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Actinobacterial research from the mangroves of India

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Abstract

Microbial diversity comprises a wider range of microbes than any other living group of organisms of the world. This rich diversity is due to existence of microbes in all niches including those of oceans where life is possible. Among the microbes, actinobacterial populations in the estuarine and marine sediments vary in density with varying regions and even among the different sites within an ecosystem and actinobacteria are being continuously reported from the marine sub habitats such as marine sediments and marine soils of almost all parts of the world. Thus, they have worldwide distribution which indicates their plasticity and adaptability to extremely varied environmental conditions. The actinobacteria found in the marine and coastal ecosystems may be viewed as a rich gene pool, possibly containing isolates capable of producing useful metabolites of unexhausted reserve of bioactive compounds having excellent commercial applications.

Mangroves are of great ecological, economic and social significance. Further, mangroves occurring along the estuaries, backwaters and the deltas function as the most important links between the land and sea. They are also unique intertidal ecosystems of the tropics, which support genetically diverse groups of aquatic and terrestrial microorganisms. As the mangrove environments differ greatly from terrestrial habitats, distribution and biological characteristics of the mangrove microbes including actinobacteria are expected to be different from those of the terrestrial ones. Hence, studies on the diversity of mangrove actinobacteria are important not only in terms of basic research, but also for the biotechnological exploitation of such organisms. This review highlights the works on marine actinobacteria isolated from the mangrove environment of India.

Key Words: Actinobacteria, Biodiversity, Biotechnological exploitation, Mangroves

INTRODUCTION

Mangroves are coastal ecosystems that are found in tropical and subtropical regions and they cover between 60 and 75% of the world's coasts. These ecosystems have unique characteristics, including brackish water, particulate sediments (muddy soil), and a specific population of plants and animals. Further, they represent a complex and dynamic system that varies in terms of salinity, water levels and nutrient availability during different seasons and they are one among the most productive and biologically important environments. They play a vital role in establishing and expanding the coast line and they are of fundamental importance in maintaining the food-chain and carbon cycle for human, coastal and marine communities.

Such mangrove ecosystems are largely the uncaged source for the isolation of newer microbes having rich potential to produce important bioactive secondary metabolites. In fact, mangrove environment is rich in organic matter because of various microbial activities including those of actinobacteria and the latter are the important resources for deriving antibiotics and they are most effective against human pathogens that are resistant to the existing antibiotics. Hence, research on actinobacteria of the mangroves of India has received good attention and the important works are given below.

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Review

Actinobacterial research from Indian mangroves was started in late seventies and Laksmanaperumalsamy *et al.* (1978) isolated 518 *Streptomyces* strains from the coastal areas of Porto Novo including the mangrove environment. Later, Kala and Chandrika (1995) isolated actinobacteria from mangrove sediments, using different media. Patil *et al.* (2001) isolated 20 actinobacterial strains from the sediment samples of the mangrove area of Tuticorin. Sivakumar (2001) sequenced two strains of actinobacteria for 16S rRNA isolated from the Pitchavaram mangrove environment. Kathiresan *et al.* (2005) isolated 160 strains of actinobacteria from the sediments from different locations of Cuddalore in which mangroves proved to be the rich source for actinobacteria with high anitifungal properties. Out of 160 isolates, 10 showed potent activity against four phytopathogenic fungi (*Rhizoctonia solani, Pyricularia oryzae, Helminthosporium oryzae and Colletotrichum falcatum*). Sivakumar *et al.* (2005) suggested Kuster's agar as the suitable medium for the isolation of actinobacteria from the Pitchavaram mangroves and found that *Streptomyces roseolilacinus* showed prominent activity against various human

pathogens. Sivakumar *et al.* (2007) reviewed the works related to the marine actinobacteria of India.

