Potential microbial diversity in mangrove ecosystems: A review

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Mangroves provide a unique ecological niche to different microbes which play various roles in nutrient recycling as well as various environmental activities. Mangrove forests are large ecosystems distributed in 112 countries and territories comprising a total area of about 181,000 km$^2$ is over a quarter of the total coastline of the world. The highly productive and diverse microbial community living in mangrove ecosystems continuously transforms nutrients from dead mangrove vegetation into sources of nitrogen, phosphorous and other nutrients that can be used by the plants and in turn the plant-root exudates serve as a food source for the microbes. Analysis of microbial biodiversity from these ecosystems will help in isolating and identifying new and potential microorganisms having high specificity for various applications. The present study consists literature on diversity of predominant microbes such as bacteria, fungi and actinomycetes from mangrove ecosystems.

Keywords: mangrove, actinomycetes, nutrient recycling and diversity.

Introduction

Mangroves are coastal wetland forests mainly found at the intertidal zones of estuaries, backwaters, deltas, creeks, lagoons, marshes and mudflats of tropical and subtropical latitudes. The specific regions where mangrove plants grow are termed as “mangrove ecosystem”. Mangrove forests occupy several million hectares of coastal area worldwide and distributed in over 112 countries and territories comprising a total area of about 1,81,000 km$^2$ in over one fourth of the world’s coastline$^{1,2}$. According to Forest Survey of India (FSI)$^3$, out of 4,87,100 ha of mangrove wetlands in India, nearly 56.7% (2,75,800 ha) is present along the east coast, and 23.5% (1,14,700 ha) along the west coast and the remaining 19.8% (96,600 ha) is found in the Andaman and Nicobar islands. The largest single area of mangroves in the world lies in the Bangladesh part of the Sunderbans, covering an area of almost 6,00,000 ha including waterways. There are about 6.9 million ha in the Indo-Pacific region, 3.5 million ha in Africa, 4.1 million ha in the Americas including the Caribbean. Mangroves also survive in some temperate zones but there is a rapid decrease in the number of species with increasing latitude$^{4,5,6}$.

Microbial Diversity in Mangrove ecosystems

Although microbial diversity is one of the difficult areas of biodiversity research, extensive exploration is required for understanding the biogeography, community assembly and ecological processes which will for isolating and identifying new and potential microorganisms having high specificity for recalcitrant compounds$^7,8$. The present review highlights on the diversity study of potential bacteria, fungi and actinomycetes in mangrove environments.

Bacteria

The importance of microbially generated detritus in mangrove areas that acts as the major substrate for bacterial growth in mangrove ecosystems was outlined in a conceptual model by Bano et al$^9$. The abundance and activities of bacteria are controlled by various physical and chemical factors such as tannin, leached from mangrove litter of the mangrove ecosystem. Increasing tannin concentration is associated with decreasing bacterial counts. Thus, tannin plays a role not only in keeping the bacterial counts low but also keeping the harmful activities of virulent pathogens down$^{10}$.

