetes are well-known producers of bioactive compounds, the genus *Frankia* has largely gone uninvestigated for this potential. In the present study, the young and mature root nodules of *Casuarina* spp. plants were collected from different habitats such as hyper-saline, saline, estuarine and terrestrial, in Tamil Nadu, India. The root nodules were surface-sterilized, dried, homogenized, and inoculated on DPM medium with sodium propionate as carbon source and biotin as vitamin source. Colonies appeared in both solid and liquid medium on 7th days afterward incubation which were characterized by unique cultural, morphological characteristics. A total of 22 *Frankia* isolates were obtained in solid and liquid medium 8 from hyper-saline, 6 from saline, 5 from estuarine and 3 from terrestrial habitats. Wherein the largest number of isolates was recorded in the hyper-saline and saline habitats compared to estuarine and terrestrial habitats. Finally 4 isolates were selected, each habitat from unique isolated were molecular characterized and identified as *Frankia* sp. DDNSF-01, DDNSF-03 and *Frankia casuarinae* DDNSF-02, DDNAF-04. Moreover, the biological active compounds of *Frankia* spp. were extracted by ethyl acetate extraction method as well as MIC assay was performed against multi-pathogens of *Staphylococcus* sp., *Pseudomonas* sp., *Candida* sp.. Furthermore, the antibiofilm activity was done against above the pathogens. The hyper-saline habitat of *Frankia* sp. isolate was highly effective against above multi-pathogens when compared to other isolates. The biofilm inhabitation was confirmed by CLSM microscopic analysis. The present findings conclude *Frankia* spp. as efficient in agricultural as well as biomedical approach.

Keywords: *Casuarina* spp., *Frankia* spp., bioactive metabolites, multi-pathogens, antibiofilms, CLSM microscope.

7. Endophytic Actinomycetes as a Source of Bioactive Compounds

PANKAJ KUMAR, VISHAL GHADGE, SANJU SINGH, DONIYA ELZE MATHEW, PRAMOD B. SHINDE*

Natural Products & Green Chemistry Division (NPGC), CSIR-Central Salt & Marine Chemicals Research Institute (CSIR-CSMCRI), Bhavnagar, Gujarat

pankajkumar@csmcri.org; *pramodshinde@csmcri.res.in

Endophytic actinomycetes are promising repositories of novel bioactive compounds. These endophytes live in symbiosis with healthy plants and help the plant to fight against pathogens and extreme biotic- & abiotic-conditions. *Salicornia brachiata* is a halophyte found in mostly arid coastal area and have been reported to possess medicinal properties. This plant is also reported to exhibit antituberculosis activity. Hence, an attempt has been made in order to study possible role of endophytes in medicinal properties of the *Salicornia brachiata* plant and biosynthetic potential of the obtained endophytic isolates. *Salicornia brachiata* was collected from different locations of coast of Gujarat. Different treatments were given to the plant parts followed by incubation on three different media for the isolation of endophytic actinomycetes. Purified isolates were screened for bioactivity against a selected panel of human, plant, & marine pathogens. Different actinomycetes belonging to genera *Isopetricola*, *Streptomyces*, *Rhodococcus*, *Nocardiopsis*, *Micromonospora*, etc. were obtained. The biosynthetic potential of isolated endophytic actinomycetes was also assessed in terms of PKS and NRPS genes using primers of conserved regions.

8. Isolation of Antifungal Metabolites from *Streptosporangium nondiastaticum* TBG-75A20

GAYATHRI VALSALA AND SHIBURAJ SUGATHAN*

Division of Microbiology, Jawaharlal Nehru Tropical Botanic Garden and Research Institute,
Palode, Trivandrum, Pin-695562, Kerala, India

* Corresponding author. Tel: +91 9495826669, E-mail: drshiburaj@gmail.com

Streptosporangium nondiastaticum is a rare actinobacteria which was reported to have antifungal activity by Shiburaj (2011). To determine the reason for this activity, the secondary metabolites from this organism were extracted using different solvents and their antimicrobial properties were studied.

S. nondiastaticum TBG-75A20 was grown in ISP2 media at 28°C in a rotary shaker at 140 rpm for 3 days. The culture was centrifuged at 12000 rpm for 15 minutes and the supernatant was filtered using Whatman No. 1 filter paper. The metabolites from culture filtrate were extracted using the solvents chloroform, ethyl acetate, n-butanol and n-hexane separately by liquid-liquid extraction using a separating funnel. The solvent fractions were collected and concentrated using a rotary evaporation and stored at -40°C. All extracts were dissolved in dimethyl sulfoxide (DMSO) to obtain solutions of 100 µg/µl concentration. Methanol extract of the cell pellet was obtained by mechanical crushing using a mortar and pestle and the extract was filtered, dried and resuspended in DMSO. Antimicrobial activity of different solvent extracts against bacterial and fungal cultures were analyzed using disc diffusion method (for bacteria and *Candida*) and agar plate cup method (for mycelial fungi).

