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Diversity of Actinobacteria in Mangrove Ecosystem of Muthupet, India

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Abstract

Totally 57 actinobacteria isolates were obtained from four sediment samples collected from four different seasons (pre monsoon, post monsoon, summer and monsoon) of Palk Strait region situated along the, South East coast of India. Among them, 6 dominant isolates in all seasons were found to be morphologically identified on the basis of color of front mycelium and reverse side color formation and sporophore morphology. The primary identification of this isolates were based on biochemical and physiological characteristics of the isolated strains. Furthermore, the analysis of nucleotide sequence of the 16SrRNA sequencing. Six isolates were assigned to the actinobacteria Streptomyces niveoruber, S.heliomycini, S.flavomacrosporus, Lechevalieria aerocolonigenes, L.flava and Dactylosporancium vinaceum.

Keywords: Diversity, actinobacteria, mangrove ecosystem,16S rRNA sequencing.

Introduction

The mangrove forest and associated with water bodies are together called mangrove wetland. The mangrove forest is rich in biological diversity and it is a globally significant habitat for wildlife[1]. Muthupet mangrove ecosystem is located at the South east Coast of India. Mangroves of muthupet (10° `-20° N, 79° 35`E), Tamil Nadu. Mangrove has a salty ecosystem and it is known to be large sources of organic matter due to various microbial enzymatic and metabolic activities[2]. Actinomycetes are all Gram-positive, filamentous and are facultatively anaerobic. All species grow best under anaerobic conditions. Actinomycetes play an important role among the mangroves bacterial communities, because of its diversity and ability to produce novel chemical compounds of high commercial value[3]. Actinomycetes participate in many important biochemical processes in the soil[4]. Actinobacteria are a well known source of various secondary metabolites such as lignocellulose, hemicellulose immunomodulators, anti-infective and anticancer agents[5,6]. The present work highlights on the diversity study of potential actinobacteria in mangrove ecosystem.

Material and Methods

Collection of sample: Sediment soil sample collected randomly from Muthupet mangroves (Lat.10°20'N & Long.79°35' E) South East Coast, Tamil Nadu, at different seasons (pre monsoon, monsoon, post monsoon and summer) (figure 1).

Media and culture conditions: The starch casein agar medium used for the isolation and cultivation of actinobacteria. The sterilized 50ml of sea water and 50ml distilled water. After autoclaving, the medium was supplemented with nylidixic acid and amphotercin B (Himedia, Mumbai) 10 µg/ml respectively as antibacterial and antifungal agents to inhibit the bacterial and fungal contamination. The diluted sediment samples (0.1 ml) were spread over the medium with a sterilized bent (L) rod and plate spinner. The inoculated plates were incubated at 30º C for 3 to 7 days. After incubation, colonies were recorded. The pure colonies were transferred on agar slants and preserved for further analysis[7].

Identification of isolated cultures: The growths of the 6 isolates were inoculating in to various culture media such as ISP1, ISP 2, ISP3, ISP 4, ISP 5, ISP6 and ISP7 incubated at 28ºC for 14 days. After growth, the slide cultures were examined under light microscope. Color of spore mass visually estimated by using the colour chart[8]. The isolates to determine the production of acids by utilizing the different sources of carbohydrates like adonitol, arabinose, glucose, galactose, glycerol, fructose, lactose, mannitol and xylose were tested by inoculating the isolates in ISP1 broth supplemented respective sugars and incubated for 7 day at 30°C[9].

Sodium chloride concentrations (5 and 9%) were poured to the ISP2 medium. The plates were incubated at 28º C for 7 days. After incubation growth was recorded. Physiological characterization of isolates carried out by performing the growth at different temperatures range from 15,20,28,37 and 45°C, pH range from 5 to 12 and the growth under anaerobic condition.