Nitrogen fixation in mangrove ecosystem

Nitrogen fixation is a process of conversion of gaseous forms of Nitrogen (N$_2$) into combined
forms i.e. ammonia or organic nitrogen by some bacteri and cyanobacteria. Free living as well as symbiotic microbes known as diazotrophs which fix N\textsubscript{2} into proteins. Nitrogen-fixing microorganisms can colonize in both terrestrial as well as marine environments. N\textsubscript{2} fixation in mangrove sediments is likely to be limited by insufficient energy sources. The low rates of N\textsubscript{2} fixation by heterotrophic bacteria detected in marine water are probably due to lack of energy sources. Nitrogen fixation by heterotrophic bacteria can be regulated by specific environmental factors such as oxygen, combined nitrogen and the availability of carbon source to support energy requirement. Energy for N\textsubscript{2} fixation can also be derived from leaves and roots decomposed by nondiazotrophic microflora that colonize dead mangrove leaves\textsuperscript{11,12}. Nitrogen-fixing bacteria such as members of the genera Azospirillum, Azotobacter, Rhizobium, Clostridium and Klebsiella were isolated from the sediments, rhizosphere and root surfaces of various mangrove species. Several strains of diazotrophic bacteria such as Vibrio campbelli, Listonella anguillarum, V. aestuarianus, and Phyllobacterium sp. were isolated from the rhizosphere of the mangroves in Mexico\textsuperscript{13}. In a mangrove in Florida, biological N\textsubscript{2} fixation could supply up to 60% of the nitrogen requirement\textsuperscript{11}. The main factors influencing N\textsubscript{2} fixation are light intensity and water temperature\textsuperscript{14}. It is also possible that microorganisms associated with Languncularia racemosa including Pseudomonas stutzeri\textsuperscript{15} could also be responsible for the fixation of atmospheric nitrogen\textsuperscript{16,17,18}. N\textsubscript{2} fixing bacteria are efficient at using a variety of mangrove substrates despite differences in carbon content and phenol concentrations\textsuperscript{19}. However, their abundance may be dependent on physical conditions and mangrove community composition. N\textsubscript{2} fixing Azotobacter, which can be used as biofertilizers, were abundant in the mangrove habitats of Pichavaram\textsuperscript{20}. Two halotolerant N\textsubscript{2} fixing Rhizobium strains were isolated from root nodules of Derris scandens and Sesbania species growing in the mangrove swamps of the Sunderbans\textsuperscript{21}. When the non N\textsubscript{2} fixing bacteria were removed from the rhizosphere it was found that the N\textsubscript{2} fixing activity dropped, indicating that other rhizosphere bacteria could also contribute to the fixation process\textsuperscript{13}. The non-N\textsubscript{2} fixer, Staphylococcus sp., isolated from mangrove roots also promotes N\textsubscript{2} fixation by Azospirillum brasilense\textsuperscript{22}.

**Phosphate solubilising bacteria**

Phosphate solubilizing bacteria which act as potential suppliers of soluble forms of phosphorus have a great advantage for mangrove plants. Certain bacteria exhibit high phosphatase activity, capable of solubilizing phosphate\textsuperscript{23}. In an arid mangrove ecosystem in Mexico, nine strains of phosphate-solubilizing bacteria such as Bacillus amyloliquefaciens, B. atrophaeus, Paenibacillus macerans, Xanthobacter agilis, Vibrio proteolyticus, Enterobacter aerogenes, E. taylorae, E. asburiae, and Kluyvera oëryocrescens were isolated from black mangrove (Avicenia germinant) roots. Further three strains viz. B. licheniformis, Chryseomonas luteola and Pseudomonas stutzeri were isolated from white mangrove (Languncularia racemosa) roots. This is the only report of the phosphate-solubilizing capacity of bacteria belonging to the genera Xanthobacter, Kluyvera and Chryseomonas, and of their presence in mangrove roots. The mechanism responsible for phosphate solubilization, in at least six of the above bacterial species, probably involved production of organic acids. Some of the organic acids might act as chelators displacing metals from phosphate complexes\textsuperscript{15}.

**Sulfate reducing Bacteria**

Mangrove sediments are mainly anaerobic with an overlying thin aerobic sediment layer. Degradation of organic matter in the aerobic zone occurs principally through aerobic respiration whereas in the anaerobic layer decomposition occurs mainly through sulfate-reduction\textsuperscript{24,25}. Sulfate reduction accounts for almost 100% of the total emission of CO\textsubscript{2} from the sediment\textsuperscript{26}. Sulfate-reducing bacteria isolated from a temperate coastal marine sediment from shallow, brackish water in Denmark could degrade up to 53% of the total organic matter\textsuperscript{27}. In Goa’s mangroves, eight species of sulfate-reducing bacteria such as Desulfovibrio desulfuricans, Desulfovibrio desulfuricans aestuarii, Desulfovibrio salexigens, Desulfovibrio sapovorans, Desulfitomaculum orientis, Desulfitomaculum acetoxidans, Desulfosarcina variabilis, and Desulfofoccus multivorans were isolated and tentatively classified within four different genera. These strains are nutritionally versatile and they have the ability to metabolize a wide range of simple compounds including lactate, acetate, propionate, butyrate, and benzoate. The ability to use several different substrates may allow these microbes to compete effectively for nutrients in the mangrove environment\textsuperscript{28}. In mangrove sediments, availability of iron and phosphorus may depend on the activity of sulfate-reducing bacteria\textsuperscript{25,27}.
All sediments (associated or not associated with the plants) in Florida's mangroves contained a significant population of sulfate reducing bacteria that were able to fix N\textsubscript{2}\textsuperscript{11}.