Among the different solvent extracts studied, of n-butanol and ethyl acetate extracts of culture supernatant and methanol extract of cell mass were found to have antifungal activity. n-butanol and methanol extracts inhibited the growth of both *Candida* (*C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei*) and phytopathogenic mycelial fungi (*Fusarium oxysporum*, *Fusarium solani*, *Alternaria solani*, *Aspergillus niger* and *Colletotrichum acutatum*).

Further analysis of the antifungal extracts has to be carried out to identify the metabolites responsible for the antifungal activity. The mechanism of antifungal action also needs to be explored to develop possible application in agriculture for protecting crops from fungal infections.

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9. Production and Optimization of chitinase from *Streptosporangium nondiastaticum* TBG75A20 through Semi-solid-state fermentation

PRASEETHA¹, RAJEEV KUMAR SUKUMARAN², SHIBURAJ S.^{1*},

1 Division of Microbiology, Jawaharlal Nehru Tropical Botanic Garden and Research Institute,
Palode, Trivandrum, Pin-695562, Kerala, India

2 Microbial Processes and Technology Division, CSIR-National Institute for Interdisciplinary Science and Technology
Industrial Estate PO, Trivandrum 695019.

* Corresponding author. Tel: +91 9495826669, E-mail: drshiburaj@gmail.com

Chitinases are a group of hydrolytic enzymes that catalyse depolymerisation of chitin, which have gained tremendous importance in the past two decades. Chitin is a major constituent of the shells of crustaceans, exoskeletons of insects, and cell walls of a variety of fungi (Bhattacharya *et al.*, 2007). *Streptosporangium nondiastaticum* TBG-75A20 (MTCC 4149) is a rare actinomycete isolated from the forest soil of Neyyar WLS with high antifungal property (Shiburaj, 2011).

In the present study, the production of chitinase by *S. nondiastaticum* grown under semi-solid state fermentation conditions was investigated. This type of fermentation is a sort of SSF in which the free liquid content has been increased in order to facilitate nutrient availability and fermentation control. Various low cost chitin rich residues such as crab, shrimp shells, fish scales and agricultural residues powder were studied in semi-solid state fermentation process. The classical one-variable-at-a-time approach determined moistening media, inoculum type and inoculum volume, pH, incubation days and inducer which significantly influenced the chitinase production. Analysis of hydrolytic products of colloidal chitin, NAG was done by HPLC. Further optimization of significant variables using response surface methodology (RSM) was done with three significant variables (pH, incubation days and % biomass) identified from one variable at a time approach. The chitinase activity was increased significantly. Therefore, it can be concluded that semi-solid-state fermentation holds great promise for chitinase production.

Keywords: Chitinase, NAG (N-Acetyl-d-glucosamine), *Streptosporangium nondiastaticum*, semi-solid-state-fermentation

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10. Tannin Acyl Hydrolase Activity of *Streptomyces mirabilis* TBGS10

¹SOUMYA KM, ²SABU ABDULHAMEED AND ¹*SHIBURAJ S

¹Division of Microbiology, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Karimancode PO, Pin-695562, Thiruvananthapuram, Kerala, India

²Department of Biotechnology and Microbiology, Kannur University, Thalassery Campus, Thalassery, Kerala, India

*Corresponding author: drshiburaj@gmail.com, Mob: +919495826669

Tannin acyl hydrolase commonly referred as Tannase finds wide application in various sectors such as cosmetics, pharmaceutical, animal feeds and leather industries. Tannase catalyze the hydrolysis of ester and depside bonds in tannins liberating glucose and Gallic acid. The significance and utility of Tannase enzymes has prompted great research prospective. The most important source of Tannase is microbes due to their ability for consistent production of stable enzymes. For the past years, most screening programs for microorganisms with tannase activity were restricted mainly to fungi. The search for new and different tannase-degrading microorganisms has been increased. In recent years, the possibility of obtaining high yields of commercially useful tannase from actinomycetes has attracted the attention of researchers; however hardly a few investigations have reported from actinomycetes for the same.

The present work aimed to isolate, screen and characterize tannase producing Actinobacteria. A total of 105 actinomycetes isolates were obtained and were subjected for qualitative screening on 1% tannic acid agar plates. The tannin degradation ability was revealed by a zone of hydrolysis around the colonies. Strain TBGS10 showing high tannin hydrolyzing percentage were selected for further study tannase production from the potential strain TBGS10 was performed in tannic acid broth with maximum enzyme activity observed at 48 h of incubation. Morphological, Cultural, biochemical and molecular characterization (polyphasic identification) of strain was carried out, 16S gene amplification and sequencing revealed identity of TBGS10 as *Streptomyces mirabilis* and at the same time tannase gene from this strain was isolated, translation of its amino acid sequence evidenced the identity of tannase. Thus, the present work demonstrates the production of tannase using the newly isolated strain of *Streptomyces mirabilis* TBGS10 and also suggests that this strain open avenues for various industrial applications.