Arifuzzaman *et al.* (2010) isolated 55 actinomycetes comprising *Actinomyces* (27), *Nocardia* (14), *Streptomyces* (11) and *Micromonospora* (3) from the soil sample of Karanjal region of the Sundarbans, which showed potential for the production of antibiotics of pharmaceutical interest. Twenty actinomycete isolates produced antibiotics against one or more gram negative pathogenic bacteria such as *Shigella boydii, S. flexneri*-AN-31153, *S. sonnei, S. dysenteriae* type-1, *Pseudomonas sp*, *Vibrio cholerae*-0139, *Vibrio cholerae*-OGET, *Salmonella typhi*-Ao-12014, *Plesiomonas sp., Hafnia* spp., and *Escherichia coli*-186LT. Antagonistic activity of the actinomycetes of Karangakadu mangroves was reported by Ravikumar *et al.* (2010). They identified 17 actinomycete isolates from the leaves of 5 different halophytic plants viz. *Avicennia marina, Bruguiera cylindrica, Rhizophora mucronata, Salicornia brachiata* and *Suaeda monoica* in which 10 isolates showed high sensitivity against *Klebsiella* sp. with a maximum zone of inhibition of 13±1.12 mm.

Vijayakumar *et al.* (2010) reported on the larvicidal activity of the marine actinomycetes, *Streptosporangium* sp. and *Streptomyces* sp., isolated from the mangroves of Muthupet, Tamil Nadu against the *Anopheles* mosquito larvae. Thirty strains were isolated by serial dilution plate technique, in which two isolates exhibited notable larvicidal activity. Raja *et al.* (2010) have reported on the actinobacteria, producing amylase inhibitors against both prokaryotic and eukaryotic amylases and seawater requirement test was carried out for all the actinobacterial strains and the results indicated that the strains SSR-3, SSR12 and SSR-16 required at least 50% seawater for their growth in the media, whereas the strain SSR-6 isolated from the rhizosphere soil of *Rhizophora mucronata* of the Vellar estuary, east coast of India required 25% seawater.

Arumugam *et al.* (2011) reported on the novel species, *Streptomyces sundarbansensis* (designated strain MS1/7^T) from the Sundarbans which produced 2-allyloxyphenol. This strain showed greater than 99% 16S rRNA gene sequence similarity to the type strains of several recognized species of the genus *Streptomyces*, but in the phylogenetic tree based on 16S rRNA gene sequences, it formed a distinct phyletic line and demonstrated closest relationships to viomycin-producers (*Streptomyces californicus* NRRL B-1221T, *S. floridae* MTCC 2534T and *S. puniceus* NRRL B-2895T). However, strain MS1/7T could be distinguished from these and other closely related species based on low levels of DNA–DNA relatedness (44 %) and different physiological features, principally amino acid utilization and growth in NaCl. Strain MS1/7T was therefore suggested to represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces sundarbansensis* sp. nov. was proposed. Baskaran *et al.* (2011) obtained 22 antibacterial metabolite producing actinobacteria from the mangroves of the Andaman island. *Streptomyces* sp. strain A107 showed the maximum activity against all the tested pathogens. Among the 42 isolates tested, 22 were active against *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and *Klebsiella pneumonia* particularly, actinomycete strains viz. A101, A102, A107, A116, A121, A125, A130, F101, F102, F104, F106, De101 and De102 significantly inhibited the growth of all bacteria tested and it was suggested that the actinomycetes from the mangroves of the Andaman and Nicobar islands could be the pursued for the synthesis of antibiotics.

Govindasamy *et al.* (2011) reported about the biochemical characterization of a few actinobacterial species obtained from the mangrove soil of the Muthukuda mangroves, southeast coast of India. They showed that the actinobacteria are primarily saprophytic and are best known from soils, where they contribute significantly to the turnover of complex biopolymers such as lignocellulose, hemicellulose, pectin, keratin and chitin. Several groups of actinobacteria including species from the genera, *Micrococcus* and *Corynebacterium* can synthesize cyclic and acyclic C45 and C50 carotenoids.