**Photosynthetic anoxygenic bacteria**

Photosynthetic anoxygenic bacteria use hydrogen sulfide (or other reduced inorganic sulfur) instead of water as an electron donor in the photosynthetic reaction. Photosynthetic bacteria of the mangrove sediments include two major groups viz., purple sulfur bacteria (family Chromatiaceae, strains of the genera Chromatium) and purple non-sulfur bacteria (family Rhodospirillaceae, strains belonging to Rhodopseudomonas sp.). Sulfur rich mangrove ecosystems, which have mainly anaerobic soil environments, provide favorable conditions for the proliferation of these bacteria. It has been reported in few papers about the presence of anoxygenic photosynthetic bacteria in mangrove environments and one reason behind this may be that some of these bacteria are slow growers and difficult to handle in the laboratory. Nevertheless, representatives of the families Chromatiaceae (purple sulfur bacteria) and Rhodospirillaceae (purple nonsulfur bacteria) were found in Indian mangrove sediments\textsuperscript{29,30}. The predominant bacteria in the mangrove ecosystem of Cochin (India) were identified as members of the genera Chloronema, Chromatium, Beggiatoa, Thiopedia, and Leucothiobacteri\textsuperscript{31,32}. Large populations of Chromatium grew in enrichment cultures made of Florida's mangrove sediments. In mangroves on the coast of the Red Sea in Egypt, 225 isolates of purple nonsulfur bacteria belonging to ten species, representing four different genera, were identified. The strains were isolated from water, mud, and roots of Avicena marina samples. Nine of the ten species inhabited the rhizosphere and root surface of the trees. The most common genera such as Rhodobacter and Rhodopseudomonas were detected in 73% and 80% of the samples respectively\textsuperscript{33}. Some of the photosynthetic anoxygenic bacteria were also diazotrophic. Although there is yet no published evidence, one can hypothesize that photosynthetic anoxygenic bacteria, the predominant photosynthetic organisms in anaerobic environments, may contribute to the productivity of the mangrove ecosystems.

**Methanogenic Bacteria**

Methanogenic bacteria are probably an important component of the bacterial community in mangrove ecosystems. In an Indian mangrove ecosystem, the methanogenic bacteria population in the sediments fluctuated during the year from 3.6×10\textsuperscript{7} to 1.1×10\textsuperscript{5} cfug\textsuperscript{-1} wet sediment, depending on temperature, pH, redox potential, and salinity of the water and sediments\textsuperscript{34}. The presence of sulfate-reducing bacteria limits the proliferation of these bacteria\textsuperscript{35}, a strain of the methanogenic bacterium, Methanococcoides methylutens\textsuperscript{36}, and four strains of unidentified thermotolerant methanogenic bacteria\textsuperscript{37} were isolated from sediment of a mangrove forest. Methane may have been oxidized under anoxic conditions as occurred in hypersaline microbial mats\textsuperscript{38} and anoxic marine sediment\textsuperscript{39}. Another mangrove ecosystem cleared for aquaculture also showed significantly more methanodynamic activity (a dynamic system of methane production, oxidation, and emission)\textsuperscript{40}. These results suggest that the potential of mangrove soils to emit methane was higher when there is anthropogenic activity\textsuperscript{41}.

A methanogenic bacterium, Methanococcoides methylutens, was isolated and characterized from the sediment of mangrove environment of Pichavaram, Southeast India\textsuperscript{42}. Methanogenic bacteria were high during summer and pre-monsoon and low during monsoon and post-monsoon\textsuperscript{43}.

**Enzyme producing bacteria**

About 71% of bacterial strains produce L-asparaginase. It is an enzyme drug of choice used in combination therapy for treating acute lymphoblastic leukemia in children. Since extraction of L-asparaginase from mammalian cells is difficult, microorganisms have proved to be a better alternative for L-asparaginase extraction, thus facilitating its large scale production. Mashburn and Wriston successfully purified Escherichia coli L-asparaginase and demonstrated its tumouricidal activity. Halophilic bacteria (Halococcus) isolated from mangrove sediments, produce L-asparaginase\textsuperscript{44}. Arylsulfatase, an important enzyme that participates in the metabolism of sulphuric acid esters, produced predominantly by Bacillus, followed by Vibrio. One hundred and eight strains of bacteria show chitin degrading activity\textsuperscript{45}.