11. Qualitative Identification of Bioactive Compounds from Unicellular Filamentous Bacteria

YALPI KARTHIK¹ ISHWARI K MANJULA²

Unicellular filamentous bacteria are unique group of bacteria with high GC content having both the characteristics features of fungi and bacteria. The existence of unicellular filamentous bacteria in a particular ecosystem offers an advantage to isolate novel compounds. This type of exploring new compounds depends on the environmental factors of an ecosystem. One such ecosystem is mangrove ecosystem that offers diversified microbes that are capable of producing metabolites with a variety of applications in industry and medicine. The present study was carried out samples from mangroves in Karnataka state, India. The mangrove of Western Ghats (Mangalore region) was mainly studied to understand the prevalence of halo-tolerance, metabolites, Diversity of unicellular filamentous bacteria. Isolation was done on selective media followed by morphological studies, All the samples had both streptomycete and non-streptomycete populations. Due to their ability to survive in conditions of stress and adapt to the environment could be chance for novel strains. The Unicellular filamentous bacteria are potential producers of antibiotics and of other therapeutically useful compounds. The bioactive secondary metabolites produced by them include antibiotics, immunosuppressive agents, antitumor agents, and enzymes. These metabolites are known to possess antimicrobial, antioxidant, neuritogenic, anti-cancer, anti- algal, anti-helminthic, anti-malarial and anti-inflammatory. They exhibit a range of life cycles which are unique among the prokaryotes and appear to play a major role in the cycling of organic matter in the soil ecosystem. It has proved their ability to produce a variety of bioactive secondary metabolites and for this reason, the discovery of novel antibiotic and non-antibiotic lead molecules through microbial secondary metabolite screening is becoming increasingly important.

Keywords: - Unicellular filamentous bacteria, mangrove, Primary and Secondary Metabolites.

12. Mycobacterial Siderophore, Exochelin Production By An Indigenous Salt Tolerant Isolate *Pseudomonas stutzeri* SGM-1

SWAPNIL G. MAHAJAN^{A,B}, VINOD S. NANDRE^A, KISAN M. KODAM^A AND MOHAN V. KULKARNI^{A,B}

^aDivision of Biochemistry, Department of Chemistry, Savitribai Phule Pune University, Pune-07, Maharashtra, India

^b Design Innovation Centre, Savitribai Phule Pune University, Pune-07, Maharashtra, India

Mycobacterium is one of the genus of *Actinobacteria* phylum and it is classified into its own family *Mycobacteriaceae*. This genus of *Actinobacteria* consists of over 190 different species. Serious diseases causing pathogens like *Mycobacterium tuberculosis* causing tuberculosis and *Mycobacterium leprae* causing leprosy are the members of this genus. Requirement of iron is universal in all living forms but, the solubility of metal ions at biological pH is less. Salicylic acid, citric acid, mycobactin and exochelin are the four different types of iron chelating molecules involved in iron acquisition in mycobacteria. Exochelin is primarily produced by and restricted to saprophytic mycobacterial species like *M. neoaurum* (Exo-MN) and *M. smegmatis* (Exo-MS). In this study the indigenous salt tolerant *Pseudomonas stutzeri* isolate SGM-1 was found to produce this Exochelin siderophore in various iron depleted and minimal growth medium. Succinate medium was found to yield maximum siderophore units and it was used for siderophore production. The produced siderophore was estimated qualitatively by CAS (chrome azurol sulphonate) assay. The siderophore was further subjected to purification and characterization. It was identified by HRMS using METLIN library for peaks obtained at [M + H] **663.4511 a.m.u.** for proton adduct and [M + Na] **685.4351 a.m.u.** for sodium adduct. From METLIN library search it was confirmed that the isolate produced the non-polar siderophore which is the Mycobacterial signature peptide 'Exochelin'. Also, the result is matching with previously reported HRMS data for Exochelin from *M. smegmatis*. This is the first report that though the isolate SGM-1 belongs to *Proteobacteria* and not to the *Actinobacteria* still it has the ability to produce mycobacterial siderophore Exochelin. The application of siderophores like exochelin can be further studied as a potential drug or drug delivery vehicle against resistant mycobacterial species.

13. Bioactive Potential of Actinobacteria

ABUJUNAID H. KHAN^{1, 2} SYED G. DASTAGER^{1, 2}

¹Academy of Scientific and Innovative Research (AcSIR), New Delhi, India;

²National Collection of Industrial Microorganisms (NCIM), CSIR-National Chemical Laboratory, Pune-411008 India. Email: abujunaidkhan1213@gmail.com