16S rRNA Sequencing of the Isolates: Genomic DNA was separated from isolate cultures following Pospiech and Neumann, 1995. The 16S rRNA was amplified using universal primers FD1 (5'-AGAGTTTGAT- CCTGGCTCAG-3') and RP2 (5-ACGGCTACCTTGTT- ACGACTT-3')[10]. The PCR products were purified and sequenced by Sri Bio Tech Company Limited (Hyderbad).

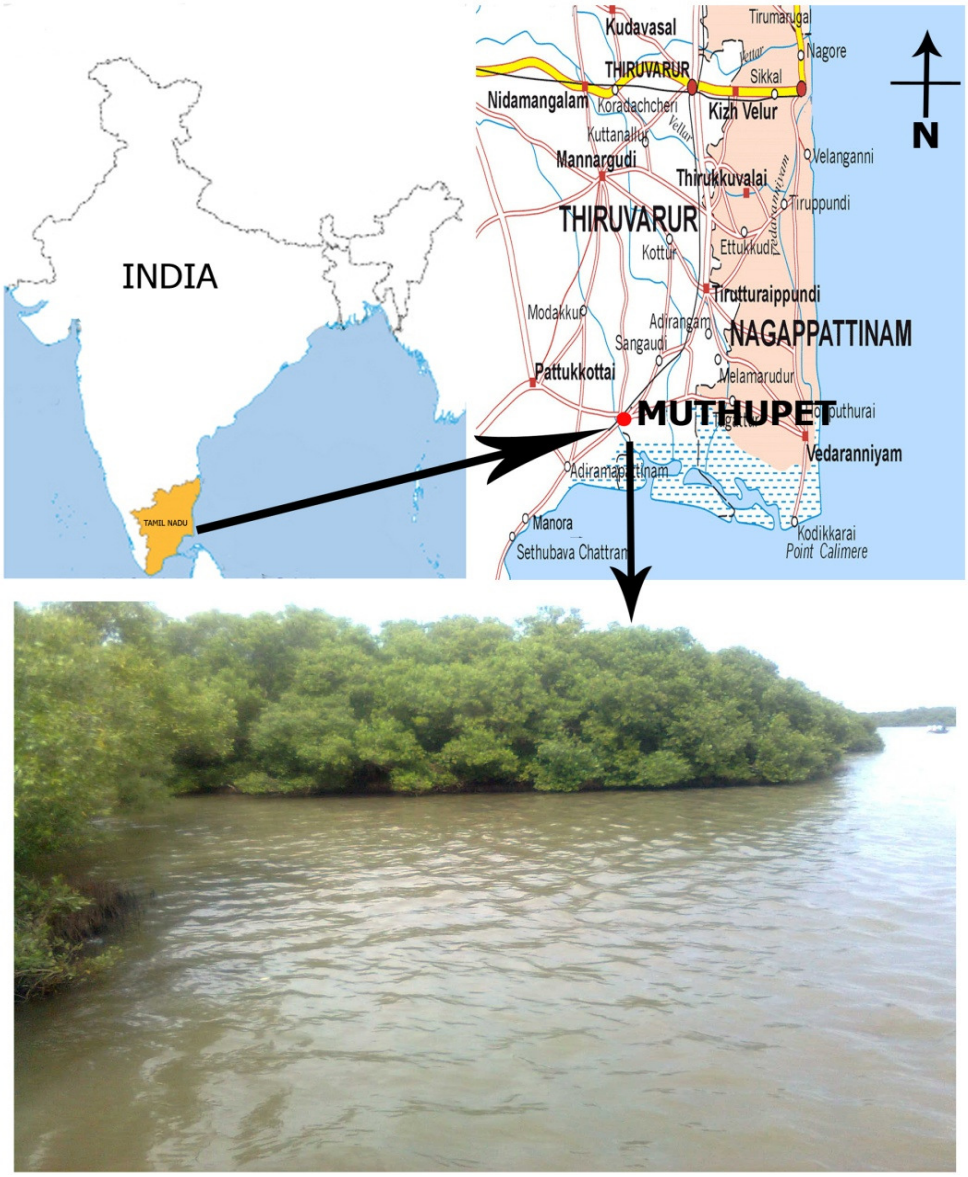

Figure-1
Map showing the study area of Muthupet mangrove

Phylogenetic analysis of isolates: 16S rRNA sequences of 6 strains were aligned with 16S rRNA sequences of other actinomycetes retrieved from the EMBL/GenBank database. Multiple alignments were performed manually using Clustal x[11]. Neighbor-joining phylogenetic tree and molecular evolutionary analyses were conducted using MEGA version 4[12].

