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Distribution and extracellular enzyme production of cultivable bacteria isolated from pneumatophores of Ayiramthengu mangrove ecosystem of Kerala coast

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Original Article

Abstract

The present study focuses on the distribution of cultivable heterotrophic bacteria and their extracellular enzyme production. The study was conducted during 2018 at three sampling stations of Ayiramthengu mangrove ecosystem. The bacteria were isolated from two distinct regions such as upper and lower pneumatophore surface from each sampling sites. The different bacterial genera isolated were *Pseudomonas* sp., *Acinetobacter* sp., *Moraxella* sp., *Streptoccoccus* sp., *Deinococcus* sp., *Micrococcus* sp., *Paenibacillus* sp. and *Staphylococcus* sp., of which *Pseudomonas* sp. was the dominant one. Mean bacterial population in upper region of pneumatophores was less than the lower region in all the studied sites. Additionally, there was a significant difference ($p < 0.05$) in the bacterial population in both upper and lower regions of pneumatophores between different stations. Production of extracellular enzymes namely amylase, cellulase, lipase and phosphatase were screened in the isolated strains. The study showed that phosphatase producing bacteria (78%) was predominant, followed by lipase (75%), cellulase (71%) and amylase (52%). The study indicated the fact that the dynamic characteristics of mangroves provides a nurturing site for diverse bacteria, which were even capable to produce various enzymes.

Keywords: *Mangrove, pneumatophores, heterotrophic bacteria, enzymes*

Introduction

Mangroves are unique productive ecosystem that caters to numerous organisms ranging from micro to macro level. Microbial communities present in the mangroves play significant role in photosynthesis, decomposition, methanogenesis, agarolysis, nutrient cycling, production of metabolites like antibiotics, exopolysaccharide, and enzymes (Lakshmipriya and Sivakumar, 2013). Bacteria and fungi stand for 91% of the total biomass in tropical mangroves whereas algae and protozoa are 7% and 2% respectively (Alongi, 1988). Ability of microbes to exist in mangroves with peculiar ecological condition marks its wide range of adaptability.

Mangrove species have many specialized root systems to overcome adverse conditions. Pneumatophores are aerial roots which arise vertically from cable roots (Tomlinson, 1986). They are slender, cone shaped structures standing erect with 1-20 m or more in length and spreads horizontally in the soil (Yanez-Espinosa and Flores, 2011). It helps in continuous oxygen diffusion, which is deficient in its habitat. Photosynthetic reaction occurs at the surface of pneumatophore and plays an important role in gaseous exchange. The oxygen produced by photosynthesis diffuses to the root in the sub soil and it is used for root respiration.

Additionally, respiratory induced carbon dioxide diffuses to the pneumatophore and is used for photosynthesis in the pneumatophore (Yabuki *et al.*, 1990; Scholander *et al.*, 1955). Pneumatophores have been found to support rich flora of bacteria and plays a significant role in nitrogen fixation (Naidoo *et al.*, 2008).

Microorganisms isolated from mangrove ecosystem have biotechnological significance due to the presence of enzymes, proteins, antibiotics and salt tolerant genes (Thatoi *et al.*, 2013). Microbes are important for enzymatic production due to their high production capability, low cost and susceptibility to gene manipulation (Castro *et al.*, 2014). Increasing demand in various fields like food industry, detergent and textile production, agriculture, pharmaceuticals, therapeuticals and molecular biology for microbial enzymes are high in demand (Quecine *et al.*, 2011). The bacterial study in pneumatophores of mangrove ecosystem is handful which draws attention to this investigation. The present study focuses on the distribution of cultivable heterotrophic bacteria from two distinct regions of pneumatophores of Ayiramthengu mangrove and their production of extracellular enzymes.

Material and methods

Study Area

The samples were collected from Ayiramthengu mangrove ecosystem (Fig. 1). Three stations were selected for study–Station 1 (near to the land area of the mangrove), Station 2 (towards the middle of the mangrove) and Station 3 (near to the estuary). The study was conducted from June to September, 2018.