A demand for new antibiotics with broad-spectrum activity is increasing due to the rapid spread of antibiotic-resistant pathogens causing numerous infectious diseases un-curable. Considering the need of the society for new antibiotics, the present work is focused on the screening of actinobacteria from the un-explored region of Western Ghats and Northeast India for the potential antimicrobial compounds. Around 200 actinobacterial strains were isolated from both the sites using different media and were identified by the 16S rRNA gene sequencing, which reveals the majority of the isolates were of *Streptomyces* genera, the most common among the actinobacteria genera. The extraction of secondary metabolites from actinobacteria using different media and their antimicrobial study was carried out by agar well diffusion method against three test organisms i.e., *Escherichia coli* (NCIM 2065), *Staphylococcus aureus* (NCIM 2127) and *Candida albicans* (NCIM 3102). One hundred and twenty one isolates were showed having activity against one of the test organism. *Streptomyces sp.* MS21 was evaluated further, as it was showing broad-spectrum antimicrobial activity and there were no antimicrobial compounds reported from the closest strain of MS21. Fermented broth of 30 litres was extracted three time repeatedly by an equal volume of ethyl acetate. The crude extract was partially purified by silica gel column chromatography. Further identification and characterization of the active compounds will be characterized with the help of preparative HPLC followed by NMR and LC-MS. The study reveals that the exploitation of actinobacteria from the unexplored region can still be a new hope and source for the novel antimicrobial compounds.

14. A Non Synthetic Protease Inhibitor from *Streptomyces griseoincarnatus* HK12 with Active Potency Against Chikungunya virus nsP2

NEETHU KAMARUDHEEN*, K V BHASKARA RAO
Department of Biomedical Sciences, Vellore Institute of Technology,
Vellore, Tamil Nadu, India
*neethz01@gmail.com

Chikungunya virus (CHIKV) is one of the six major vector borne diseases endemic to India which caused severe morbidity during the recent outbreak. Viral proteases represent an attractive target for novel antiviral agents. Protease inhibitors from *Streptomyces* are an emerging source of antagonists against various pathologies. Efficient isolates resistant to the digestion of casein were retained and the trypsin & papain inhibitory activities were recorded in percentage value. From the 85 isolates screened, 8 efficient isolates' Trypsin-BAPNA, Papain-BAPNA quantification of protease inhibitor activity was recorded. The higher inhibitory activities were found as $83.473 \pm 1.280\%$ and $78.006 \pm 0.731\%$ against trypsin and $76.456 \pm 2.81\%$ and $64.563 \pm 2.450\%$ against papain by isolates HK12 and HK19 respectively. However, HK19 was found to be cytotoxic against BHK 21 cells with an IC_{50} value as low as $7.81 \mu\text{g/mL}$, whereas HK12 exhibited a value of $62.5 \mu\text{g/mL}$. HK12 at a concentration of $31.25 \mu\text{g/mL}$ displayed no-cytopathic effect exerted by CHIKV. The cells were intact and minimal morphological changes were observed in comparison to the control, which were rounded and observed to be lifting off from the surface. The isolate HK12 was identified as *Streptomyces griseoincarnatus*, strain HK12. The cell free supernatant was purified by CM-Sepharose Ion exchange chromatography. The protease inhibitor exhibited one clear band around 66kDa in SDS-PAGE, by both native and denaturing SDS.

15. Diversity of Actinobacteria From South Coastal Regions of Andhra Pradesh, India and Screening Their Antimicrobial Potential

M. MARY SWAPNA AND M. VIJAYALAKSHMI*

Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur - 522510, Andhra Pradesh, India. Email-id: profmvijayalakshmi@gmail.com

Actinobacteria are a group of bacteria with high G+C content in the genomic DNA and well known for producing abundant active secondary metabolites. However, the increasing emergence of new diseases from pathogens and the antibiotic resistance in recent years caused a resurgence of interest in finding new biologically active compounds for drug discovery. Hence, the novel actinomycetes from marine sediments have been pursued as sources of antibiotics and other useful biologically active agents. In the present study an initiative has been taken up to isolate culturable halophilic actinobacteria and to screen their bioactive potential. To assess the diversity of actinobacterial taxa, the marine sediment samples collected from 20 different locations of south coastal regions of Andhra Pradesh, India were pretreated with calcium carbonate. Isolations were carried out using yeast extract malt extract dextrose agar medium by employing dilution plate technique. Overall 50 actinobacterial strains were isolated, of which 15 strains exhibited good antimicrobial activity against bacteria and fungi tested. Based on cultural, morphological, biochemical, physiological and molecular characteristics (16S rRNA gene sequences), nine of them are identified as species of *Streptomyces* while the rest belong to the genera *Nocardiopsis* and *Kocuria*. Attempts were made to optimize the cultural parameters to enhance the yield of antimicrobial metabolite produced by the strains. Attempts are in progress to explore the novel actinobacterial strains from different marine habitats using different types of isolation strategies.

