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Distribution and enzyme production of litter bacteria from Ayiramthengu mangrove ecosystem, south west coast of India

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Original Article

Abstract

The present study focuses on the screening of litter bacteria from decomposing leaves for enzyme production. The study was conducted at three different sampling stations of Ayiramthengu mangrove. Bacteria belonging to various genera were isolated. *Acinetobacter* sp. was the dominant genus followed by *Pseudomonas* sp. and *Micrococcus* sp. Thirty-four isolates were selected for enzymatic study and extracellular enzymes such as phosphatase, lipase, cellulase and amylase were estimated. About 31% of isolates showed positive for lipase production, followed by phosphatase (28%), cellulase (23%) and amylase (18%). The enzymatic indices of isolates were calculated. The highest enzymatic index was observed for lipase enzyme (2.264). The result revealed that decomposing leaf litter of mangrove can harbour potential bacteria with enzymes production and maintains the stability of ecosystem at various trophic levels.

Keywords: *Mangrove, litter, enzymes, enzymatic index*

Introduction

Mangrove ecosystems are well known for its productivity as well as for its vital role in food chain. The unique characteristic features like organic matter, sulphur and nitrogen content of the ecosystem caters to the growth of bacteria (Hong *et al.*, 2009). At higher trophic levels, bacteria act as an intermediate link in energy flow, which helps them to directly acquire carbon and nutrients like nitrogen and phosphorous from leaf litter (Zhao *et al.*, 2017).

In tropics and subtropics, mangroves are distributors of organic material in the form of litter (Ogbonna, 2011). Mangrove litter consists of leaf litter which has the highest total litter production (70%), followed by flower litter (15%), twigs (10%), and propagules (5%) (Ake-Castillo *et al.*, 2006). Litter degradation is a multifaceted process in which degradation is commenced and mediated by various microbes that colonizes on the litter (Van der Heijden *et al.*, 2016). When the microbes colonize on litter they convert the nutrients to available form which can be absorbed by plants. Degradation also depends on several other factors like mangrove species, season, and its position in the intertidal zone (Mfilinge *et al.*, 2002; Kristensen *et al.*, 2017). Various fungi and bacteria were known for litter degradation (Krishna and Mohan, 2017). Mangrove litter has a pivotal role

in the food web by providing food to fish and invertebrates and thus they form a detrital food chain. During litter decomposition, the organic carbon hence produced may be exported to the nearby aquatic environment. This land-water-atmosphere interactions in these regions helps in the biogeochemical cycles, thereby influences not only the regional ecosystem but also the global climatic system (Mukherjee and Ray, 2012). Carbon dioxide produced is utilized by microorganisms and animals for respiration (Chandrasekhara, 1997).

Extracellular enzymes produced by microbes, help in the breakdown of larger compounds into simpler ones (Chrost, 1991). Several enzymes produced by the mangrove bacteria are industrially important. They are widely used in food, fermentation, pharmaceuticals, medical applications, cosmetics, textile, detergent, pulp and paper industries (Gurung *et al.*, 2013). However, there is a dearth of information on bacteria found on decomposing mangrove leaf litter and its enzyme study. Hence, the present study attempts to understand the distribution of bacteria in decomposing mangrove leaf litter and explore its enzyme activity.

Material and methods

Study area

The study was carried out in the Ayiramthengu mangrove ecosystem. The study area was divided into three sampling stations. Station 1 lie close to the land area; Station 2 situated in the middle of the mangrove ecosystem and Station 3 was the area adjoining the estuary (Fig.1). Sampling was done from June to September 2018.

