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Identification and phylogeny of potential bacteria isolated from mangroves of Kadalundi, Kerala, India

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Short communication

Abstract

Mangroves are among the most productive ecosystems in the world and are of ecological, economic, and societal importance. Microbes play a key role in maintaining this productivity. Microorganisms from the mangrove ecosystem contain many useful enzymes, proteins, and antibiotics all of which have biotechnological significance. The present study focuses on the identification and bioactivity of bacteria isolated from mangrove sediments of Kadalundi community reserve, Kozhikode. 76% of isolates produced lipase followed by protease (75.5%), amylase (72%), cellulase (61.5%), and ligninase (20%). 38 potential strains of bacteria were identified using 16S gene sequencing. The potential isolates belonged to the genus, *Bacillus, Pseudomonas, Ralstonia, Serratia, Enterobacter, Shewanella,* etc.

Keywords*: Diversity, mangrove sediments, phylogeny, soil microbes, hydrolytic potential*

Introduction

Microbes have diverse ability to degrade the substances and this capability is used in bioremediation projects like oil spills to acid drainage and sewage waste. Chemical and physical properties of soils like pH, quality and amount of organic matter, and redox condition influence the microbial communities of soil (Lombard *et al*., 2011).

Mangroves are the tidal forests of coastal wetlands existing in the intertidal zone of sheltered shores, estuaries, tidal creeks, backwaters, lagoons, marshes, and mud-flats of the tropical and subtropical region of the world. In the Indian Ocean region, nearly about 84984 square kilometers (km) area is covered by mangroves which contribute to 47 percentage (%) of the total area of world mangroves (Kadhireshan and Rajendran, 2004). Mangroves are adapted to grow in harsh environmental conditions such as high salinity, high temperature, extreme tides, high sedimentation, and muddy anaerobic soils (Spiers and Finlayson, 1999). Mangrove provides a unique niche to a variety of microorganisms ranging from bacteria, fungi, algae, yeast, and so on (Kathirvel, 1996). Microbes constitute an important part of the mangrove ecosystem; they help in creating and maintaining this biosphere and also serve as a source of biotechnologically valuable and important products. They participate in various steps of decomposition and mineralization of leaf litter, together with playing a critical role in the productivity of the mangrove ecosystem (Thatoi *et al*., 2013).

Unveiling the diversity and structure of microbial communities in

mangrove environments denotes the first step towards a better understanding of their specific role in ecosystem functioning. Analysis of microbial biodiversity from these ecosystems will help in identifying and isolating undescribed and potential strains having high specificity for various applications. The present study focuses on the isolation, enzyme production, and identification of microbes that are present in the mangrove sediments of Kadalundi.

Material and methods

The study area is the mangrove forest inside Kadalundi Community Reserve (10°51'42'' N and 75°48'21'' E). This is the first community reserve of Kerala, declared in 2007 which spread across 1.5 square km. A large portion of the shallow wetland is exposed to intertidal fluctuations which attribute to the unique characteristics of this wetland.

The sub-surface sediment was collected by hand core, transferred aseptically to sterilized polythene bags, and transported to the laboratory at 4ºC. A total of five sub-samples were collected from the location and pooled for the analysis. The temperature of sediment was noted as 27ºC and pH as 6.8. The collected soil samples serial diluted (10^{-1} to 10^{-3}) and plated to nutrient agar medium employing the spread plate method. The plates were incubated at 28 ± 2°C for 24 hours. The developed colonies were purified and transferred to nutrient agar slants for further analysis.

The bacterial isolates were screened for their capacity for the production of hydrolytic enzymes *viz.* protease, amylase, lipase, cellulase, and ligninase as per standard methods. Nutrient agar supplemented with casein (2%), starch (1%), and tributyrin (1%) were used for detection of protease, amylase, and lipase respectively. Cellulase agar and Crawford's agar supplemented with 0.5% tannic acid were used for the detection of cellulase and ligninase activity respectively. The plates were spot inoculated and incubated at 28± 2°C overnight. Formation of clearance/ halo zone or brown colour around the colonies indicated enzyme activity.

The genomic DNA was extracted from the isolated colonies by using the Ultraclean Soil DNA isolation kit (MoBio, USA) (Gray and Herwig, 1996). 16S rRNA gene was amplified using 27F and 1496R primers (Jiang, 2006), and the purified PCR product was sequenced from both ends using forward and reverse primers by Sanger's dideoxy chain termination sequencing method (Sanger and Coulson, 1975). The forward and reverse sequences were assembled by using Clustal W and the consensus was taken for the analysis (Thompson *et al.*, 1994). Residue and pair-wise distances were estimated using the MEGA6 software. The multiple sequence alignment was done using ClustalX 2.1 program (Larkin *et al.*, 2007). A taxonomical hierarchy was assigned to the sequences using the Ribosomal Database Project (RDP) Naive Bayesian rRNA Classifier Version 2.5 (Wang *et al.*, 2007).