16. Biogenic Synthesis of Functionalised Antibiotic Nanoparticles from Actinomycetes

MANJUNATH B K, DIVAKARA R*, VALARMATHY K, FLAVIA CATHERINE V V, NIGI PAUL C, JESNA DANIEL, PREETHA R

Department of Biotechnology, The Oxford College of Engineering, Bommanahalli, Bengaluru-560068
Email: divakarabdt@gmail.com

The present work describes a simple, rapid and ecofriendly method for the synthesis of silver nanoparticles by actinomycetes isolates, isolated from the soil samples of Western Ghat region. A total of 7 actinomycetes isolates were isolated in pure form from the soil samples. These isolates were subjected to preliminary antibacterial activity screening by perpendicular streaking method. Based on the broad spectrum of antibacterial activity in perpendicular streaking method, the actinomycetes isolates RHC-1 and SP 11 were selected for the biogenic synthesis of AgNPs by using cell free extract and biomass of the actinomycetes. The synthesized nanoparticles were characterized by UV-Vis spectroscopy, Fourier Transform Infrared analysis (FT-IR) and confirmed by SEM. The synthesized AgNPs sizes were found to be in the dimensions ranging between 50-200 nm. These nanoparticles were screened for antibacterial activity by using well diffusion method. These nanoparticles showed promising antibacterial activity against *K. pneumoniae*, *E. coli*, *S. typhi*, *S. aureus* pathogenic species with MIC ranging from 50 to 100 µg/ml. The synergistic activity of the antibiotic-silver nanoparticle conjugates showed enhanced potency against the test pathogens *K. pneumoniae*, *E. coli*, *S. typhi* and *S. aureus* indicating that AgNPs can be used alone or in formulation with antibiotics as an effective antimicrobial agent. Further molecular characterization of the isolate RHC-1 was carried out by using 16S rDNA analysis. The strain was identified as *Streptomyces sp. 13636G*. However further purification and characterization of bioactive metabolites from *Streptomyces* species are in progress.

Key words: Actinomycetes, silver nanoparticles, biogenic synthesis, functionalised antibiotics

17. Marine Rare Actinobacteria: A Potential Source of Anticancer, Antimicrobial and Antihaemolytic Compound Activities

RAGALATHA RAGIREDDYPALLEM, VIJAYALAKSHMI MUVVA*, KOKKANTI MALLIKARJUNA

Department of Botany and Microbiology, Acharya Nagarjuna University Guntur -522510, Andhra Pradesh, India

The underexplored marine ecosystem has been focused for the discovery of novel actinobacteria for their versatile medicinal, industrial and agriculture applications utilizing advanced techniques in genomics, proteomics, and bioinformatics for identification. Marine rare actinobacteria a potent provenance for new metabolites which marked an era recently for the developments in antibiotic chemotherapy.

The present study aimed to isolate and screen the bioactive potentiality of marine rare actinomycetes which could suppress dreadful diseases and antibiotic resistant pathogens. A total of fifteen actinomycetes strains were isolated from the marine sediments of Kakinada, Andhra Pradesh, India. Characterization of the strains was conducted through 16S rRNA gene sequencing and phylogenetic tree construction. The organic extracts of actinobacterial isolates were screened for anticancer by MTT assay, antimicrobial (agar well diffusion method), and antihaemolytic activities. Among them one isolate identified as *Nocardiopsis terrae* VJRM-udi3 crude extract showed cytotoxicity against the cell lines Hela (cervical carcinoma), SKNSH (Neuroblastoma), one mouse cancer cell line B16 F10 (Mouse melanoma) and one normal mouse cell line NRK 49F (normal rat kidney fibroblast cells) in 96 well plates on testing and doxorubicin was used as positive control.

The VJRM-udi3 crude extract exhibited thrombolytic activity on caseinolysis followed by blood clot dissolving assay respectively using streptokinase 15,000IU as standard and normal saline as negative control. *In vitro* antimicrobial activity of crude ethyl acetate extract from strain VJRM-udi3 revealed antagonistic activity against some Gram positive, Gram negative bacteria and fungi.

18. Bioactive Compounds from *Streptomyces cavourensis* Isolated from Vermicompost.

T. UMA, C. SRINIVAS* AND RADHA D. KALE.

Department of Microbiology & Biotechnology, Bangalore University, Jnanabharathi Campus, Bangalore- 560056.
Corresponding author: srinivasub@gmail.com

Actinomycetes found in vermicompost play a significant role in decomposition of the organic materials and produce secondary metabolites. Colonisation of actinomycetes in the rhizosphere can provide biological control of soil-borne fungal plant pathogens and plant growth promotion. In the present investigation *Streptomyces cavourensis* isolated from vermicompost was evaluated for the production and identification of bioactive compounds. Solvent extraction method was performed using ethyl acetate. Identification of various bioactive compounds were analysed through GC-MS. The GC-MS result revealed the presence of twenty major bioactive compounds. Most of the bioactive compound *viz*: Cetane, E-15-Heptadecenal and n-Hexadecanoic acid were found to be antimicrobial, nematicide, antioxidant and anticancer. Hence, actinomycetes established from vermicompost can be used as plant growth promoting rhizobacteria (PGPR) and for suppression of pathogens in soil.

Keywords: Vermicompost, *S. cavourensis*, Bioactive compounds.

19. Antibacterial Activity of Soil Actinomycetes from the Coffee Plantation of The Less Explored Region of Western Ghats

BANU SAMEERA AND MONNANDA SOMAIAH NALINI

Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore – 570 006, Karnataka, India.