**Fungi**

Mangrove areas or mangals are home to a group of fungi called “mangicolous fungi”. These organisms are vitally important for nutrient cycling in these habitats\textsuperscript{46,47} and are able to synthesize all the
necessary enzymes to degrade lignin, cellulose, and other plant components. Hyde listed 120 species from 29 mangrove forests around the world. These included 87 Ascomycetes, 31 Deuteromycetes, and 2 Basidiomycetes. In mangrove communities, over a hundred species of fungi were identified. About 48 fungal species were found in decomposing Rhizophora debris in Pichavaram, South India. Most of the studies involving fungi are of a descriptive nature, designed for taxonomic and inventory interests. Fungal hyphae are commonly found on and in decomposing mangrove leaves and wood. In a mangrove from the coast of the Indian Ocean, Hyde identified 67 species of marine fungi and found an additional 20 unidentified species associated with mangrove roots and dead branches.

In addition to degrading lignin and cellulose, the fungi Cladosporium herbarum, Fusarium moniliforme, Cirrenalia basiminuta, an unidentified hyphomycete and Halophytophthora vesicula isolated from the dead leaves of Rhizophora apiculata also show pectinolytic, proteolytic, and amylolytic activity. These fungi begin the decomposition of vegetative material and thereby allow secondary colonization by bacteria and yeasts that further decompose the organic matter. In an Indian mangrove, the first colonizers of fallen mangrove leaves were fungi and thraustochytrids (fungi-like unicellular protists). It is possible that both thraustochytrids and fungi tolerate high levels of phenolic compounds in the leaves of mangroves that inhibit the growth of other microorganisms. Despite the great wealth of systematic information, there is a little knowledge about the role of mangrove fungi in nutrient recycling. A few researchers have studied the physiology and biochemistry of manglicolous fungi. Many of the species produce interesting compounds. For example, most of the soil fungi produce lignocellulose-modifying exoenzymes like laccase. Preussia aurantiaca synthesizes two new depsidones (Auranticins A and B) that display antimicrobial activity.

**Actinomycetes**

Actinomycetes play a quite important role in natural ecological system and they are also profile producers of antibiotics, antitumor agents, enzymes, enzyme inhibitors and immunomodifiers which have been widely applied in industry, agriculture, forestry and pharmaceutical industry. The actinomycetes population density is less common in marine sediments relative to terrestrial soils. In the past, the research work on actinomycetes was mainly concentrated on that of common habitats. Actinomycetes resources under extreme environments (including extreme high and low temperature, extreme high or low pH, high salt concentration etc.) have received comparatively little attention from microbiologists. The mangrove environment is a potent source for the isolation of antibiotic-producing actinomycetes. An antibiotic compound-beta-unsaturated gama-lactone from Streptomyces grisebrunneus, showing wide-range anti-microbial activity, was isolated and identified. Besides this, the Streptomycetes produce cellulase that degrades cellulolytic waste materials. The actinomycetes, inhabiting the sediments and molluscs of Vellar estuary are the potential sources of extracellular enzymes involved in the marine environment. This would suggest that bioprospecting of enzymes of industrial needs can be made from these marine actinomycetes. There is a scope for the use of S. galbus as an ideal organism for the industrial production of extracellular L-glutaminase and this can be pursued further. Different strains of actinomycetes isolated from the sediments of the Vellar estuary, viz. S. alboniger, S. vastus, S. violaceus, S. moderatus and S. aureofasciculus elucidates interesting information on the antibiotic producing properties and possesses bioactive properties isolated from the Pichavaram mangrove environment. Actinopolyspora sp. isolated from the west coast of India, showed good antimicrobial activity against gram positive bacteria like S. aureus, S. epidermis, B. subtilis and fungi such as A. niger, A. fumigatus, A. flavus, F. oxysporum, Penicillium sp. and Trichoderma sp. where as it did not show any antimicrobial activity against gram-negative bacteria such as E. coli, P. aeruginosa, S. marcescens, E. aerogens and fungi like C. albicans and Cryptococcus Sp.