Results and discussion

Microbial activity is responsible for major nutrient transformations within a mangrove ecosystem (Alongi, 2014; Holguin *et al.*, 2001). The diverse microbial communities can continuously transform nutrients from dead mangrove vegetation into sources of nitrogen, phosphorus, and other nutrients that can be used by mangrove trees. They include methanogenesis, phosphate solubility, sulfate reduction, and production of other substances, including antibiotics and enzymes, and are reservoirs of products of biotechnological interest as, for example, bacteria that produce bio emulsifiers (Wu and Lu, 2015). As a result, bacteria play a key role in the productivity, conservation, and rehabilitation of mangrove ecosystems.

Different enzymes from terrestrial microbes have been proved to have potential applications in various industries (Chi *et al.*, 2009). Marine environments are also proved to be a good source of enzymes with unique properties. The hydrolytic potential of the isolated bacteria was checked. 76% of isolates produced lipase followed by protease (75.5%), amylase (72%), cellulase (61.5%) and ligninase (20%) (Fig.1). The list of identified bacterial isolates obtained in the present study and their NCBI GenBank accessions are given in Table 1. The potential isolates belonged to the genus, *Bacillus, Pseudomonas, Ralstonia, Serratia, Enterobacter, Shewanella,* etc. The majority of the isolates belonged to the genus *Bacillus.* The phylogenetic relationship of bacterial strains isolated in the present study was analysed using the neighbor-joining algorithm. The dendrogram showing the phylogenetic relationship between the bacterial strains inferred based

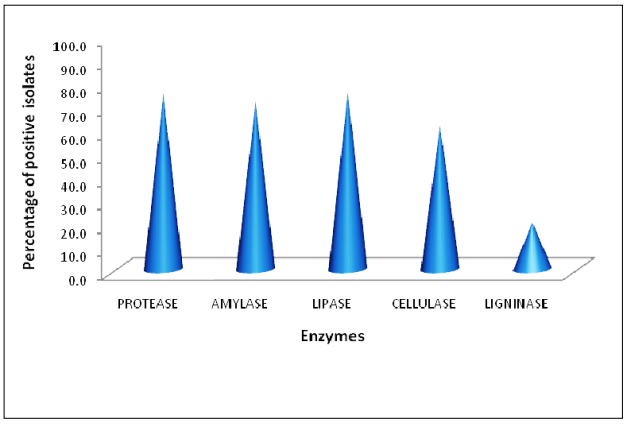

Fig. 1. Hydrolytic potential of bacterial isolates from the mangrove sediments of Kadalundi

Table 1. The list of bacterial isolates identified with their NCBI GenBank Accessions

Sl. No.	Isolate strain	Organism	GenBank Accession
1.	CUMB KDY-22	*Anurinibacillus aneurinilyticus*	MG 252692
2.	CUMB KDY-34	*Bacillus albus*	MT 994599
3.	CUMB KDY-70	*Bacillus altitudinis*	MG 238593
4.	CUMB KDY-04	*Bacillus amyloliquefaciens*	MG 238546
5.	CUMB KDY-17	*Bacillus anthracis*	MG 252498
6.	CUMB KDY-24	*Bacillus anthracis*	MK 294236
7.	CUMB KDY-60	*Bacillus cereus*	MG 238592
8.	CUMB KDY-48	*Bacillus lichiniformis*	MK 294233
9.	CUMB KDY-09	*Bacillus* sp.	MG 238551
10.	CUMB KDY-51	*Bacillus* sp.	MG 238564
11.	CUMB KDY-53	*Bacillus* sp.	MK 294229
12.	CUMB KDY-63	*Bacillus* sp.	MG 238552
13.	CUMB KDY-41	*Bacillus subtilis*	MT 994519
14.	CUMB KDY-64	*Bacillus subtilis*	MK 294226
15.	CUMB KDY-20	*Enterobacter asburiae*	MG 252629
16.	CUMB KDY-80	*Enterobacter cloacae*	MG 238594
17.	CUMB KDY-14	*Enterobacter* sp.	MG 238556
18.	CUMB KDY-50	*Geobacillus stearothermophilus*	MG 238548
19.	CUMB KDY-12	*Klebsiella aerogenes*	MG 238557
20.	CUMB KDY-71	*Lysinibacillus macroides*	MT 994559
21.	CUMB KDY-66	*Lysinibacillus* sp.	MK 294235
22.	CUMB KDY-05	*Lysinibacillus xylanilyticus*	MT 994589
23.	CUMB KDY-06	*Pseudomonas fulva*	MT 994551
24.	CUMB KDY-01	*Pseudomonas stutzeri*	MT 994548
25.	CUMB KDY-02	*Psuedomonas fulva*	MK 294222
26.	CUMB KDY-03	*Psuedomonas stutzeri*	MK 294234
27.	CUMB KDY-07	*Ralstonia pickettii*	MG 238595
28.	CUMB KDY-08	*Ralstonia pickettii*	MG 238596
29.	CUMB KDY-10	*Ralstonia pickettii*	MG 238560
30.	CUMB KDY-19	*Ralstonia pickettii*	MG 238555
31.	CUMB KDY-90	*Ralstonia pickettii*	MG 252552
32.	CUMB KDY-16	*Ralstonia* sp.	MG 238553
33.	CUMB KDY-18	*Ralstonia* sp.	MG 238554
34.	CUMB KDY-11	*Serratia marcescens*	MG 252371
35.	CUMB KDY-13	*Serratia marcescens*	MG 238562
36.	CUMB KDY-15	*Serratia* sp.	MG 238561
37.	CUMB KDY-30	*Shewanella bicestrii*	MG 252372
38.	CUMB KDY-21	*Shewanella seohaensis*	MG 238563