Actinomycetes are noteworthy producers of secondary metabolites with an array of bioactivities. Although there are reports available on the antimicrobial activity of soil actinomycetes, the studies of newer sources of antimicrobial agents are in need as the pathogens become resistant with time to the available antibiotics. So far, the exploration of actinomycete populations is least in the coffee plantation areas. Thus, the objective of this study was to isolate and characterize the actinomycetes from the coffee plantation soils of Chikkamagalur and preliminary evaluation and characterization of their extracts as antibacterial compounds. A total of thirty soil samples were collected successively diluted and inoculated into the Starch casein agar media for the actinomycete isolations. Molecular characterization aided the identification of recovered isolates. Antibacterial activity and Minimum Inhibitory Concentration (MIC) of the actinomycete extracts were evaluated. Furthermore, the TLC fraction of active strain was characterized by bioautography and GCMS analysis. Based on molecular characterization, thirty-two actinomycete strains were isolated from the samples and amongst them, 17 strains were active against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri* and *Xanthomonas oryzae*. The strain *Streptomyces rubrogriseus* (MH393288) exhibited broad-spectrum antibacterial activity against all the tested pathogens. *S. rubrogriseus* extract showed MIC at a range of 0.04 to 0.16 mg/ml. The bioautography conducted against *B. subtilis* with the least MIC (0.04 mg/ml) was confirmed by the formation of clear zone. The GCMS analysis of the band corresponding to this zone identified six antibacterial compounds. This study revealed that coffee plantation is a habitat for potential actinomycetes with promising bioactive metabolites.

Keywords: Coffee plantation soils, 16S rRNA, *Streptomyces rubrogriseus*, GCMS, antibacterial compounds

20. Bioactive Antibacterial Compounds from *Streptomyces indiaensis*, An Endophyte of The Endemic Medicinal Plant, *Zingiber nimmonii* (J. Graham) Dalzell.

M. S. NALINI*, MADHUCHHANDA DAS* AND H. S. PRAKASH §

*Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore- 570 006, India

§UGC-BSR Fellow, Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore- 570 006, India

Actinomycete endophytes are being isolated from plants of medicinal value due to their immense pharmacological and agricultural benefits in the form of antimicrobial, antioxidant and anticancer agents. This study is directed towards the isolation and molecular identification of endophytic actinomycetes from *Zingiber nimmonii* (J. Graham) Dalzell., an endemic plant species of the 'Western Ghats', southern India and characterization of the secondary metabolites responsible for the antimicrobial properties. Seven actinomycetes were isolated from the rhizome (13.5%), roots (2.5%) and leaves (2.0%) of *Z. nimmonii*. The strains belonged to four genera viz., *Streptomyces*, *Arthrobacter*, *Curtobacterium* and *Corynebacterium* and seven species. The secondary metabolites of the strains extracted with ethyl acetate were evaluated for the antibacterial assay by the disc diffusion method against six pathogenic bacteria namely, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*. The minimal inhibitory concentration (MIC) was determined by the modified broth dilution method. All the strains were positive against four test bacteria, while *Streptomyces indiaensis* (MF083723) showed significant antibacterial potential against test organisms with the MIC in the range of 0.02 to 0.16 mg/ml. Therefore, the extract of *S. indiaensis* was further characterized for the identification of antimicrobial compounds by TLC and bio-autography. GC-MS of the TLC fraction revealed six major peaks corresponding to the presence of plasticizer, alkylphenols, saturated fattyacids, heterocyclic and dihydrotriterpenic hydrocarbon compounds. This study is the first report on the characterization of antibacterial compounds from the actinomycete endophyte of *Z. nimmonii*.

Keywords: endophytic actinomycetes, *Zingiber*, Western Ghats, *Streptomyces indiaensis*, antibacterial, GCMS

21. Isolation, Molecular Characterization of *Stenotrophomonas sp.* strain NS-24 from Non-Rhizosphere Soil Samples and Their Biological Synthesis of AgNPs as PGPR for Soyabean Root Colonization.

DATTATRAYA AIRODAGI, BIDHAYAK CHAKRABORTY, PALLAVI S S, MEGHASHYAMA P BHAT, MUTHURAJ R, DHANYAKUMARA S B, HALASWAMY H M, SHASHIRAJ K N AND SREENIVASA NAYAKA*

P.G. Department of Studies in Botany, Karnatak University, Dharwad, Karnataka, India.