Ballav *et al.* (2012) have reviewed the biotechnological significance of actinobacterial research in India and enlisted the prolific metabolites of the culturable actinobacteria. They have briefly explained about the enzymes, enzymes inhibitors, anti-cancer compounds, carotenoids, extra alkaloids, nano particles, bioemulsifiers, biosurfactants and other applications of actinobacteria like bioremediation of xenobiotics, biosorption of heavy metals and metagenomics. Ravikumar *et al.* (2012) obtained metabolite extracts from the actinomycetes isolated from the mangrove sediments of the Manakkudi mangrove ecosystem, Kanyakumari, Tamilnadu for the cytotoxic assays against breast cancer cell lines viz. MCF-7 and MDA-MB-231. Ethyl acetate extracts from the crude culture broth showed the IC_{50} value less than 30 µg/ml which can be considered as promising for anticancer drug development. Extracts also showed the presence of alkaloids and quinines as chemical components having anticancer property. Rajkumar *et al.* (2012) reported actinobacterial diversity from 30 mangrove environments of the Bhitharkanika, Orissa and obtained 116 actinobacterial colonies; among them, 67 isolates were morphologically distinct on the basis of colour of spore mass, reverseside colour, aerial and substrate mycelia, production of diffusible pigment, sporophore and spore morphology; 43 isolates were assigned to *Streptomyces, Saccharopolyspora, Nocardiopsis,*

Micromonospora, Actinomadura, Actinomyces and *Actinopolyspora.* Mangamuri *et al.* (2012) obtained the potent bioactive metabolite producing strain VUK-10 (*Pseudonocardia endophytica*) by plating on asparagine – glucose agar medium. Identification of the strain was carried out by employing the polyphasic taxonomical studies including the 16S rRNA sequence based analysis and the phylogenetic tree was constructed using the Molecular Evolutionary Genetic Analysis (MEGA) version 5. This strain was collected from the mangrove ecosystem of the Nizampatnam south coastal region of the Andhra Pradesh and it inhibited Gram positive and Gram negative bacteria and fungi.

Antagonistic activities of *Streptomyces* isolated from the mangrove sediments of Vishakhapatnam were studied by Rao *et al.* (2012a). Out of the 20 isolates, only 12 isolates showed antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, B. cereus, Escherichia coli, Pseudomonas aeruginosa , P. vulgaris, Saccharomyces cerevisiae, Candida albicans, Aspergillus niger,* and *Aspergillus flavus* during the preliminary screening. These active isolates were morphologically distinct on the basis of spore mass, spore colour, reverse side colour, aerial and substrate mycelial formation, production of diffusible pigment and biochemical characterization. Rao *et al.* (2012b) also isolated 20 actinobacterial strains from the mangrove sediments. Of these, four active isolates were identified as *Streptomyces* species based on the morphological, physiological, biochemical and cultural characteristics. These isolates were subjected to shake flask fermentation and the secondary metabolites were extracted with ethyl acetate and screened for their antimicrobial activities against selected bacterial and fungal pathogens which showed that among the active isolates, four isolates (BC 01, BC 02, BC 03 and BC 04) showed promising activities against the selected test pathogens and were further analyzed for UV Spectrophotometric and HPLC. Spectral data of the extracted compound revealed its antimicrobial nature. The UV spectrum of the methanol extracts for the active isolates showed absorbance peaks ranging between 207-223 nm. Two to three bioactive regions were detected on the HPLC which proved that *Streptomyces* strains from the mangrove sediments of Vishakhapatnam produced potential antibacterial, antifungal and broad spectrum antibiotic compounds.

