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Depth integrated variation in the distribution of soil bacteria and its enzyme production from Ayiramthengu mangrove ecosystem of Kerala coast, India

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Abstract

The present study focused on the distribution of bacteria at three different depths of soil column at three different sampling stations. Total heterotrophic bacteria population revealed that bacterial count decreased from upper zone to lower zone. Percentage composition of gram negative bacteria (70%) was abundant than gram positive forms. The overall generic composition revealed that *Micrococcus* sp. and *Acinetobacter* sp. (13% each) were the dominant genera. Enzyme production of bacteria revealed that phosphatase producers were comparatively higher than other studied enzymes at three different depths. Hence it could be inferred that Ayiramthengu mangrove ecosystem harbours diverse novel bacterial composition that differs along various depth of soil column that have the ability in enzyme production.

Keywords: Soil, Heterotrophic bacteria, Mangrove, Depth, Enzyme

Introduction

Mangroves are unique ecosystem adapted to saline coastal soil. Mangroves comprise a substantial portion of tropical coastal biodiversity that occupy less than 1% of the world's surface and are mostly found between the Tropic of Cancer and the Tropic of Capricorn. Mangrove ecosystems have high habitat heterogeneity as they are in the ecotones of land-sea-estuary (Chen et al., 2016).

Microorganisms have fundamental role in mangrove environment, with special importance in controlling geochemical habitat (Holguin et al., 2001). The productivity, conservation and recovery of mangroves maintained by the diversity and activity of microbes (Alongi, 1996). Bacteria have a pivotal role in carbon, nitrogen, sulphur and phosphorous cycles within the mangrove (Rojas et al., 2001). Several groups of bacteria were present in mangrove performing various activities like nitrogen fixation, phosphate solubilisation, photosynthesis, cellulose degradation, sulphur oxidization and methanogenesis (Thatoi et al., 2013).

It has been found that mangrove ecosystems are a possible source for discovering a variety of bacterial species that generate enzymes, proteins, antibiotics, and contain genes for salt tolerance. Most of these bacterial species have the potential to be used in the future. Because of their enormous capacity for production, low cost of use, and ease of genetic modification, bacteria are crucial for the manufacture of enzymes (Thatoi et al., 2013). Therefore, the present study deals with the isolation of bacteria and its enzyme production from three depths of soil from Ayiramthengu mangrove ecosystem, Kerala, India.

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Materials and Methods

Study Area

Ayiramthengu mangrove ecosystem (lat. 9° 07' 30"- 9° 07' 40" N and long. 76° 28' 40"- 76° 28' 50" E) was the study area, which was divided into three sampling stations. Station 1 lies close to the land area; Station 2 situated in the middle of the mangrove and Station 3 was the area adjoining the estuary.

Sampling

Soil samples (1gm) were collected aseptically from three sampling stations at three different depths namely, upper zone (0-5 cm), middle zone (5-10 cm) and lower zone (10-15 cm) using a pipe core sampler (2 cm diameter). Samples (1gm) were transferred into sterile labelled containers with 25% v/v of glycerol prepared in sterile 50% sea water and were immediately transported in an ice box. Samples were immediately taken to the laboratory for the further analysis.

Cultivation and enumeration of Bacteria

The samples were serially diluted in sterile sea water and plated on to ZoBell marine agar (Hi Media, India) using standard plate count method. Then incubated at 28 ± 2°C for 48-72 hours to estimate the Total Heterotrophic Bacteria (THB) present in the sample. The colonies were counted and expressed in colony forming units per gm weight of sample.

CFU/g = Number of colonies counted × (1/dilution factor) × (1/volume plated(ml))

Isolation and purification of heterotrophic bacteria

The individual bacterial colony were isolated and purified for the study. The individual bacterial colonies that developed were isolated and purified. The purified isolates were identified up to generic level based on cell morphology and biochemical reactions as per Bergey's Manual of Determinative Bacteriology (Holt et al., 2000).

Enzyme Production

The isolates which were fast growing, and which could produce at least one of the four evaluated enzymes were chosen. All the pure isolates were tested for various enzymes like phosphatase, lipase, cellulase and amylase. Phosphatase enzyme production was carried out by the method of Baird-Parker (1966). The plate assay for lipase enzyme production was carried out by following Sierra (1957). Cellulase enzyme production was screened according to the method of Hankin and Anagnostakis (1977). Amylase enzyme production was done using plate assay method following Mac Faddin (1980).

