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Diversity of Culturable Bacterial Isolates from Mangroves of Kadalundi – Vallikkunnu Community Reserve, Kerala, India

Sreedevi N. Kutty[1], Nijisha Suresh, A.[2], Anjali, M.[2], Bhavitha, M.K.[2] and Sebastian, C.D.[2]*

[1]Department of Zoology, N. S. S. College, Nemmara, Palakkad, Kerala 678 508
[2]Division of Molecular Biology, Department of Zoology, University of Calicut, Kerala 673 635
**Email: drcdsebastian@gmail.com*

Abstract

Mangroves are salt tolerant trees, also called halophytes, and are adapted to live in harsh coastal conditions. They contain a complex salt filtration system and complex root system to cope with salt water immersion and wave action. 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 mangrove ecosystem contain many useful enzymes, proteins, and antibiotics all of which have biotechnological significances. The present study focused on the identification of bacterial strains isolated from mangrove forest inside Kadalundi – Vallikkunnu community reserve, Kozhikode. Molecular identification of the isolates was carried out. 25 strains of bacteria were identified.

Keywords: Diversity, Mangrove sediments, Soil microbes, 16S rRNA gene sequencing

1. Introduction

Microorganisms are considered as the first forms of life on earth shaping the evolution of the planet and are capable of exploiting a wide range of energy sources thriving in almost every habitat [10]. Multi-cellular life on earth would not have been evolved and the biology as we know today would not be sustainable in the absence of microbes. Microbes have diverse ability to degrade the substances and this capability is used in bioremediation project like oil spills to acid drainage and sewage waste. Microbes are the pillars of life on earth (Gibbons and Gilbert, 2015). Chemical and physical properties of soils like pH, quality and amount of organic matter, redox condition have influence on 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 km area is covered by mangroves which contribute to 47% of the total area of world mangroves (Kadhireshan and Rajendran, 2004). Mangroves are adapted to grow in harsh environmental condition such as high salinity, high temperature, extreme tides, high sedimentation and muddy anaerobic soils (Spiers and Finlayson, 1999). Mangrove provide unique niche to variety of microorganisms ranging from bacteria, fungi, algae, yeast and so on (Kathirvel, 1996). Microbes constitute an important part of the mangrove environment, they have role in creating and maintaining this biosphere but 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 focused on the isolation and identification of microbes that are present in the sediments of mangrove for the assessment of potential strains.

2. Methodology

The mangrove forest inside Kadalundi – Vallikkunnu Community Reserve (Kerala: Kozhikode and Malappuram districts, 10°51’42’’ N and 75°48’21’’ E) was the area selected for the present study. This is the first community reserve of Kerala, declared in 2007 which spread across 1.5 square km and this area hosts a total of 7 species of mangrove plants belonging to 5 families (Myrsinaceae, Avicenniaceae, Rhizophoraceae, Euphorbiacea and Sonneratiaceae). A large portion of the shallow wetland is exposed with intertidal fluctuations which attributes to the unique characteristics of this wetland.

The sediment samples (five sub-samples from each defined locations and pooled together) were collected by hand core, removed surface leaf litter, transferred aseptically to sterilized polythene bags and transported to the laboratory at 4º C. The samples were plated on nutrient agar employing spread plate method.

The genomic DNA was extracted from the isolated colonies by using Ultraclean Soil DNA isolation kit (MoBio, USA) (Gray and Herwig, 1996). For molecular identification of the strains, 16S rRNA gene fragments were PCR 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

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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). The multiple sequence alignment was done using ClustalX 2.1 program (Larkin *et al*., 2007). Taxonomical hierarchy was assigned to the sequences using Ribosomal Database Project (RDP) Naive Bayesian rRNA Classifier Version 2.5 (Wang *et al*., 2007). The evolutionary analyses were inferred by using the Maximum Likelihood method in MEGA X software (Kumar *et al*., 2018).

3. Results and Discussion

The present study was focussed on the identification of bacterial isolates from the mangrove sediments of Kadalundi using molecular techniques (16S rRNA sequencing). Mangroves occupy the intertidal zone, interact strongly with aquatic, inshore, upstream and terrestrial ecosystem and in this way they help to support a diverse flora and fauna of marine, freshwater and terrestrial species. The higher nutritive value of mangrove sediment could possibly be one of the reasons for its selection as a breeding ground by many marine as well as fresh water microbes.

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 which 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 are important to the productivity, conservation and rehabilitation of mangrove ecosystems. The list of identified bacterial isolates obtained in the present study and their NCBI GenBank Accessions are shown in the Table 1.

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 evolutionary relationship among the 25 strains of bacteria isolated in the present study was analysed using maximum likelihood method in MEGA X. The dendrogram showing relationship among the identified bacterial strains isolated from mangrove sediments of Kadalundi, Kerala inferred on the basis of aligned 16S rRNA gene sequences is given in Figure 1. The tree with the highest log likelihood (-8609.53) is shown and is drawn to scale, with branch lengths measured in the number of substitutions per site. The dendrogram shows two separate monophyletic clades. Among this one clade is further clustered as two. One of the clusters has *Bacillus* strains and the other has *Ralstonia* strains. The second clade is divided into three clusters, one having *Shewanella* strains, another one with *Serratia* strains and the third one with *Enterobacter* and *Klebsiella* strains. The difference between within same species of isolates is less than 0.05, which is negligible when taken the difference between different species. Hence they can be regarded as same species.

Mangroves provide many ecological, environmental and socioeconomic benefits to the 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. The mangrove forests are a treasure that promotes biodiversity and even helps protect coastal areas. Although these natural wonders have so many benefits that they provide in their natural habitat humans have found other uses for them that endanger their species and harm the environment around them. 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 reduction in biodiversity, species decline, genetic erosion, extinction, increased flooding and decline in water quality.