Sample Collection

Pneumatophore surface were washed with sterilized sea water to remove the surface contaminants. Biofilms from the outer surface of pneumatophores were collected using sterile knife and aseptically transferred into sterile bottles. Two distinct areas of pneumatophores were chosen as upper region (above the tidal range) and lower region (within the tidal range). The samples were immediately preserved at -20°C and analysed in laboratory. Water quality parameters like temperature (using standard mercury thermometer), pH, dissolved oxygen, salinity, phosphate and nitrate were determined following standard

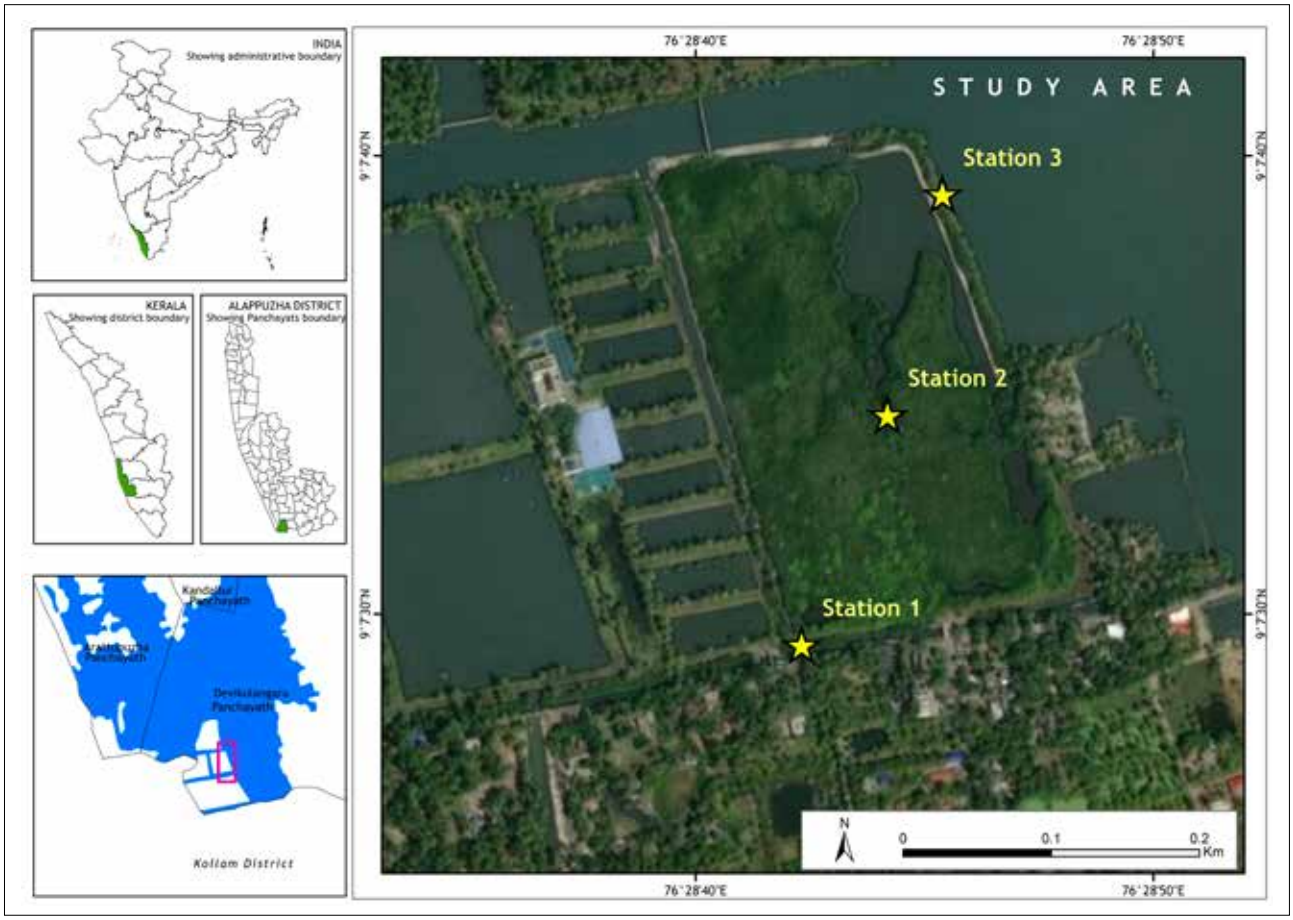

Fig. 1. Sampling Site in the present study (Ayiramthengu mangrove ecosystem)

methods (APHA, 2005) and average monthly values from different stations were taken for the study.

Estimation of Total Heterotrophic Bacteria (THB)

THB estimation was done by standard plate method using Zobell Marine Agar (Hi- Media). The plates were incubated for 48 hours at 28±2° C. The colony forming units (cfu) were calculated. The colonies were isolated and purified on saline nutrient agar medium. The cultures were identified up to generic level using Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 2000).