Isolation and identification of bacteria

Biofilm from decomposing litter was collected using sterile blade from three sampling stations and aseptically transferred into sterile bottles. Surface contaminants were removed using sterilized sea water. Samples were immediately preserved at -20°C and analysed in the laboratory. The estimation of Total Heterotrophic Bacteria (THB) was done by standard plate method using Zobell Marine Agar (Hi Media). The plates were incubated for 48 hours at 28±2° C and number of colony forming units (cfu) per gram wet weight were calculated. Colonies were

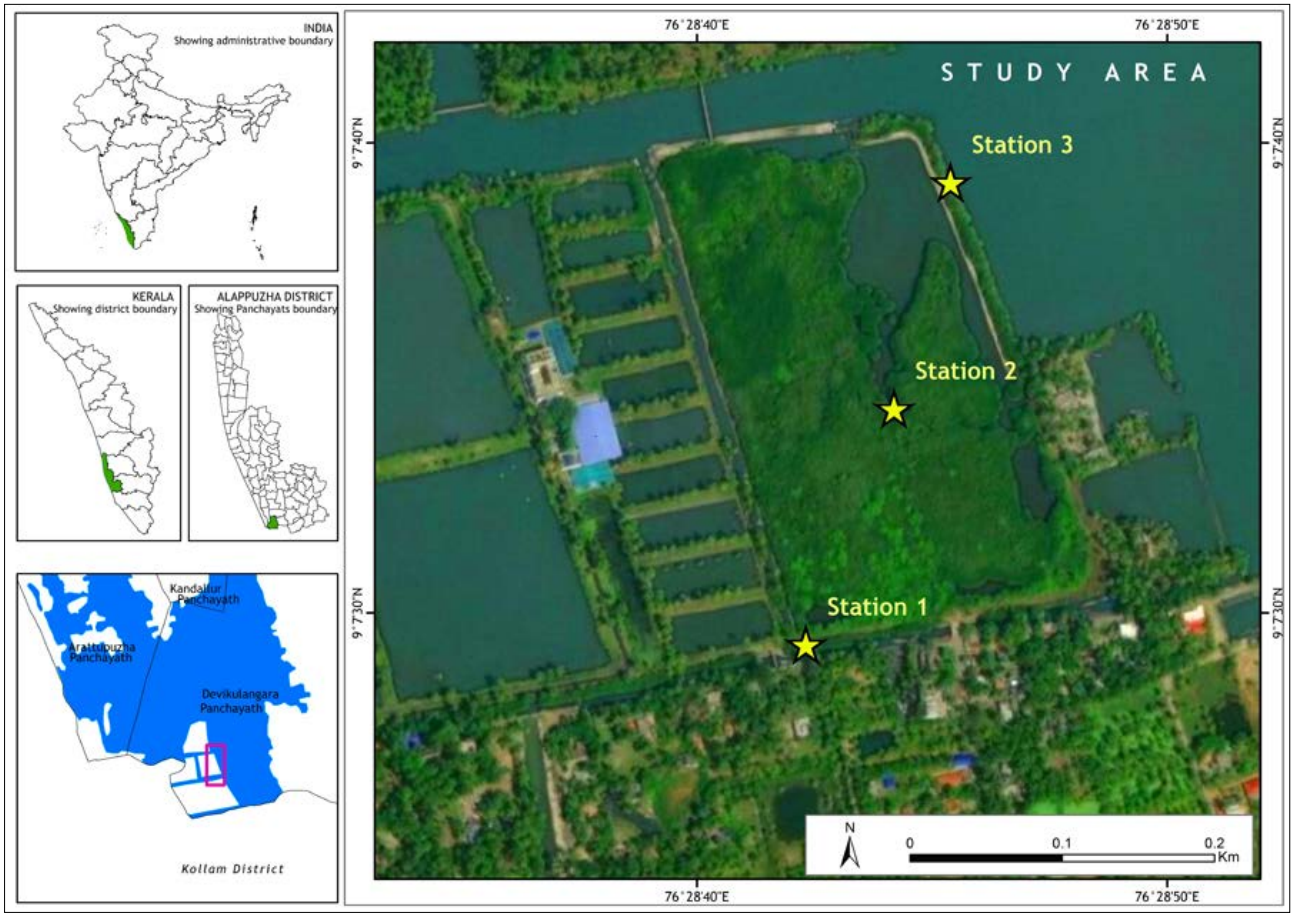

Fig.1. Sampling Sites in the Ayiramthengu mangrove ecosystem

isolated and purified on saline nutrient agar. The isolates were morphologically studied and biochemically tested for Gram staining, spore staining test, catalase test, oxidase test, citrate utilization test, sulphide test, nitrate test, indole test, motility test, mannitol test, and oxidation/fermentation test. All the identification was done upto generic level by following Bergey's Manual of Determinative Bacteriology (Holt *et al*., 2000).