Statistical Analysis

Statistical calculations were done for Total Heterotrophic Bacteria (THB) at various depths using Anova and Post- Hoc test by SPSS software.

Result

Soil sample was studied at three various depths (upper, middle and lower) along three different sampling stations. The result revealed that THB count showed a decreasing trend from upper layer to the lower layer. One-way ANOVA result revealed that the three layers showed a significant difference in bacterial density ($p < 0.05$) (Table 1) and the post hoc analysis showed that a significant difference was found between upper and lower layers and also between upper and middle layer ($p < 0.05$) but not significant between middle and lower layer ($p > 0.05$) (Table 2).

During the study, Gram-negative bacteria (GN) were relatively abundant, being grouped under 19 genera (70%), while Gram positive bacteria (GP) contributed only 8 genera (30%).

Table 1 One-way ANOVA for soil heterotrophic bacteria at various depths

ANOVA

Total Heterotrophic Bacteria

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	101.630	2	50.815	14.089	.000
Within Groups	378.694	105	3.607		
Total	480.324	107			

Table 2 Post hoc analysis for soil heterotrophic bacteria at various depths

Multiple Comparisons

Dependent Variable: Total Heterotrophic Bacteria

Tukey HSD

(I) Element	(J) Element	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Soil Upper	Soil Middle	1.5556*	.4476	.002	.491	2.620
	Soil Lower	2.3333*	.4476	.000	1.269	3.398
Soil Middle	Soil Upper	-1.5556*	.4476	.002	-2.620	-.491
	Soil Lower	.7778	.4476	.196	-.286	1.842
Soil Lower	Soil Upper	-2.3333*	.4476	.000	-3.398	-1.269
	Soil Middle	-.7778	.4476	.196	-1.842	.286

*. The mean difference is significant at the 0.05 level.

The overall percentage dominance of soil heterotrophic bacteria isolated during study was presented in Fig. 1. The result showed that *Micrococcus* sp. and *Acinetobacter* sp. (13% each) was the dominant genera. This was followed by *Bacillus* sp. (9%), *Pseudomonas* sp. (7%), *Acetobacter* sp. (6%), *Moraxella* sp., *Beijernckia* sp. and *Alcaligenes* sp. (5% each), *Alteromonas* sp. (4%), *Staphylococcus* sp., *Xanthomonas* sp., *Vibrio* sp., *Rhizobacter* sp., *Deinococcus* sp. and *Planococcus* sp. (3% each), *Streptococcus* sp., *Flavobacterium* sp., *Klebsiella* sp., *Derxia* sp. and *Proteus* sp. (2%), *Arthrobacter* sp., *Escherichia* sp., *Salmonella* sp., *Citrobacter* sp. and *Enterococcus* sp. (1% each).

About 37% of isolates were isolated from the upper region followed by lower region (32%) and middle region (31%) for enzyme production study. The isolates from the upper region showed 78% phospholytic and 70% lipolytic forms were predominant in relation to cellulolytic (52%) and amylolytic (48%) forms. The middle region showed 80% phosphatase enzyme production followed by lipase (77%), amylase (55%) and cellulase (49%) enzymes production. The lower region characterized by the predominance of phosphatase producers (74%) on considering the lipase (70%), cellulase (54%) and amylase (47%) producers. The highest percentage of phosphatase, lipase and amylase enzyme production was ob-