*Corresponding author: Email ID: sreenivasanayaka06@gmail.com

The biological method for the synthesis of silver nanoparticles (AgNPs) from bacteria (*Stenotrophomonas sp.* strain NS-24) is a non-toxic, eco-friendly and can be used for large scale productions. Many bacterial species have been used to synthesize the nanoparticles, mainly because of their medicinal values. Biologically synthesized nanoparticles are with a unique different efficiency, size and shape. The present goals of our study are biological synthesis, characterization of silver nanoparticles, and to evaluate its antimicrobial activity against microbial pathogens like *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus pneumoniae* and *Staphylococcus aureus*. The characterization of silver nanoparticles was done by UV-Visible spectroscopy, AFM, TEM, SEM with EDX, X-ray Diffraction, FTIR, and HR-TEM. The molecular characterization of AgNPs is done by DNA extraction, 16s rRNA gene sequencing. The UV-visible spectrophotometric observation of *Stenotrophomonas sp.* strain NS-24 extract showed a maximum absorbance at 371nm. The AFM data confirmed that the particles are polydispersed and spherical in shape. Further, the FTIR analysis revealed the IR spectral band patterning. TEM analysis showed that the size of the biogenic AgNPs were in the range of 12.56 nm to 20.32 nm, with an average of 18.06 nm in size. The antimicrobial activity of AgNPs was studied on different gram negative and gram positive bacterial strains like *Escherichia coli* (MTTC 40), *Enterococcus faecalis* (MTTC 6845), *Streptococcus pneumonia* (MTTC 8874) and *Staphylococcus aureus* (MTTC 2825) for inhibition of their growth at varying concentrations of AgNPs. Finally, biologically synthesized AgNPs from *Stenotrophomonas sp.* strain NS-24 was used as a PGP ton Soyabean to conduct the Bio-root assay. The Bio-root assay revealed that the *Stenotrophomonas sp.* strain NS-24 had the ability to colonize plant roots as an endophyte.

keywords: Bacteria (*Stenotrophomonas sp.* strain NS-24); AFM; SEM with EDX; Antimicrobial; Bio-root assay;

22. *In Vitro* Cytotoxic Activities of Mangrove Actinomycetes From Andhra Pradesh

SABA YASMEEN AND VIJAYALAKSHMI MUVVA

Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, A.P.

Despite of much progress in the diagnosis and treatment of cancer, it constitutes one of the main reasons of deaths worldwide. Natural products have been proven to be promising sources of novel anticancer drugs. Actinomycetes produce biomolecules useful for cancer treatment and immunomodifiers that enhance immune response. As mangrove actinomycetes are known to produce novel anticancer compounds, an attempt has been made to isolate actinomycetes from Gilakaladindi mangrove sediments of Andhra Pradesh. Four potential strains designated as VJSY-1, VJSY-2, VJSY-3 and VJSY-14 are isolated and identified by polyphasic taxonomy as species of *Nocardioopsis* and *Streptomyces*. The *in vitro* anticancer activity of the actinobacterial strains were evaluated by performing MTT (3-(4, 5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against human cervical cancer (HeLa) cell line, neuronal (SKNSH) cell line, mouse melanoma (B16F10) cell line and mouse normal (NRK49f) cell line. The ethyl acetate extracts of four strains were inoculated on different human and mouse cancer cell lines. The extracts of four strains exhibited good cytotoxicity with IC_{50} value ranges from 13.1 to 47.2. VJSY-1 exhibited pronounced cytotoxic potency against human cervical cancer cell line with less toxic effect on mouse normal cell line as compared to crude extracts isolated from the rest of selected strains. Hence, attempts are being made to purify the compounds produced by VJSY-1.

23. The role of Actinomycetes in Termite Hills: A case study in Western Ghats - Nagarhole Region, Karnataka

J.S. CHANDRASHEKAR*¹, DEEPAK, B¹. AND PAVAN, R².

¹Department of Studies and Research in Environmental Science,
Karnataka State Open University, Mysuru - 570006, India

² Senior Scientist, Central Research Laboratory, Saptagiri Institute of Medical Sciences and Research Centre, Chikkabanavara,
Bengaluru - 560090

*Correspondence: +91-9663061978, Email: chandra.ksou@ka.gov.in

The continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health. *Actinomycetes* are one of the major groups of soil population and are widely distributed and play an important role in degrading organic matter. Although termite hill and specific bacterial taxa, such as the *Actinomycetes*, have been studied extensively in various habitats, few studies have examined them simultaneously, especially in the termite hill soil of Western Ghats - Nagarhole region. The soils were tested for its physicochemical parameters and *Actinomycetes* were isolated from the soil samples by screening, and isolation from the termite hill soil using Yeast Mannitol Agar medium. The soils were slightly acidic to near neutral, organic carbon ranged between 1.52 and 2.03% and nitrogen between 0.040 and 0.067%. Gram positive usually form branching vegetative filaments, rod shaped organisms belonged to genus *Nocardioform actinomycetes*. The average height of the termite hill in the region is 5 m in height and 3 m in diameter with a average distance of 3 to 40 m for 1 acre of land. The termite hill numbers were more during the monsoon season. The termite hill witnessed the growth of Mushroom (*Odontotermes sp.*) which usually consumed by the local people. A wide variety of bacteria, especially those belonging to Actinobacteria and Proteobacteria, degrade soluble organic molecules such as organic acids, amino acids, and sugars.

Keywords: *Actinomycetes*, Termite Hill, Isolation, Yeast Mannitol Agar, Gram positive.