Extracellular enzyme study

The extracellular enzyme production of bacteria was studied using 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. Phosphatase test showed a pink coloration around the colonies on phenolphthalein agar when the plates were exposed to liquid ammonia after an incubation of 2-3 days. The enzymatic index was calculated using a semi quantitative approach by using the value obtained by the ratio of diameter of halo size and diameter of bacterial colony (Soares Junior *et al*., 2013).

Results and discussion

The total heterotrophic bacterial count was the highest at Station 3 (80.50 ± 1.29) followed by Station 1 (79.25 ± 2.21) and Station 2 (75.75 ± 1.708) (Fig. 2). Statistical analysis using one-way ANOVA showed that the total heterotrophic bacterial population were significantly different between stations ($p = 0.011$, $F = 7.658$). Generally, total heterotrophic bacteria count was the highest at maximum decomposition (Rajendran and Kathiresan, 2007). In the important phase of degradation of litter, 40 to 70% hydrolysis of original material occur with the help of microbes (Valiela *et al*., 1985). A total of twenty genera were isolated from the decomposing litter *viz*.,*Pseudomonas* sp., *Azotobacter* sp., *Acinetobacter* sp., *Alteromonas* sp., *Moraxella* sp., *Alcaligenes* sp., *Acetobacter* sp., *Proteus* sp., *Derxia* sp., *Escherichia* sp., *Flavobacterium* sp., *Bacillus* sp., *Streptococcus* sp., *Micrococcus* sp., *Aeromonas* sp., *Planococcocus* sp., *Beijerinckia* sp., *Xanthomonas* sp., *Rhizobacter* sp. and *Citrobacter* sp. Among these, *Acinetobacter* sp. was the dominant group (14%) followed by *Pseudomonas* sp. (9%) (Fig. 3).

Ogbonna (2011) reported *Pseudomonas*, *Acinetobacter*, *Escherichia*, *Flavobacterium*, *Bacillus* and *Micrococcus* from decomposing mangrove leaf litter. Rajendran and Kathiresan (2007) observed *Flavobacterium, Pseudomonas, Acinetobacter* and *Azotobacter* from Pichavaram mangrove litter. *Acinetobacter* involved in pathways for degradation of different long-chain dicarboxylic acids and aromatic and hydroxylated aromatic compounds (Weelink, 2008). *Pseudomonas* has a significant role in the turnover of certain nutrients like nitrogen, carbon and phosphorous present in the leaf biomass (Robertson and Daniel, 1989; Mumby *et al*., 2004; Romero *et al*., 2005). The presence of *Escherichia* might be due to the human wastes from the nearby settlement that gets interacted with the mangrove ecosystem. Nitrogen fixation has been reported in *Pseudomonas*, *Derxia* and *Azotobacter,* whereas *Bacillus* and