Nucleotide sequence accession numbers: The 16S rRNA sequences of 6 isolates have been deposited in the GenBank library under accession numbers KF414963–KF414968.

Results and Discussion

In study area situated at muthupet mangroves ecosystem, South East coast of India. During the study period the seasonal distribution of actinobacteria counts were found maximum during pre monsoon season in (18.0 x 10^{-6}CFU /g) when compared to other season and minimum during post monsoon season in (10.0 x 10^{-6}CFU /g). Moreover, the maximum number of actinobacteria were recorded in pre monsoon season is due to the rich amount of organic substrate, light, temperature and other variable of the limiting factors determine the diversity of actinobacteria. The occurrence of microbial populatiom, plant species, nutrient status and salinity, and other environmental variables[13,14]. This might be attributed to nutrient accumulation, precipitation of inorganic compounds and settlement of organic matter in the mangrove sediments[15]. In Similar report by Sivakumar, 2001, the, counts of actinomycetes diversity were found maximum during monsoon season in mangrove forest[13].

Totally 57 isolates of different color colonies of actinobacteria were isolated from the mangroves soil. Many actinomycetes reported by the present study are white colour morphology. In a study by Vanajakumar *et al*., 1991, reported that, out of 192 colonies belong to 68 isolates white colour series actinobacteria were the dominant forms than the gray and pink colour series[16]. The results obtain by the present studies correlated by the finding of Sivakumar and suganthi, 2011, the 107 actinomycetes isolated from different mangrove[17]. Among the isolates, six were found to be dominant in all seasons. These organisms grew well on the other growth media. The actinobacteria isolates like GACMPT4, GACMPT9, GACMPT57, GACMPT8, GACMPT44 and GACMPT 34 showed good growth was observed on different media (table1). Good growth were observed various carbon sources, pH 7 and 9, temperature 28ºC and 37ºC and salinity 5 and 9 % (table 2). After PCR amplification from strains GACMPT4, GACMPT9, GACMPT57, GACMPT8, GACMPT44 and GACMPT34, the 16S rRNA gene partical sequenced. All six Strains were deposited in Genbank, USA and the strains were assigned the accession numbers *Streptomyces niveoruber* (KF414963), *S.heliomycini* (KF414964), *S.flavomacrosporus* (KF414965), *Lechevalieria aerocolonies* (KF414966), *L.flava* (KF414967) and *Dactylosporangiun vinaceum* (KF414968).

Table - 1
Cultural characteristics of GACMPT4 (*Streptomyces niveoruber*) on different media

Media	Front pigmentation	Reverse side pigmentation
Yeast extract agar (ISP1)	Grey	Yellow
Yeast extract malt extract agar (ISP2)	Whitish grey	Orange
Oat meal agar (ISP3)	Whitish grey	White
Inorganic salt agar (ISP4)	Whitish grey	Violet
Glycerol aspergine agar(ISP5)	Whitish grey	Pale yellow
Peptone yeast extract iron agar (ISP6)	Whitish grey	Orange
Trysine agar (ISP7)	Whitish grey	Pale yellow

Cultural characteristics of GACMPT9 (*Streptomyces heliomycini*) on different media

Medium	Front pigmentation	Reverse side pigmentation
Yeast extract agar (ISP1)	White	Orange
Yeast extract malt extract agar(ISP2)	Dull white	Orange
Oat meal agar (ISP3)	White	Pale yellow
Inorganic salt agar (ISP4)	White	Pale yellow
Glycerol aspergine agar (ISP5)	Yellowish white	Pale yellow
Peptone yeast extract iron agar(ISP6)	White	Pale yellow
Trysine agar (ISP7)	Yellowish white	Pinkish white