Extracellular Enzyme Production

The bacterial strains were studied for extracellular enzyme production by agar diffusion method (Gerhardt *et al.,* 1981). Nutrient agar medium in saline (10%) supplemented with 1% tributyrin for lipase, 0.5% starch for amylase, 0.5% carboxy methyl cellulose for cellulase and 0.01% phenolphthalein diphosphate for phosphatase test was spot inoculated and incubated for 3-5days. Cultures positive for lipase showed a clear zone around the spot of inoculation after 2-3 days. After incubation for 24-48 hours, cultures positive for amylase showed clear zone when treated with Lugol's iodine solution. The cellulase activity was determined after the incubation by flooding Congo red on carboxy methyl cellulose agar and further incubated for 15 minutes at room temperature. A clearance zone against the bright red background indicated the production of cellulase. For phosphatase test, after an incubation of 2-3 days, a pink coloration developed around the colonies on phenolphthalein agar when the plates were exposed to liquid ammonia.

Statistical Analysis

One-way ANOVA was used to find out and compare the means between the bacterial population among different stations with $p < 0.05$ to represent significant difference and t-test was used to test the significant difference in bacterial population between lower and upper region using SPSS software (version 21). Similarity between different stations were computed using Primer software package for Windows.

Results and discussion

The bacterial population at upper and lower region of pneumatophore in all three stations were normally distributed and there was homogeneity of variance. There was significant difference ($p < 0.05$) in the bacterial population of upper and lower regions of pneumatophores between three stations (Table 1). This difference in bacterial population might be due to the influence of human activities and runoff from the nearby land area. But the absolute difference in bacterial population between upper and lower surfaces was not significantly different ($p > 0.05$) among the stations. Tam (1998) and Cotano and Villate (2006) observed that the release of effluents contributes greatly to the increase of bacterial population. High nutrient concentration favours high mangrove bacterial abundance. The lower level of pollutants might be responsible for decrease in bacterial population at Station 2. Grisi and Lira (2010) reported a study in mangrove habitat of Paraiba do Norte estuary as the microbial population decreases with lower nutrients.

The total heterotrophic bacteria revealed that the mean bacterial population of upper region sample contained lower count compared to the sample of lower region of pneumatophore. The upper region recorded the bacterial count as 68×10^4 cfu/

Table 1. Analysis of variance (one-way ANOVA) for bacterial population in different stations

	Descriptives							
	Station	Mean	Std. Deviation	Std. Error	Minimum	Maximum	F Statistic	P-Value
Bacterial Population Upper (x 10^4 cfu/ml)	1	71.00	2.944	1.472	68	74	4.571	0.043
	2	68.25	1.708	0.854	66	70		
	3	72.00	2.160	1.080	69	74		
	Total	70.42	2.678	0.773	66	74		
Bacterial Population Lower (x 10^4 cfu/ml)	1	75.00	2.944	1.472	71	78	4.348	0.047
	2	71.75	2.630	1.315	68	74		
	3	74.25	4.500	2.250	68	78		
	Total	73.67	3.447	0.995	68	78		
Absolute Difference in Bacterial Population (x 10^4 cfu/ml)	1	4.00	2.582	1.291	1	7	2.785	0.114
	2	3.50	1.291	0.645	2	5		
	3	2.75	2.363	1.181	1	6		
	Total	3.42	2.021	0.583	1	7		

ml to 72×10^4 cfu/ml whereas the lower region ranged from 72×10^4 cfu/ml to 75×10^4 cfu/ml (Fig. 2). The mean bacterial load was maximum in the lower region of pneumatophore at Station 1 and minimum in the upper region of pneumatophore at Station 2.

