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Screening of novel actinobacteria and characterization of the potential isolates from mangrove sediment of south coastal India

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ABSTRACT

The importance of the current research is to investigate the different types of samples from the various mangrove sediments; as source of actinobacteria from the mangrove wet soil. Potential isolate screening by antimicrobial activity and identified actinobacteria was characterized based on cultural morphology, physiological and biochemical characteristics. Three different types of media were used to isolate actinobacteria from various geographical region of mangrove soil sediment and the genotype locus was recognized by 16S rDNA. Totally 144 actinobacteria isolates were recovered from 10 samples using three media. The most active culture media in the isolation of actinobacteria were ISP2 and Glycerol Yeast Extract Agar. Among 144 isolates, 38 isolates (26.38%) exhibited antimicrobial activity. Out of 38 isolates, potentially active 2 cultures were further supported for morphological and biochemical characterization analysis. Most of the isolates were produced pharmaceutically important enzymes such as protease, amylase, lipase, cellulose and also revealed antimicrobial activity against tested microorganism. The enriched salt, pH and temperature tolerance of the actinobacteria isolates to discharge commercially valuable primary and secondary bioactive metabolites. The present results functionally characterize novel mangrove actinobacteria and their metabolites for commercial interest in pharmaceutical industry.

1. Introduction

The mangrove ecosystems are specialized environments widely spread along with the coastal region, which is support biologically different group of organisms and microbes [1]. Actinobacteria represent a group of microbes which are the most economically important and biotechnologically valuable prokaryotes with 78% G + C content [2]. They are predominant in dry alkaline soil [3]. They are being exploited for various application biomedical, environmental and industrial purposes [4]. Mangrove ecosystem shows a potential natural resource of new actinobacteria due to their high species diversity [5]. Actinobacteria have been continuously reported as the prolific producers of microbial bioactive secondary metabolites for pharmaceutical aspect and agricultural applications [6] and more than 70% of the commercially available active compounds isolated from actinobacteria [7]. Mangrove ecosystem is the primary producer of biological diversity and microorganism are recognized to be rich sources of bioactive compound [8]. Mangrove actinobacteria are accomplished of producing variety of enzymes with good stability at higher temperature and alkaline state [9]. Actinobacteria are responsible for the digestion of resistant carbohydrate such as chitin and cellulose [10]. Mangrove actinobacteria are potential source of bioactive compounds as the mangrove ecosystem conditions entirely different from terrestrial conditions and several researchers have isolated novel antibiotic from mangrove ecosystem [11] [12]; [13]. The mangrove actinobacteria produced a variety of antibiotic, anticancer compounds and enzyme inhibitors [14]. Genetic methods are most reliable methods to identify the potential organism; to target specifically the 16s rRNA sequence of the actinobacteria [15]. The present studies on actinobacteria are very limited ecological niche. The actinobacteria have been stated incidentally on the microbial diverse of mangrove habitats. Hence, study was considered to evaluate different types of samples in different region and screen the antimicrobial activity. The identified actinobacteria was characterized based on culture morphology, physiological and biochemical characteristics of the isolates for pharmaceutical industrial application.

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2. Materials and method

2.1. Sample collection

The mangrove soil samples were collected from different location of mangrove forest along with the south coat of India. Samples were collected randomly, labelled and bring to the laboratory in sterile polythene bags and stored in refrigerator for further study.

2.2. Sample processing

The soil samples were collected at mangrove forest of south coastal of India, November 2015. Topsoil mangrove sediment of the upper 15 cm layer (removing the top 2–3 cm) was collected and labelled into sterile polythene bag using an aseptic metal trowel and stored at −20 °C. Air dried mangrove soil samples were ground with mortar and pestle. The pre-treatment of soil samples were treated using wet heat sterilized with de-ionized water (15 min at 50 °C; [16]). The mangrove soil sample was dried at 50 °C for 5 h and used further for actinobacterial isolation. Take five gram of the pre-treated soil was dissolved with 45 ml of sterilized de-ionized water, and then placed on shaker for 150 rpm at 1 h.

2.3. Measurement of physicochemical parameters

The pH of samples was determined by Meena et al. [17]. Briefly, 10 g of each mangrove sediment samples were suspended in 20 ml of deionized water and it was allowed to stand for 15 min, then the pH was measured by using digital pH meter (Elico LI617). Conductivity of the samples was recorded with an Electro-conductivity meter. Temperature, Dissolved Oxygen Demand (DOD), Biological Oxygen Demand (BOD) and soil nutrient of the sampling sites were measured by Grasshoff et al. [18].