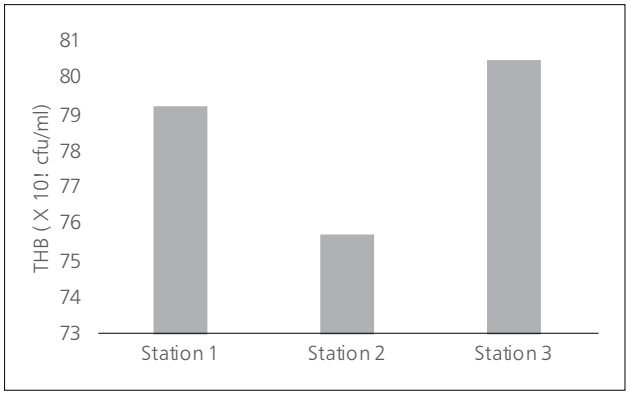

Fig. 2. Enumeration of total heterotrophic bacteria along different stations

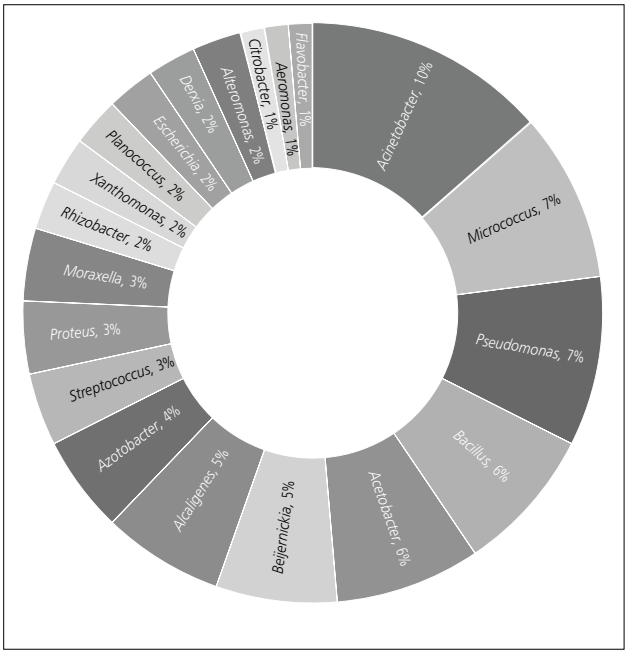

Fig. 3. Relative abundance of isolated bacteria

Alcaligenes were responsible for phosphate solubilisation in mangrove (Sahoo and Dhal, 2009; Zhang *et al*., 2008; Behera *et al*., 2017). *Bacillus*, *Micrococcus*, *Alteromonas*, *Escherichia*, *Xanthomonas*, *Moraxella, Alcaligenes*, *Proteus*, *Citrobacter*, *Acetobacter*, *Streptococcus*, *Aeromonas* and *Planococcus* were reported from different mangroves (Grisi Lima de and Liri, 2010; Essien *et al*., 2013; Castro *et al*., 2014; Saseeswari *et al*., 2016; Ambeng *et al*., 2019).

The isolates which were fast growing and which could produce at least one of the four evaluated enzymes were chosen for the study. Lipase activity was observed by 31%, phosphatase activity by 28%, cellulase activity by 23% and amylase activity by 18% of isolates (Fig.4). During the study, most of the bacteria produce lipase except *Flavobacterium* sp. and *Escherichia* sp. These enzymes play peculiar function

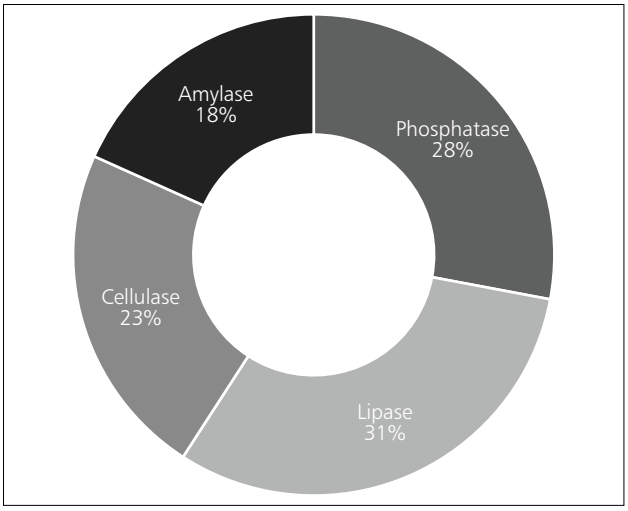

Fig. 4. Percentage composition of bacterial enzyme production

Table 1. Enzymatic activity of the isolated bacteria from mangrove litter in the study area

Genus	Isolate Code	Phosphatase	Lipase	Cellulase	Amylase
Pseudomonas	ML1	++	++	++	++
	ML2	+	++	+++	+
	ML3	++	++	++	++
	ML4	+	++	+++	+
Acinetobacter	ML5	-	++	+	+
	ML6	-	++	+	-
	ML7	-	++	+	-
	ML8	-	+++	++	+
	ML9	+	-	-	-
Alteromonas	ML10	+	++	+++	++
Alcaligenes	ML11	++	++	++++	-
	ML12	++++	++	++++	++
	ML13	++	++	+++	-
Beijernickia	ML14	++	+++	++	+++
	ML15	++	+++	++	+
Acetobacter	ML16	+++	-	+	++
	ML17	+	+++	-	-
	ML18	+	++	-	-
Citrobacter	ML19	+	-	++	+++
Proteus	ML20	++	+++	-	-
	ML21	+	+++	-	-
Derxia	ML22	+	+++	-	+
	ML23	+	+++	-	+
Escherichia	ML24	+	-	-	-
Flavobacterium	ML25	++	-	-	+
Bacillus	ML26	+	++	-	-
	ML27	+	++	-	-
	ML28	+	++	-	-