Janardhan *et al.* (2012) isolated five potential actinomycete strains from the rhizhosphere region of the mangrove plants of Pennar, Nellore district in Andhra Pradesh which produced bioactive compounds with an excellent wound healing property. These compounds also exhibited effective antibacterial activity against both Gram positive and Gram negative bacteria (*Bacillus, Pseudomonas, Staphylococcus* and *E.coli).* Amrita *et al.* (2012) opined that the diverse actinobacteria derived from the mangrove environment are potentially rich sources of antibiotics, anticancer, antifungal and antiviral agents, enzymes and enzyme inhibitors and also they are a prolific but underexploited source for the discovery of novel secondary metabolites. Bhattacharya *et al.* (2012) isolated 12 actinomycetes along with 28 bacteria and three fungi from the soil samples of Vallapattanam and Pappinishery mangroves, Kerala. Majority of the bacterial isolates were gram positive rods. The actinomycetes showed antagonistic activity against the bacteria isolated from the same soil samples, with the highest activity shown by the strains A11 and A16. Whereas, the strains A16, A18, A24 and C14 showed significant fungicidal activity (inhibitory zones ranging from 16 mm to 39 mm) against three native fungi, *Aspergillus flavus, Penicillium sp. and Trichoderma sp.* A17 and A24 showed excellent antibiosis against hospital isolates of *Candida albicans, C. parapsilosis, Trichophyton rubrum* sp., *T. mentagrophytes* and *Cryptococcus* sp. These results indicated that these mangrove microbes possess promising antimicrobial properties and they can be studied in details for their novel chemotherapeutic properties.

Recently, Karthikeyan *et al.* (2013) identified 21 actinobacteria of different genera viz. (*Actinokineospora, Actinopolyspora, Amycolata, Glycomyces, Microbispora, Microtetraspora, Micropolyspora, Nocardia, Nocardiopsis, Promicromonospora, Saccharothrix, Saccharopolyspora, Streptomyces, Streptoverticillium, Spirillospora* and *Thermomonospora*) from the mangroves of Ennoor, Tamil Nadu. Karthikeyan *et al.* (2014) also isolated 21 actinobacteria belonging to *Actinomadura, Actinokineospora, Catellospora, Kitasatospora, Nocardia, Nocardiopsis, Planobispora, Planomospora, Terrabacter, Saccharothrix, Streptomyces, Streptosporangium, Streptoverticillium* from the Palaverkadu mangroves, Tamil Nadu. Mangamuri *et al.* (2014) have made seasonal enumeration of actinobacteria and the physico-chemical properties of the mangrove sediments of Nizampatnam and Coringa located along the southeast coast of Andhra Pradesh. Pretreatment with calcium carbonate and plating on starch casein agar yielded maximum number of actinobacteria. The strains were identified based on the morphological characteristics such as aerial mycelium, substrate mycelium, diffusible pigments and micro morphological features which revealed that majority of the mangrove actinobacteria (69%) belong to *Streptomyces* spp. Among the 55 isolates screened for antimicrobial compounds, 28 were found to be the potential producers. The isolates could also produce commercially important enzymes such as L-asparaginase, cellulase and amylase. In addition,

statistical study revealed the positive correlation between the distribution of the actinomycetes as influenced by the physico-chemical parameters and the organic matter of the soil. Authors concluded that the unexplored regions like Nizampatnam and Coringa mangrove ecosystems will serve as potential sites for the isolation of antimicrobial and industrial enzyme producing actinobacteria.

CONCLUSION

Actinobacteria have evolved as a group with greatest genomic and metabolic diversity. *Bona fide* actinobacteria not only exist in the oceans but are also widely distributed in different marine ecosystems. In India, majority of the surveys for the actinobacteria have been conducted in the coastal areas, collecting the littoral sediments from the states of West Bengal, Maharashtra, Orissa, Andhra Pradesh, Karnataka, Tamil Nadu, Goa, Kerala and Andaman & Nicobar group of Islands (Sivakumar *et al*., 2007). There is a tremendous scope for the isolation of novel secondary metabolites from these marine actinobacteria. Actinobacteria have the proven potential as chemically creative species which could be sustainably utilized for human welfare (Periera and Kamat, 2013). In this respect, future success relies on our ability to isolate more novel actinobacteria from the marine environments. Although isolation strategies directed towards new marine-derived actinobacteria have been lacking, some progress has recently been made in this area. From this article, it can be understood that the mangrove environment is the best source for the isolation of potential actinobacteria which can lead to the discovery of many new and novel antibiotics and secondary metabolites. There has been already considerable exciting ground work set, for the exploration and exploitation of marine actinobacteria including those of the mangroves as the next new source of novel secondary metabolites.

Further developments in improving the existing isolation strategies for the recovery of more novel marine actinobacteria would be of utmost importance for ensuring success in this arena of research.

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