2.4. Isolation of actinobacteria and maintenance

Three different type of culture media was used such as ISP2 medium (Yeast extract 0.8 g, Malt extract 2.0 g, Glucose 0.8 g, Agar 3.0 g, deionized water 200 ml), Starch Casein Agar (SCA) (Soluble starch 2.0 g, Casein 2.0 g, Meat extract 0.6 g, NaCl 1.0 g, Disodium phosphate 0.4 g, Bromo thymol blue 0.01 g, Agar 3.0 g, deionized water 200 ml, pH 7.3) and Glycerol Yeast Extract (Glycerol 1.0 g, Yeast Extract 0.4 g, $K_2HPO_4$0.02 g, Peptone 3.0 g, Filtered sea water 50% and deionized water 50%). The mediums were added with sterilized nystatin (20 μg/ml) and nalidixic acid (100 μg/ml). The sediment samples were dissolved in 1 ml of deionized sterile water and diluted up to 10^{-4} then the samples were spread on plate of above media. The plates were incubated at 28 °C for 3 weeks. The isolate was preserved on ISP2 agar plate.

2.5. Characterization of actinobacteria

Morphological, physiological and biochemical characterization of actinobacterial isolate strains were initially characterized according to Jicheng et al. [19]. The smears of isolated actinobacterial cultures were prepared and fixed, then the gram's staining was carried out with using Gram's staining kit (Himedia, Cat no: K001-1 KT). Properties and formation of aerial, substrate mycelium and arrangement of spores on mycelium was observed under the microscope at 100× magnification.

2.6. Molecular identification

Genomic DNA was isolated according to the method described previously by Xu [20]. Selected isolate was subjected to 16S rRNA sequence analysis for establishing of their genotypic location. The 16S rRNA was amplified with specific primers (27F 5′-AGAGTTTGATCCTGGCTCAG-3′) and (1492R 5′-TACGGYTACCTTGTTACGACTT-3′). PCR reaction cycling included an initial denaturation at 94 °C for 2 min and then followed by 35 amplification cycle of denaturation at 94 °C for 45 s, annealing at 55 °C for 1 min and extension at 72 °C for 1 min, followed by a final extension at 72°Cfor a period of 10 min. Amplified PCR products were checked with on 1% Agarose gel in 1× TAE buffer, then purified with a mixture of 2.5 Nacl mol/Land 20% polyethylene glycol (PEG). The obtained sequences were compared with 16S rRNA gene sequences cultured species using BLAST. The phylogenetic tree and homology was prepared with Clustal W and Tree view software.

2.7. In vitro antimicrobial assay

The antimicrobial assay was determined by the Agar plate diffusion method [21]. The samples were assayed against following test organisms [Gram negative bacteria *Pseudomonas aeruginosa* (MTCC-741), *Klebsiella pneumonia* (MTCC-4030) and *Escherichia coli* (MTCC-443); Gram positive bacteria *Staphylococcus aureus* (MTCC-902), *Bacillus subtilis* (MTCC-441), *Staphylococcus epidermidis* (MTCC-435), *Proteus vulgaris* (MTCC-742); Fungal stain *Candida albicans* (MTCC-227), *Aspergillus flavus* (MTCC-871), *Aspergillus nigar* (MTCC-282)] were chosen as test microbes. The isolates were incubated on the modified ISP2 medium at 28 °C for one week and discs (6 mm) were cut and placed on Luria Bertani (LB) agar medium (for bacterial culture) or Potato Dextrose Agar (PDA) medium, then this was seeded with appropriate teat organism. Agar plates were incubated at 37 °C for 48 h (for bacterial) and 72 h (for fungal). Inhibition of zone was measured after incubation of plate.

3. Screening of mangrove actinobacteria for surfactant production

3.1. Lipase production

Ability of the isolated strain to synthesis extracellular lipase were measured using ISP2 medium (Yeast extract 0.8 g, Malt extract 2.0 g, Glucose 0.8 g, Agar 3.0 g, deionized water 200 ml) with 1% (w/v Try-butyrin and pH 7.4). A loopful of inoculum (strain) were streaked on to the agar plates and incubated at 30 °C for 1 week. After incubation the plates were observed for lipase production and measure the clear zone around the colonies.

3.2. Hemolytic activity

Isolates collected from mangrove sediments were screened for hemolytic activity was determined by Carillo et al. [22]. Blood agar medium contain (Yeast Extract 5% (w/v), Peptone 3% (w/v), Nacl 5% (v/v) and Human Blood 5% (v/v)). Plates were observed for hemolytic activity after incubation at 37 °C for 7 days and presence of zone clearly around the colonies. The potential of isolate shown surfactant production was recorded.

3.3. Emulsification test

Emulsification assay were examined by the method reported by Menna et al. [17]. Cell free supernatant and kerosene was mixed with the final ratio of 1:1, then vortex for 2 min and incubated at room temperature for 24 h. The height of emulsified layer and emulsification index were calculated as the following formula;