Genus	Isolate Code	Phosphatase	Lipase	Cellulase	Amylase
Streptococcus	ML29	+	++	-	+++
Micrococcus	ML30	-	++	+++	-
	ML31	-	++	++	-
	ML32	+	++	++	-
	ML33	-	++	+++	-
Aeromonas	ML34	++	+++	+	+

Enzymatic index represents – no enzyme production, + represents between 1 and 2mm, ++ represents between 2 and 3mm, +++ represents between 3 and 4mm, ++++ represents above 4mm

in litter decomposition. Many lipase producing bacteria including *Bacillus* sp., *Azotobacter* sp., *Acinetobacter* sp. and *Pseudomonas* sp. were reported from mangrove ecosystem (Kathiresan *et al.*, 2011; Santos *et al.*, 2016). Cellulolytic enzymes produced during the decomposition have intense capability to degrade cellulolytic materials for the enrichment of nutrients in the leaf (Nakagiri, 1998). These enzymatic reactions convert the plant debris into a nutritious matter for the consumption of organisms at higher trophic levels. Therefore, as far as the primary and subsequent secondary productivity of mangrove ecosystem was concerning, the litter bacterial community and its enzyme production duly indicated the pivotal role played by these bacteria in Ayiramthengu mangrove and elsewhere.

The enzymatic index is a quick and useful tool for choosing and evaluating the enzyme production of various bacterial isolates (Carrim *et al.*, 2006). This study revealed that the enzyme production differs among bacteria and the type of enzymes produced. The average enzymatic index value for the four enzymes were evaluated. The highest enzymatic index was observed for lipase (2.264), followed by cellulase (1.644), phosphatase (1.469) and amylase (1.068) (Fig.5). However, if the enzymatic index value equals to 1, then the isolate was considered as negative and when the value was greater than 1 then the isolates were positive to the test (Chaves *et al.*, 2003). Based on the value of enzymatic index, certain enzymes were classified into four groups as no enzyme activity with value 1, low activity with value 2, medium activity with value 3 and high activity with value 4 (Lechuga *et al.*, 2016). Each selected isolate was studied for enzyme production (Table 1) and its index was represented as per Bibi *et al.*, 2017.

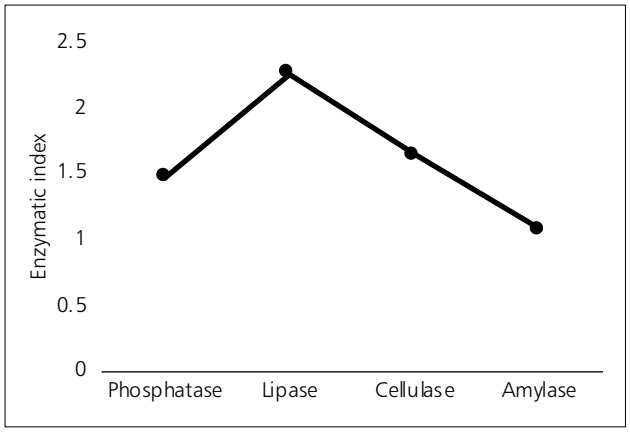

Fig. 5. Average enzymatic index of isolated bacteria capable of enzyme production

Thus the present study explored the bacterial distribution in decomposing leaf litter of Ayiramthengu mangrove and it was found that this ecosystem harbours a wide range of bacteria having potential enzyme producing capacity. Lipase enzyme was predominantly present in most isolates while amylase enzyme was the least prominent. However, further research is required to understand the efficiency of potent bacterial strains in its ecosystem.

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