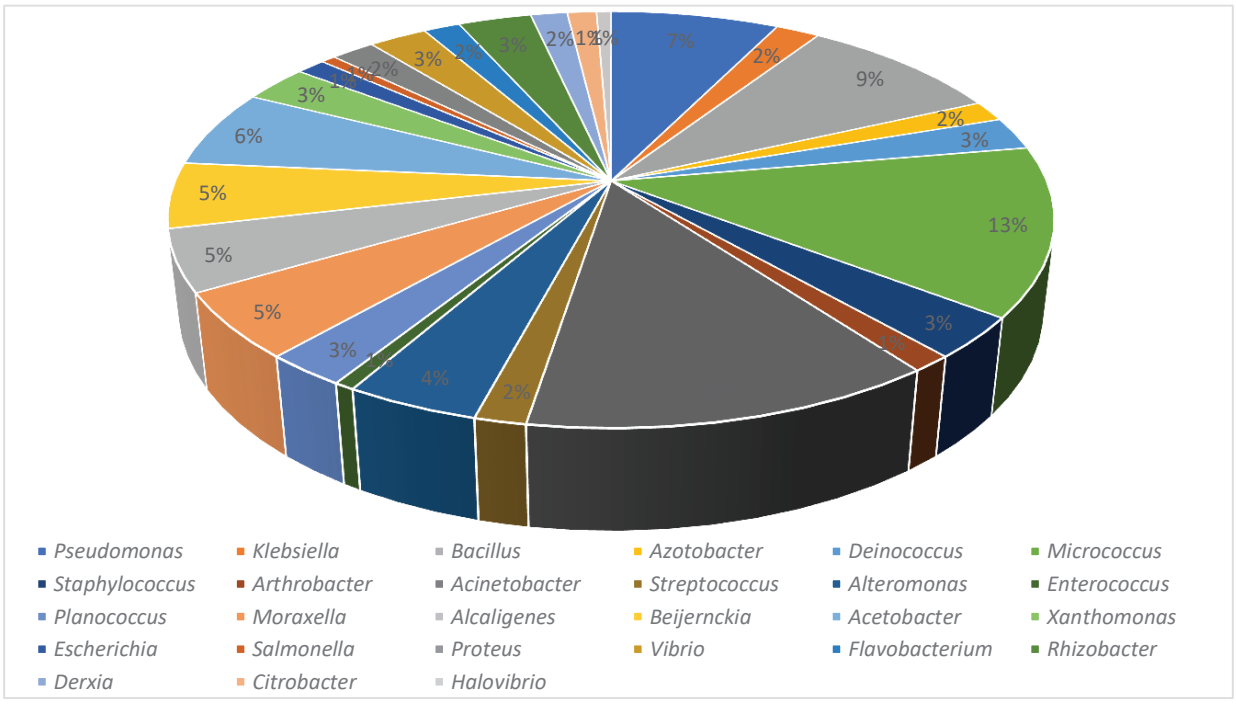

Figure 1. Percentage dominance of soil heterotrophic bacteria

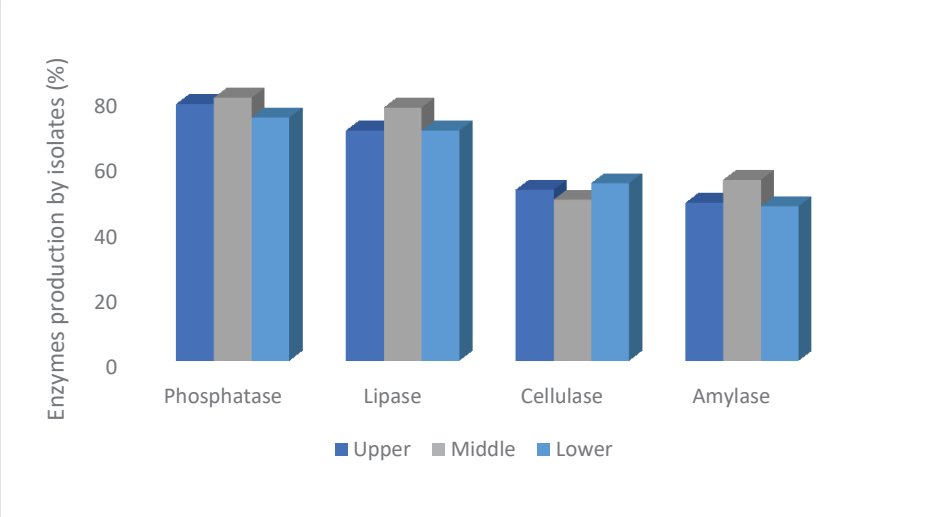

Figure 2. Extracellular enzyme production of soil heterotrophic bacteria (Mean) in different depth ranges

served in the middle region whereas cellulase enzyme in the lower region (Fig. 2).