24. Anti-Infective Potential of Endophytic Actinomycetes Associated with *Madhuca insignis* Radlk., A Critically Endangered Riparian Medicinal Plant from Western Ghats Of India

SOMA MONDAL AND VITTAL RAVISHANKAR RAI*

Department of Studies in Microbiology, Manasagangotri, University of Mysore, Mysore, Karnataka, India - 570 006

*Correspondence: Vittal Ravishankar Rai, Email: raivittal@gmail.com

Endophytic actinomycetes as a source of bioactive metabolites have explored in industrial and therapeutical applications. The search for new biologically active antimicrobial secondary metabolites synthesized by endophytic actinomycetes continues to be an important arena in modern drug discovery. The present study has been initiated to explore endophytic actinomycetes inhabiting *Madhuca insignis* (Radlkofer) H.J. Lam from Western Ghats of India because of it is a critically endangered and a very narrow endemic riparian tree found in Western Ghats with rich microbial diversity which has been studied only to a limited extent. Endophytic actinomycetes were isolated from asymptomatic stem, root and leaf tissues of *Madhuca Insignis* and evaluated for their antimicrobial activity. A total of 36 endophytic actinomycetes were isolated from tissue segments. The higher recovery of strains was found in leaf tissues, followed by stem and root tissues. Determination of antimicrobial activity of endophytic actinomycetes showed that 09 strains possessed anti-infective activity against test human pathogens. Among the potential strains, *Streptomyces* sp. exhibited significant anti-infective activity against all the tested human pathogens. This research study implies that endophytic actinomycetes inhabiting *Madhuca insignis* explores beneficial bioactivity aspects which could be exploited for microbial natural product based drug discovery.

Keywords: Endophytic actinomycetes, secondary metabolites, *Streptomyces*, *Madhuca insignis*

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25. Evaluation of Antimicrobial, Enzyme Inhibitory, Antioxidant and Cytotoxic Activities of Metabolites isolated from Marine derived Actinobacteria

SAKET SIDDHARTH AND RAVISHANKAR RAI V*

Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore – 570006, INDIA

Marine actinobacteria are less explored than their terrestrial counterparts as potential source of natural products [1]. The present study was aimed to elucidate the bioactive potential of metabolites produced by marine-derived actinobacterial strains isolated from Havelock Island, Andaman and Nicobar Islands, India. The potential isolates SCA29 and S2A were identified as *Streptomyces* sp. and isolate SCA21 as *Nocardioopsis* sp. by phenotypic, genotypic (16S-rRNA) and phylogenetic analyses. Bioactive metabolites were extracted by solvent extraction method. The metabolites were assayed for antagonistic activity against bacterial and fungal pathogens, inhibition of α -glucosidase and α -amylase enzymes, antioxidant activity and cytotoxic activity against various cell lines. The compounds were subjected to separation and purification by column chromatography which led to the identification of 4-methoxyacetanilide [2], an acetamide derivative from strain SCA29 and 4-bromophenol [3], a bromophenol derivative and Bis (2-ethylhexyl) phthalate, a phthalate ester from strain SCA21. The compounds produced by strain S2A were identified using gas chromatography-mass spectrometry (GC-MS) and resulted in three constituents; pyrrolo[1-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-, being the main component (80%) [4]. The purified compounds showed significant inhibition potential against α -glucosidase and α -amylase enzymes and showed remarkable antibacterial activity against test bacterial and fungal pathogens with the MIC value ranged from 3.90 to 31.25 μ g/mL. The compound also exhibited concentration-dependent cytotoxicity on against HT-29, MDA and U-87MG cell lines without significant effect against human normal cells.

Keywords: Marine actinobacteria; bioactive metabolites; antioxidants; enzyme inhibitors

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26. Bioassisted Synthesis of Gold Nanoparticles from *Saccharomonospora glauca*: Toxicity and Biocompatibility study

RANJITHA VR AND RAVISHANKAR RAI V *

Department of Studies in Microbiology, University of Mysore, Manasagangotri,
Mysore-570 006, Karnataka, India.

*Corresponding author: Prof. Ravishankar Rai V

E-mail address: raivittal@gmail.com

Nano synthesis is an important step in the field of nanotechnology to obtain the functional material of biological interest. In the present study, bioassisted synthesis of gold nanoparticles (GNPs) was carried out using *Saccharomonospora glauca*, rare Actinomycetes isolated from soil and was tested for Haemocompatibility and cytotoxicity against NIH3T3, HT-29 and Hep 2 cells. Bioreduction was monitored by using UV-Visible spectroscopy and characterised by FTIR, XRD, SEM, DLS and zeta potential. The average particle size obtained by SEM and DLS was found to be 30nm with spherical in shape. The synthesised GNPs showed good cytotoxic activity against cancer cell lines HT-29 and Hep 2 with an IC-50 value of 49.8 μ g/ml and 96.8 μ g/ml respectively with least toxicity for normal cell line NIH3T3. This was further confirmed by staining the cells with AO and PI dual staining for Apoptosis detection. The synthesised GNPs showed excellent biocompatibility for human blood without haemolysis. The work reports for the first time, the synthesis of simple and eco-friendly GNPs from *Saccharomonospora glauca* showing excellent biocompatibility, good toxicity for cancer cells with mild toxicity for normal cell lines that can be implemented for biomedicine.

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