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Spatiotemporal Variation in Litter Bacterial Community of Ayiramthengu Mangrove Ecosystem of Kerala Coast, India

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Abstract: The present study focusses on the spatiotemporal variation of litter bacterial composition isolated from Ayiramthengu mangrove ecosystem of Kerala coast. Monthly sampling was conducted from three different sampling stations of mangrove ecosystem for various physicochemical parameters and bacterial analysis. Total heterotrophic bacteria study revealed that there was significant difference in bacteria among different seasons as well as different stations. 18 genera of gram-negative bacteria and 4 genera of gram-positive bacteria were isolated. *Acinetobacter* sp. was the dominant bacteria. This indicate that, Ayiramthengu mangrove contains an abundant number of bacteria, which were directly influenced by the different physicochemical parameters in the ecosystem.

Keywords: Seasons, Spatiotemporal variation, Total Heterotrophic bacteria, Mangrove, Litter

Mangroves are unique ecosystem known for its beneficial impact on both ecological and economical aspect. They provide nutrients to the coastal water, preserve and stabilize coastal zones. Microbial community is considered as the most important population in this ecosystem. Multitude of microbial communities inhabit the mangrove ecosystem as a result of the constant supply of carbon and other nutrients, and these communities are able to adapt to the environment's fluctuations and mild salinity (Thatoi et al 2013). The accumulation of litter in the mangrove is regulated by abscission, withering, death, or other factors such as wind or birds . Mangrove forest litter plays a significant role in its ecosystem as a source of energy and nutrients for numerous decomposing organisms, leading to a significant amount of organic matter and nutrient storage or export to both marine and terrestrial environments through tidal water exchange and freshwater inputs (Feller et al, 2009). The decomposition of litter is influenced by various factors like climatic conditions (Imgraben and Dittmann 2008, Kristensen et al 2008, Arnaud et al 2020), soil properties, organic matter quality (Chomel et al 2016), and the characteristics of microbial communities (Hattenschwiler et al 2005, Gießelmann et al 2010). The leaves and wood of mangrove were mainly composed of lignocellulose, that are degradable by microorganisms. Major nutrient transformation within these bacteria sustains mangroves' ecological balance and nutritional well-being (Holguin et al 2006). The present study is to examine the spatiotemporal variation of litter bacteria in relation to various physicochemical factors of Ayiramthengu mangrove ecosystem.

MATERIAL AND METHODS

Ayiramthengu mangrove ecosystem (lat. 9^{0}07' 30'' – 9^{0} 07' 40'' N and long. 76^{0} 28' 40''-76^{0}28' 50''E) was selected as the study area and was divided into three sampling stations as Station 1, Station 2 and Station 3. Station 1 near to the land area and influenced by humans, Station 2 within the middle of the mangrove with thick dense mangrove trees and Station 3 situated near to the estuary with narrow strip of vegetation. Monthly sampling was carried out for a period of one year (February 2018 to January 2019) and represented seasonally as pre monsoon (February- May), monsoon (June-September) and post monsoon (October-January).

Litter samples were taken from decaying leaf litter surface aseptically after the removal of surface contaminants. They were transferred to sterile labelled containers with 25% glycerol and transported to laboratory. Samples were serially diluted and plated on ZoBell Marine Agar (Hi Media, India) using standard plate count method and Total Heterotrophic Bacteria (THB) were determined. The individual bacterial colonies were isolated and purified. They were identified up to generic level based on cell morphology and various standard biochemical reactions as per Bergey's Manual of Determinative Bacteriology (Holt et al 2000). Several water quality and sediment parameters were recorded. The determination of various physicochemical parameters of water like temperature, pH, salinity, dissolved oxygen, nitrate, phosphate and sulphate was done according to the standard methods (APHA,1998). Sediment characteristics like temperature, pH, total organic carbon (TOC) content

(Walkley and Black method, 1934), total organic matter and nutrients like nitrate, phosphate and sulphate (Grasshoff et al 1983) were estimated.

Statistical analysis: Statistical analysis was carried out for seasonal analysis of total heterotrophic bacteria and post hoc test was done. Based on generic composition of bacteria, the similarity among the seasons were analysed by hierarchical agglomerative cluster analysis and non-metric-multidimensional scaling (MDS) based on Bray-Curtis similarities and the results obtained were plotted as ordination graphs.