**Other potential usefulness of microbes from mangrove ecosystems**

Besides these above microbes, there is the presence of other potential microbes in mangrove ecosystems on which much studies has not been done yet. In addition to processing nutrients, mangrove bacteria may also help in processing industrial wastes. Iron-reducing bacteria were common in mangrove habitats.
in some mining areas. Eighteen bacterial isolates that metabolize waste drilling fluid were collected from a mangrove swamp in Nigeria. Interestingly, four additional bacterial strains isolated from the same swamp depress growth rates of *Staphylococcus* and *Pseudomonas* species and could, therefore, decrease normal rates of organic decomposition. Other mangrove bacteria are parasitic or pathogenic. *Bdellovibrios* capable of parasitizing *Vibrio* sp. are common in an Australian mangrove habitat. Also in Australia, *Bacillus thuringiensis*, which showed insecticidal activity against mosquito larvae of *Anopheles maculatus*, *Aedes aegypti* and *Culex quinquefasciatus*, has been isolated from mangrove sediments. Nine species of purple non-sulfur bacteria have also been found in mangroves of Egypt. Growth of the purple sulfur bacteria in these habitats was limited by low light and sulfide. Certain bacterial strains such as *Pseudomonas mesophilica*, *P. caryophylls* and *Bacillus cereus* exhibit magnetic behaviour which may be called magnetobacteria isolated from mangrove sediments of Pichavaram, Southeast India. The total heterotrophic bacterial counts in the degrading materials such as polythene bags and plastic cups were recorded up to higher in comparison with fungi in an Indian mangrove soil. The microbial species found associated with the degrading materials were identified as five Gram positive and two Gram negative bacteria, and eight fungal species of *Aspergillus*. The species that were predominant were *Streptococcus*, *Staphylococcus*, *Micrococcus* (Gram+ve), *Moraxella*, and *Pseudomonas* (Gram-ve) and two species of fungi (*Aspergillus gloocus* and *A. niger*). Among the bacteria, *Pseudomonas* sp. degraded 20.54% of polythene and 8.16% of plastics in one-month period. Among the fungal species, *Aspergillus gloocus* degraded 28.80% of polythene and 7.26% of plastics in one-month period. This work reveals that the mangrove soil is a good source of microbes capable of degrading polythene and plastics. Hydrocarbon-utilizing microorganisms were isolated by enrichment techniques from soil and water samples collected from an oil spill site in the Niger delta area of Nigeria. The isolates included species of *Micrococcus*, *Pseudomonas*, *Bacillus*, *Aeromonas*, *Serratia*, *Proteus*, *Penicillium*, *Aspergillus*, *Candida*, *Geotrichum* and *Rhizopus*. Amanchukwu *et al.* demonstrated similarly that lower concentrations (1.5-6.0%) of hydrocarbons were highly utilized by *Schizosaccharomyces pombe* than higher concentrations (12%).

**Mangrove reforestation and conservation using Plant Growth Promoting Bacteria (PGPB)**

Mangrove greenbelts were known to offer some protection against destructive ocean events, such as tsunamis and tropical cyclones, but they have not always been valued for that function. It may be possible to use PGPB to speed up the development of mangrove plantlets for reforestation of the damaged areas or even to create artificial mangrove wetlands out of wastelands. PGPB promotes the plant growth by mechanisms such as N$_2$ fixation, phosphate solubilization, phytohormone production, siderophore synthesis, or biocontrol of phytopathogens. PGPB specific to mangrove ecosystems are unknown. Scanning electron microscope studies revealed that, in seawater *in vitro*, a dense population of *Azospirillum brasilense* and *A. alopreferens* successfully colonized black mangrove roots, establishing an association with the plant within four days. Inoculation of black mangrove plantlets with the cyanobacterium *M. chthonoplastes* yielded copious root colonization in a thick mucilaginous sheath which resulted in an increased in N$_2$ fixation and nitrogen accumulation in inoculated seedlings. Many studies of plant-growth promotion by beneficial bacteria have reported the advantage of using mixed cultures of microorganisms over pure cultures. It can be concluded that PGPB will effectively promote the growth of mangrove plantlets which will be helpful in mangrove reforestation.

**Conclusion**

Mangrove ecosystems provide shelter and nurturing sites for many marine microorganisms. Conservation strategies for mangroves should consider the ecosystem as a biological entity, which includes all the physical, chemical, and ecological processes that maintain productive mangroves. Despite of various studies on the biogeography, botany, zoology, ichthyology, environmental pollution and economic impact of mangroves, little is known about the activities of microbes in mangrove waters and sediments. Due to the presence of rich source of nutrients mangroves are called the homeland of microbes. Extensive exploration, identification, isolation and screening is suggested in search of new leads for microbial drugs.

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