In this study at Kadalundi mangrove ecosystem, some of the potent bacterial strains has been explored and identified. Studies of microbes and their interactions with mangrove ecosystem is critical for our understanding of mangrove ecosystem functioning and remediation. *Enterobacter asburiae* may prove to be very useful to human society and the environment, taking into consideration their ability to degrade polytethelyne plastics. Bacterial enzyme that could degrade tributyrine can be used to control the oil spill 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. *Bacillus amyloliquefaciens* have antifungal property which can be used in agriculture, aquaculture and hydroponics to fight against pathogens. Some of these strains also showed medical importance as they could cause diseases in humans including necrotizing fasciitis and anthrax. 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 sewages and the 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 due to the enzymatic activity of the 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. Thus, it cannot be allowed to let these wonderful habitats die out but that people are working every day to make sure that generations in the future will have these awe inspiring environments to explore.

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

Sl. No.	Isolate strain	Organism	GenBank Accession
1.	CUMB KDY-3	*Anurinibacillus aneurinilyticus*	MG 252692
2.	CUMB KDY-700	*Bacillus altitudinis*	MG 238593
3.	CUMB KDY-04	*Bacillus amyloliquefaciens*	MG 238546
4.	CUMB KDY-17	*Bacillus anthracis*	MG 252498
5.	CUMB KDY-600	*Bacillus cereus*	MG 238592
6.	CUMB KDY-09	*Bacillus* sp.	MG 238551
7.	CUMB KDY-900	*Bacillus* sp.	MG 238552
8.	CUMB KDY-500	*Bacillus* sp.	MG 238564
9.	CUMB KDY-120	*Klebsiella aerogenes*	MG 238557
10.	CUMB KDY-2	*Enterobacter asburiae*	MG 252629
11.	CUMB KDY-800	*Enterobacter cloacae*	MG 238594
12.	CUMB KDY-130	*Enterobacter* sp.	MG 238556
13.	CUMB KDY-5	*Geobacillus stearothermophilus*	MG 238548
14.	CUMB KDY-19	*Ralstonia pickettii*	MG 238555
15.	CUMB KDY-10	*Ralstonia pickettii*	MG 238560
16.	CUMB KDY-07	*Ralstonia pickettii*	MG 238595
17.	CUMB KDY-08	*Ralstonia pickettii*	MG 238596
18.	CUMB KDY-90	*Ralstonia pickettii*	MG 252552
19.	CUMB KDY-16	*Ralstonia* sp.	MG 238553
20.	CUMB KDY-18	*Ralstonia* sp.	MG 238554
21.	CUMB KDY-13	*Serratia marcescens*	MG 238562
22.	CUMB KDY-11	*Serratia marcescens*	MG 252371
23.	CUMB KDY-15	*Serratia* sp.	MG 238561
24.	CUMB KDY-300	*Shewanella bicestrii*	MG 252372
25.	CUMB KDY-200	*Shewanella seohaensis*	MG 238563

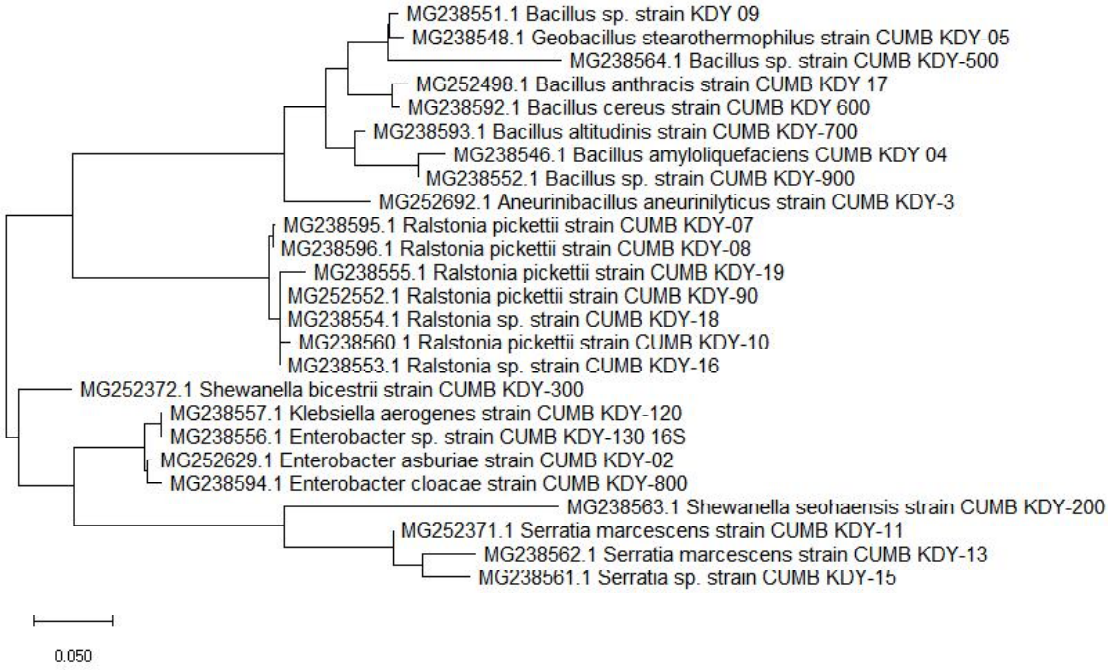

Fig. 1. The dendrogram showing relationship among the bacterial strains isolated from mangrove sediments of Kadalundi, Kerala inferred on the basis of aligned 16S rRNA gene sequences using maximum likelihood algorithm.

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