Cultural characteristics of GACMPT57 (*Streptomyces flavomacrosporus*) on different media

Media	Front pigmentation	Reverse side pigmentation
Yeast extract agar(ISP1)	White	Pale yellow
Yeast extract malt extract agar (ISP2)	White	Pale yellow
Oat meal agar (ISP3)	White	Pale yellow
Inorganic salt agar(ISP4)	White	Pale yellow
Glycerol aspergine agar(ISP5)	White	Pale yellow
Peptone yeast extract iron agar (ISP6)	White	Yellowish white
Trysine agar (ISP7)	White	Pinkish white

Cultural characteristics of GACMPT8 (*Lechevalieria aerocolonies*) on different media

Media	Front pigmentation	Reverse side pigmentation
Yeast extract agar(ISP1)	Grey	Pale yellow
Yeast extract malt extract agar (ISP2)	Whitish grey	Orange
Oat meal agar (ISP3)	Dull White	Pale yellow
Inorganic salt agar (ISP4)	Whitish grey	Orange
Glycerol aspergine agar (ISP5)	Grey yellowish White	Pale yellow
Peptone yeast extract iron agar (ISP6)	Whitish grey	Pale yellow
Tyrosine agar (ISP7)	Whitish grey	Pale yellow

Cultural characteristics of GACMPT44 (*Lechevalieria flava*) on different media

Media	Front pigmentation	Reverse side pigmentation
Yeast extract agar (ISP1)	White	Pale yellow
Yeast extract malt extract agar(ISP2)	White	Pale yellow
Oat meal agar(ISP3)	White	Pale yellow
Inorganic salt agar(ISP4)	White	Dark yellow
Glycerol aspergine agar (ISP5)	White	Pale yellow
Peptone yeast extract iron agar(ISP6)	White	Pale yellow
Tyrosine agar (ISP7)	White	Dark yellow

Cultural characteristics of GACMPT34 (*Dactylosporangium vinaceum*) on different media

Media	Front pigmentation	Reverse side pigmentation
Yeast extract agar(ISP1)	Whitish grey	Pale yellow
Yeast extract malt extract agar(ISP2)	Grayish pink	Reddish brown
Oat meal agar (ISP3)	Whitish pink	Orange
Inorganic salt agar (ISP4)	Whitish grey	White
Glycerol aspergine agar(ISP5)	White	Pale yellow
Peptone yeast extract iron agar(ISP6)	Whitish pink	Whitish pink
Tyrosine agar (ISP7)	White	Pale yellow

Table - 2
Physiological and biochemical characteristics of isolates

Tests	GACMPT 4	GACMPT9	GACMPT57	GACMPT 8	GACMPT 44	GACMPT 34
Carbon sources 1% W/V						
Adonitol	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+
Glucose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Xylose	+	+	+	+	+	+
Effect of pH						
5	_	_	_	_	_	_
7	+	+	+	+	+	+
9	+	+	+	+	+	+
12	_	_	_	_	_	_
Effect of T^0						
15ºC	_	_	_	_	_	_
20ºC	_	_	_	_	_	_
28ºC	+	+	+	+	+	+
37ºC	+	+	+	+	+	+
45ºC	_	_	_	_	_	_
Effect of NaCl concentration W/V						
5%	+	+	+	+	+	+
9%	+	+	+	+	+	+

Conclusion

The present study concludes, that the diversity of actinobacteria were maximum found in the pre monsoon season when compared to the other season due to the favorable nutrient, temperature, pH and salinity were present in this season. It contributes to the knowledge status of microbial diversity in mangrove ecosystem. Actinobacteria is most widely exploited microorganisms in terms of their capabilities in production of antibiotics and other compounds of biotechnology importance uses.

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