RESULTS AND DISCUSSION

All the studied parameters showed variations with change in season. High temperature, pH, conductivity and salinity was during pre-monsoon season, whereas high dissolved oxygen and nutrients found during monsoon season (Table 1). During pre-monsoon season, more sunlight could increase the temperature in mangrove as well as less freshwater influx could increase the conductivity. In spite of heavy rainfall during monsoon season coupled with high wind velocity resulted in freshwater mixing in mangrove ecosystem which could be the reason for high dissolved oxygen concentration. The fluctuation in nutrient content was controlled by natural sources like igneous rock, plant decay and animal debris as well as man-made sources like fertilizers, livestock, urban and agricultural runoff and wastewater discharges (Lee et al 2002, Jalali 2005, Lotfinasabasl et al 2013). High organic content could be due to the accumulated dead and decaying planktonic organisms and large amounts of humus transported by the rivers and terrestrial land drainages .

Seasonal analysis of total heterotrophic bacteria showed significant difference among various seasons observed (Fig. 1). The post hoc analysis suggested that pre monsoon and monsoon season, and both post monsoon and monsoon season showed significant difference in heterotrophic bacterial population whereas pre monsoon and post monsoon showed no significant difference. Station-wise litter heterotrophic bacterial density indicated significant difference among stations and on post hoc analysis Station 1 and Station 2 and Station 2 and Station 3 showed significant difference whereas Station 1 and Station 3 show no significant difference.

The heterotrophic bacteria were correlated with the water and soil parameters of the region (Table 2). The water parameters like dissolved oxygen, nitrate, phosphate and sulphate when corelated with the heterotrophic bacteria of litter showed positive results. The correlation of heterotrophic bacteria of litter with the soil parameters such as organic carbon, organic matter, nitrate, phosphate and sulphate was positive when compared to the other parameters. Cotano and Villate (2006) also observed that the increase in bacterial population was contributed by the release of effluents.

The litter bacterial composition appeared to be relatively heterogeneous with various stations. This was due to the

Table 1. Physicochemical parameters of Ayiramthengu mangrove ecosystem (Mean ± SD)

Parameters	Pre monsoon	Monsoon	Post monsoon
Water quality parameters			
Temperature (°C)	30.3 ± 0.57	27.5 ± 0.49	28.8 ± 0.87
pH	7.53 ± 0.10	7.47 ± 0.06	7.51 ± 0.06
Conductivity (m/s)	23.92 ± 2.75	9.42 ± 1.24	22 ± 2.21
Dissolved Oxygen (mg/l)	2.97 ± 0.69	3.3 ± 0.74	2.06 ± 0.69
Salinity	8.1 ± 2.48	3.43 ± 0.69	4.65 ± 1.55
Nitrate (mg/l)	1.13 ± 0.2	2.47 ± 0.77	2.18 ± 0.8
Phosphate (mg/l)	1.38 ± 0.14	2.28 ± 0.26	1.32 ± 0.31
Sulphate (mg/l)	75.08 ± 2.64	138.27 ± 17.35	115.1 ± 8.73
Sediment characteristics			
Temperature (°C)	26.8 ± 0.42	23.7 ± 0.42	25.1 ± 0.51
pH	6.83 ± 0.13	6.37 ± 0.15	6.38 ± 0.11
OC (%)	0.40 ± 0.09	0.41 ± 0.09	0.61 ± 0.09
OM (%)	0.65 ± 0.15	0.75 ± 0.32	1.05 ± 0.16
Nitrate (mg/g)	2.64 ± 0.0.06	2.58 ± 0.03	2.24 ± 0.06
Phosphate (mg/g)	3.04 ± 0.05	3.20 ± 0.06	3.08 ± 0.08
Sulphate (mg/g)	82.02 ± 1.35	85.78 ± 1.42	81.50 ± 1.56