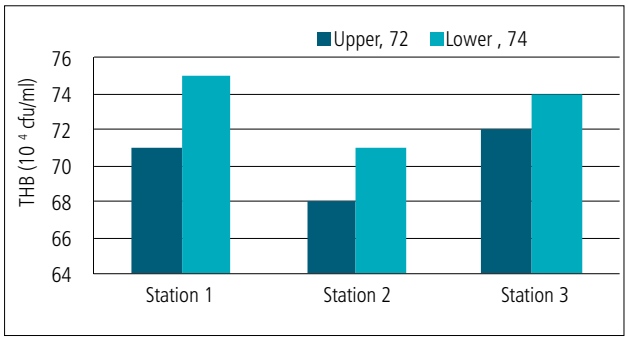

Fig. 2. Enumeration of total heterotrophic bacteria along different stations

Table 2 indicated that there was significant difference ($p < 0.05$) in the bacterial population between lower and upper regions along Station 2. But there was no significant difference ($p > 0.05$) in the bacterial population between lower and upper regions for Station 1 and 3. This might be due to the influence of flood and rain that occurred during the sampling period that can result in nutrient fluctuation causing improper distribution of bacteria and this was more observed in Station 3 as this station was very close to the estuary and land area.

Further, diversity of cultivable bacteria was studied in which the various genera isolated were *Pseudomonas* sp., *Acinetobacter* sp., *Moraxella* sp., *Streptoccoccus* sp., *Deinococcus* sp., *Micrococcus* sp., *Paenibacillus* sp. and *Staphylococcus* sp. (Fig. 3). Relative abundance of gram-negative bacteria was higher during the study. The result agreed with the work of Lakshmipriya and Sivakumar (2012) in Pichavaram mangrove and Govindasamy *et al.* (2011) in Muthukuda mangrove. In our study *Pseudomonas* sp. was the dominant genus and the dominance points out its importance in mangrove ecosystem. Sahoo and Dahl (2009) stated that *Pseudomonas* was involved in nitrogen fixation, degradation process and act as plant pathogen. *Pseudomonas* and *Paenibacillus* were also reported as phosphate solubilizing bacteria of mangrove (Vazquez *et al.*, 2000). Other genera of this study like *Streptococcus*, *Staphylococcus*, *Micrococcus*, *Moraxella* and *Pseudomonas* were reported to be involved in degradation of materials in Indian mangrove soil (Kathiresan, 2003).

A study by Alongi (1988) in tropical mangrove suggested that microbial biomass and bacterial growth were primarily regulated by physicochemical factors and tidal flushing. The physicochemical characteristics showed a remarkable variation in the different stations (Table 3). The mean surface temperature of water ranges from 26.5°C to 28.7°C. Generally, water temperature was influenced by certain factors like solar radiation, evaporation, freshwater influx and cooling and mix up with ebb and flow from adjoining neritic waters (Govindasamy *et al.*, 2000). pH was slightly alkaline with highest value (7.58) at Station 3 and dissolved oxygen was also found to have highest value (3.53 mg/l) at Station 3. Variation in pH value might be the result of sea water penetration and biological activity (Balasubramanian and Kannan, 2005). High dissolved oxygen might be the result of mixing of sea water with estuarine water

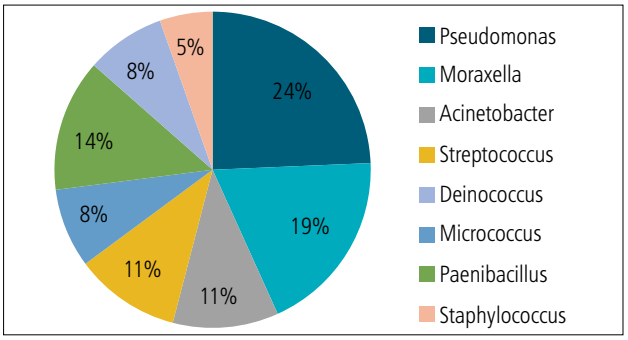

Fig. 3. Relative abundance of the observed bacterial genera

Table 3. Physico-chemical characteristics of water sample (Average Values)

	S1	S2	S3
Temperature (°C)	27.3	26.5	28.7
pH	7.45	7.39	7.58
Dissolved Oxygen(mg/l)	3.16	3.20	3.53
Salinity (ppt)	3.35	3.13	3.80
Phosphate(mg/l)	2.21	1.86	2.76
Nitrate(mg/l)	2.53	2.41	2.47

Table 2. One sample t-test for bacterial population between upper and lower region

	One-Sample t-test					
Station	Mean	Std. Deviation	Std. Error Mean	t	df	p-value
Absolute Difference in Bacterial Population at Station 1	4.00	2.582	1.291	3.098	3	0.053
Absolute Difference in Bacterial Population at Station 2	3.50	1.291	0.645	5.422	3	0.012
Absolute Difference in Bacterial Population at Station 3	2.75	2.363	1.181	2.328	3	0.102

at Station 3. The value of salinity ranges from 3.13 to 3.80 ppt. Salinity was influenced by the topography of the estuary, the state of tide (high or low, and spring or neap), the time of the year controlling rainfall, and the extent of freshwater flow (Paramasivam and Kannan, 2005). Nitrate concentration was minimum (2.41 mg/l) at Station 2 and the concentration of phosphate was also minimum (1.86 mg/l) at Station 2. The concentration of nitrate and phosphate were influenced by leaching of urea and fertilizers from soil to the wetland region through agricultural activities (Mariappan *et al.*, 2016).