on aligned 16S rRNA gene sequence is given in Fig. 2. The evolutionary histories of different taxa were inferred using the neighbor-joining method (NJ) (Saitou and Nei, 1987). The tree is drawn to scale, with branch lengths having the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were calculated using the Maximum Composite Likelihood method

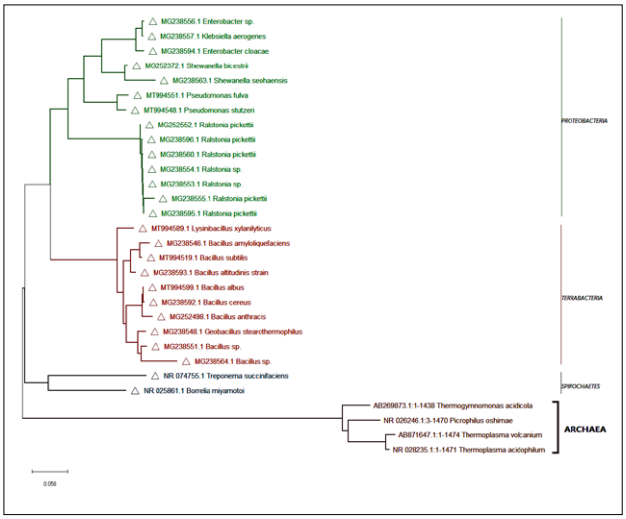

Fig. 2. Phylogenetic relationship representation of the bacterial isolates from the mangrove sediments of Kadalundi including outgroups using Neighbour Joining algorithm. Different taxa are coloured differently

(Tamura *et al.*, 2004) and were expressed as the number of base substitutions per site. All positions containing gaps and missing data were eliminated. Archeae and spirochaetes were taken as an outgroup. The 24 sequences were classified into three large clades and 10 nodes. All *Enterobacter, Pseudomonas,* and *Shewanella* were grouped into one clade, which represents the gammaproteobacteria, where all the Ralstonia isolates were grouped into one clade. The rest of the *Bacillus* isolates comprised a separate clade (Firmicutes) representing Terrabacteria.

Mangroves provide many ecological, environmental, and socioeconomic benefits to mankind. They are complex and dynamic ecosystems varying in salinity, water level, and nutrient availability; containing diverse and distinct microbial communities. Microbes play a key role in maintaining mangrove productivity; in fact they also constitute the largest pool of metabolic pathways on earth with potential biotechnological and environmental implications. Mangrove vegetation has diminished in its extent drastically and has acquired a threatened status in Kerala. The mangroves in the state are threatened with unprecedented destruction, which includes commercial exploitation of raw materials, land reclamation for agriculture, aquaculture, and housing. The deterioration of mangroves can cause serious consequences which include a reduction in biodiversity, species decline, genetic erosion, extinction, increased flooding, and decline in water quality.

In this study at the Kadalundi mangrove ecosystem, some of the potent bacterial strains have been explored and identified. Studies of microbes and their interactions with the mangrove ecosystem are critical for our understanding of mangrove ecosystem functioning and bioremediation.

A bacterial enzyme that could degrade tributyrin can be used to control oil spills which causes a multitude of problems for the environment and us. The microbes that exhibit amylase activity can be used in the sewage treatment plants which help to degrade most of the household waste as these wastes are rich in starchy material. Unveiling the diversity and structure of microbial communities in mangrove environments represents the first step towards a better understanding of their role in ecosystem functioning.

Mangrove forests are found to be naturally capable of filtering large amounts of untreated sewage and other waste materials. They filter the solid waste by trapping it with their pneumatophores. With the help of the vast bacterial diversity associated with them and the hydrolytic potential of bacteria, these waste materials crumble and deliquesce. Mangroves are among the most productive ecosystems in the world and are of ecological, economic, and societal importance. Microbes play a key role in maintaining this productivity.

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