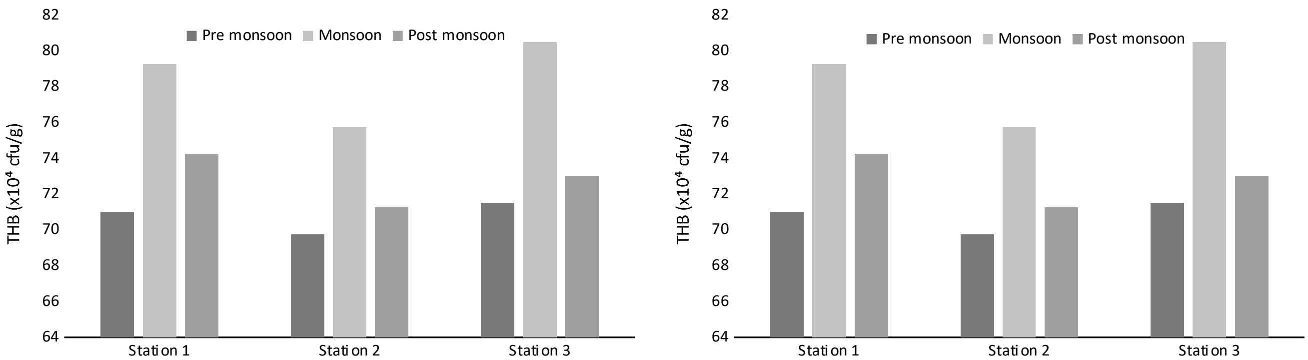

Fig. 1. Spatio-temporal variation in litter heterotrophic bacteria

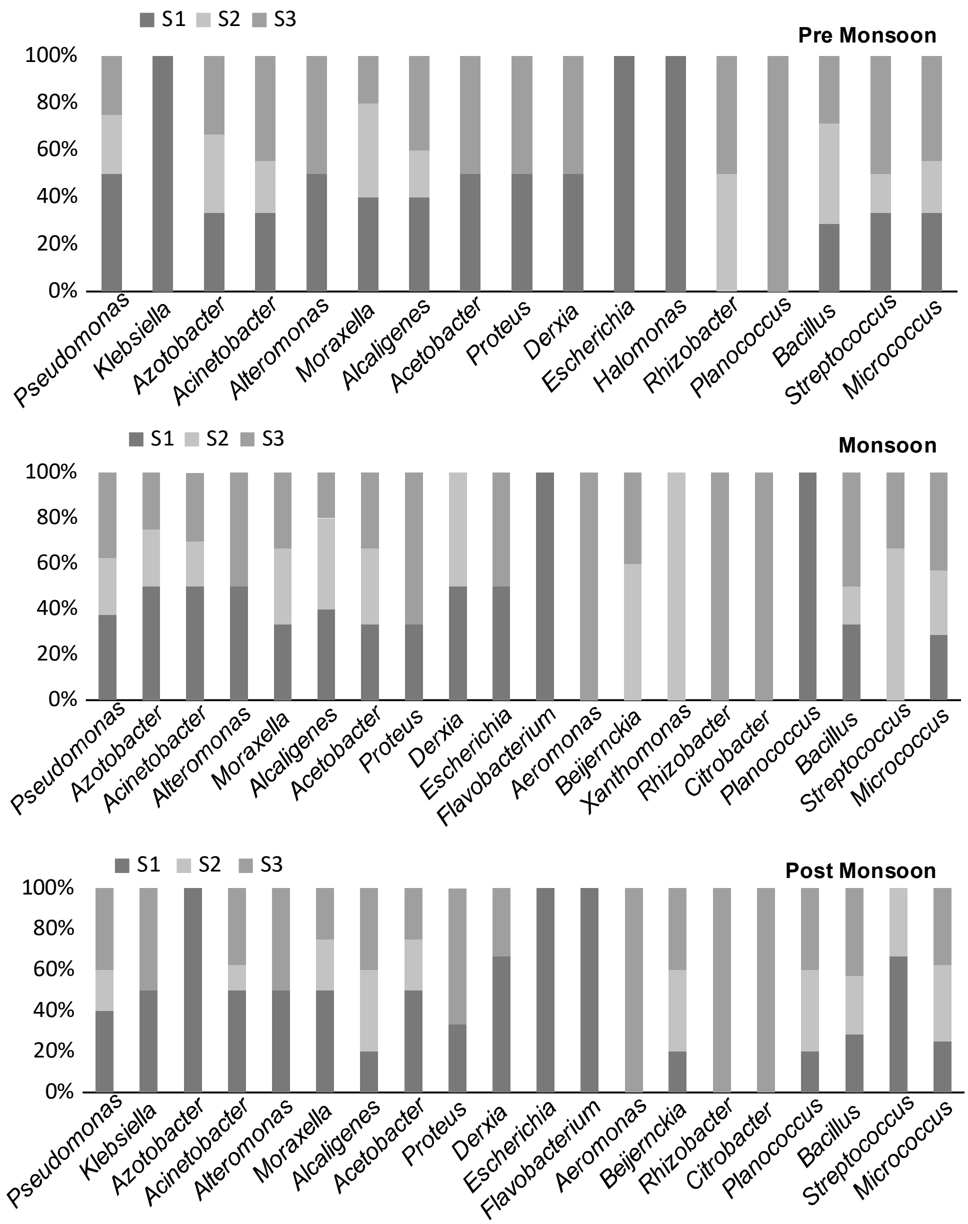

Fig. 2. Spatiotemporal variation in the generic distribution of litter bacteria (%)