Discussion

A huge composition of bacterial load was isolated from soil sample heterotrophic bacteria soil sample. These bacteria may get involved in the detritus decomposition of litter fall on the sediment surface. Studies on mangrove microbial community in the sediments are essential in recognizing the process of biogeochemical cycling (Roy et al., 2002). Bacteria may behave as a primary decomposer, which use small amount of dissolved organic substances and absorb dissolved inorganic substances such as nitrate and phosphate (Thatoi et al., 2013).

Three samples were studied from three depths of the soil and the result indicated that the bacterial density was high at the upper zone followed by middle and lower zone. The upper zone has an enormous number of bacteria that can convert the dead and decaying matter into nutrients. As the depth increases down the soil column, the dead and decaying matter become reduced thereby the aerobic culturable bacteria remain reduced. Hence the middle and lower zone under study showed a minimized density of bacteria compared to the upper region. The upper surface of sediment contains phototrophic and heterotrophic microorganisms that were responsible for sediment stabilization against resuspension, plant growth promotion and chelation of toxic metals and other contaminants (Decho, 2000; Bouchez et al., 2013). A change in bacterial pattern was reported on studying the depth integrated microbial community in mangrove soil of Sundarbans (Das et al., 2012).

One of the dominant group of bacteria observed during the present study was *Acinetobacter* sp. *Acinetobacter* involved in degradation pathways of various long-chain dicarboxylic acids and aromatic and hydroxylated aromatic compounds (Yoshida et al., 1975). Another dominant genus was *Micrococcus* sp., play an important role in degradation and nutrient cycling. Also, Venkateswaran and Natarajan (1983) reported *Micrococcus* as a phosphate solubilizing bacteria from mangrove biotopes in Porto Novo, Chennai water and sediment. *Bacillus* as a rhizosphere organism in mangroves and recommended that they should be aimed to provide microbial resolutions which improve polluted environments. The occurrence of *Bacillus* sp. in mangrove ecosystem has been well documented (Lee et al., 2014; Deivanai et al., 2014; Castro et al., 2014; Eldeen et al., 2015).

Pseudomonas has a major role in the turnover of certain nutrients like nitrogen, carbon and phosphorous appeared in the leaf biomass (Robertson and Daniel, 1989; Mumby et al., 2004; Romero et al., 2005). Nitrogen fixing bacteria included *Azospirillum* sp., *Azotobacter* sp. and *Klebsiella* sp., and phosphate-solubilizing bacteria were *Bacillus* sp., *Paenibacillus* sp., *Vibrio* sp. and *Pseudomonas* sp. (Thatoi et al., 2013). Ambeng et al. (2019) reported *Bacillus*, *Staphylococcus*, *Vibrio*, *Micrococcus*, *Alteromonas* and *Escherichia* from mangrove sediments of Pangkajene River estuary. Certain bacteria like *Streptococcus*, *Staphylococcus*, *Micrococcus*, *Moraxella* and *Pseudomonas* found associated with degrading materials in Indian mangrove soil (Kathiresan, 2003). Many bacterial genera including *Bacillus*, *Micrococcus*, *Alteromonas*, *Escherichia*, *Xanthomonas*, *Moraxella*, *Alcaligenes*, *Proteus*, *Citrobacter*, *Acetobacter*, *Streptococcus*, *Aeromonas* and *Planococcus* were reported from different mangroves (Essien et al., 2013; Castro et al. , 2014; Saseeswari et al., 2016).

The overall highest enzyme producing isolates' percentage was observed in the middle layer of soil for phosphatase, lipase and amylase enzymes. This may be due to the reason that the degradation process occurs

at this layer. Also, the enzyme producing bacteria for specific enzyme of study may be abundant in this layer. In the case of cellulase enzyme highest production of isolates found in lower layer that corroborates with the finding of Das, et al. (2012) in Sundarbans mangrove soil that cellulase bacteria increases with increase in depth. They also stated that phosphate solubilizing bacteria decreases with increase in depth which was also observed here.

Soil microbial communities perform a crucial role in the process of nutrient recycling and in the conservation of mangrove health. Hence the present study depicted that mangrove soil contain variety of bacteria. These bacteria varied along different depths of soil column based on the role played by it. Studies on microbial ecology in this complex mangrove environment are essential for the better understanding of bacterial responses to ecosystem functioning and the diverse extracellular enzymes study provide a resource for novel biocatalysts discovery and its industrial application.

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