Dias *et al.* (2010) assessed bacterial diversity of mangrove and observed that the location within the mangrove was determinant for all the fractions of community studied, which validates with our finding. The similarity analysis (Fig. 4) between three stations clearly demonstrated that maximum similarity (54%) was observed between Station 1 and Station 3 based on the presence of bacteria. Hence one cluster was formed between Station 1 and 3. An overall similarity of 46.6% was indicated in all stations. Second cluster was formed of Station 2. The r value associated with the hierarchial clustering used in the present study is 0.76839. Thus Station 1 and 3 were more similar than Station 2 to Station 1 and 3 respectively.

The extracellular enzyme study revealed that 78% of bacterial population could produce phosphatase followed by 75% lipase,

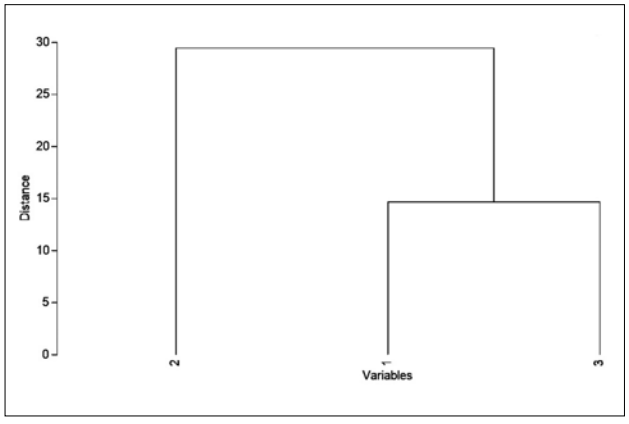

Fig. 4. Similarity between stations

71% cellulase and 52% amylase (Fig. 5). The phosphatase activity was maximum. The observation matched with the diversity results as many of the observed genera have been reported as phosphate solubilizing bacteria. Of the various bacteria capable of producing phosphatase, *Pseudomonas* sp. was found to be dominant. Sahoo and Dahl (2009) stated that *Pseudomonas* sp. isolated from white mangrove roots was capable of phosphate solubilisation. The enzymatic analysis of various bacteria in the present study points out its importance in nutrient cycling and degradation. In mangrove ecosystem, Khinngam *et al.* (2013) screened bacteria for hydrolytic enzyme

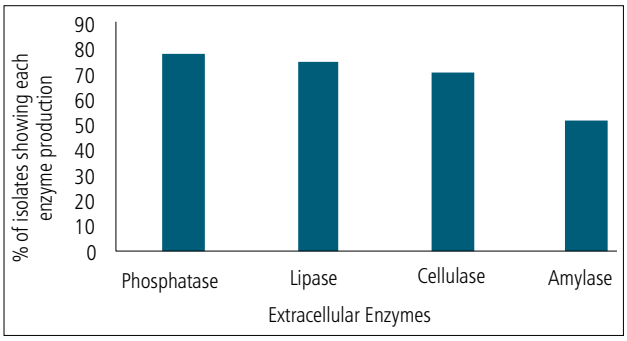

Fig. 5. Percentage of bacterial isolates capable of enzyme production

study and twenty isolates showed activities associated with protease, lipase, amylase and cellulase. Taboa and Moasalud (2010) evaluated the bioprospecting potential of the bacterial communities in the Philippines.

In conclusion the present work gave an insight into the distribution of bacteria and extracellular enzymes production on the surface of pneumatophore of Ayiramthengu mangrove. The study indicated the fact that the dynamic characteristics of mangroves provides a nurturing site for diverse bacteria and many were capable to produce various hydrolytic enzymes. More interestingly, the station was found to have significant influence on bacterial population in both upper and lower regions of pneumatophore. The property of many strains showing enzyme production deserves special attention due to their importance in industrial and biotechnological applications.

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