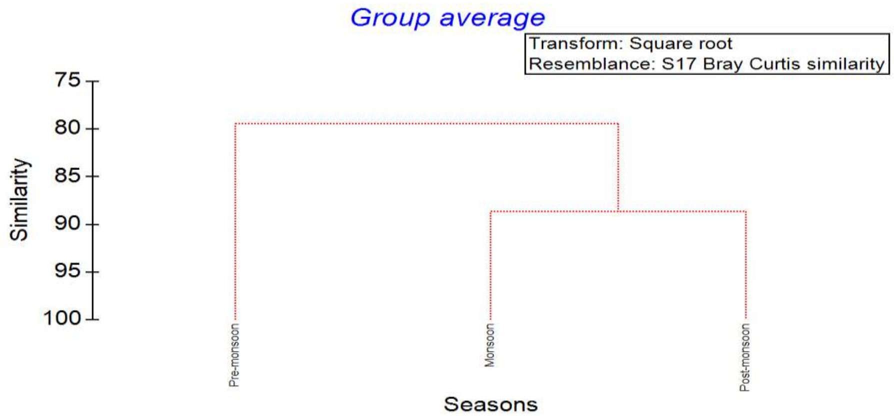

Fig. 3. Cluster diagram plotted based on similarity/dissimilarity of the various seasons in relation to the litter bacterial diversity

Table 2. Correlation analysis of litter bacteria with water and soil parameters

Parameter	Correlation	Parameters ® value)	Correlation coefficient (r)
Water	Positive correlation	Dissolved Oxygen	0.373
		Nitrate	0.466
		Phosphate	0.727
		Sulphate	0.558
	Negative correlation	Temperature	-0.379
		pH	-0.167
		Conductivity	-0.619
		Salinity	-0.480
Soil	Positive correlation	Organic carbon	0.018
		Organic matter	0.019
		Nitrate	0.172
		Phosphate	0.383
		Sulphate	0.264
	Negative correlation	Temperature	-0.58
		pH	-0.462

difference in the rate of decomposition found in different stations. Besides that, all the stations had equal importance in the detritus decomposition and were thereby actively involved in biogeochemical cycles. But the litter heterotrophic bacteria indicated changes among different seasons. However, the physicochemical variations that occurred during different seasons affected the litter production rate (Juman 2005, Zafar et al 2012). It is well known that mangrove microbial communities were strongly influenced by the environmental parameters like temperature, pH, salinity, dissolved oxygen and nutrient concentrations (Tam 1998, Li and Gu 2013). During the study, heterotrophic bacterial load from litter revealed that gram negative bacteria were relatively abundant with 18 genera (82%), while gram positive bacteria contributed only 4 genera (18%). The comparatively higher percentage of gram-negative bacteria showed higher decomposition favoured by the litter substratum for the better adapted gram-negative bacteria.

Acinetobacter sp. (13%) was the dominant followed by *Micrococcus* sp., *Bacillus* sp., *Pseudomonas* sp. (Fig. 2). The cluster analysis suggested that the monsoon and post monsoon season (88%) showed more similarity than to pre monsoon season (Fig. 3). *Acinetobacter* involved in degradation pathways of various long-chain dicarboxylic acids and aromatic and hydroxylated aromatic compounds. Dorothy et al (2003) stated that *Micrococcus* were actively involved in leaf litter decomposition in mangrove forest. *Bacillus* sp. was known for its cadmium biodegradation, hydrocarbon degradation, emulsification, phytase production, plant growth promotion and protection of plant from microbes (Holguin et al 2001, Macrae et al 2001). *Pseudomonas* had a major role in the turnover of certain nutrients like nitrogen, carbon and phosphorous appeared in the leaf biomass (Mumby et al 2004, Romero et al 2005).

CONCLUSION

The heterotrophic bacterial population was influenced by various physico-chemical parameters of soil and water. Moreover, Gram negative bacteria was comparatively high than Gram positive bacteria with a dominance of *Acinetobacter* sp. Thus, it is concluded that Ayiramthengu mangrove ecosystem have a rich litter bacterial population holding a well-organized community structure at various seasons as well as stations.

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