ANTAGONISTIC ACTIVITIES OF ACTINOBACTERIA FROM MANGROVE SEDIMENT

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INTRODUCTION

Actinobacteria are gram positive bacteria frequently filamentous and spore bearing with DNA rich in G-C from 57-75%. Some of their secondary metabolites have been employed as useful antimicrobial compounds. They were originally considered an intermediate group between bacteria and fungi then were recognized as prokaryotic organisms. They are the most widely distributed group of microorganisms in nature which primarily inhabit the soil and are found to be more in dry than wet soils.

Actinobacteria also serve as an important source of food for a variety of marine organisms. Thus, actinobacteria not only maintain the pristine nature of the environment, but also serve as biological mediators through their involvement in biogeochemical processes. Actinobacteria synthesize numerous natural metabolites with diverse biological activity such as antibiotics, herbicides, pesticides, enzymes and antiparasitic compounds. Antibiotics from Actinobacteria origin evidence a wide variety of chemical structures including aminoglycosides, anthracyclines, Beta-lactans, nucleosides, peptides, polyenes, polyketides, actinomycins and tetracyclins. A total 7899 (100%) bioactive compounds has been identified out of which from 67% are from actinobacteria, 12% from bacteria and 20% from fungi. Since the discovery of actinomycin, the first antibiotic from an Actinomycete, many commercially important bioactive compounds and antitumor agents have been produced using actinobacteria. Antibiotics have been used in many fields including Agricultural, Veterinary and Pharmaceutical industry. The genus Streptomyces is the most predominant among actinobacteria. The Screening of microorganisms for the production of novel antibiotics has been intensively pursued for many years. The number of actinobacteria which live in marine environment are poorly understood and few reports are available pertaining to actinobacteria from mangroves.

Mangrove ecosystem has a saline environment and it is known to be highly rich in organic matter due to various microbial enzymatic and metabolic activities. Distribution and occurrence of microbes are well reported from several mangrove ecosystems. Marine environments are largely untapped source for the isolation of new microorganisms with potentiality to produce active secondary metabolites. Among such Microorganisms, actinobacteria are of special interest, since they are known to produce chemically diverse compounds with a wide range of biological activities. The demand for new antibiotics continues to grow due to the rapid emerging of multiple antibiotic resistant pathogens causing life threatening infection. Now a day’s considerable progress is being continuing with in the fields of chemical synthesis and in the field of engineered biosynthesis of antibacterial compounds. So, the nature still remains the richest and the most versatile source for new antibiotics. In order to discover new antibiotics, our approach is to investigate unexplored regions of the world with the aim of isolating bioactive actinobacteria from these regions and from those organisms whose potential was neglected throughout the history. The mangrove ecosystem is a largely unexplored source for actinobacteria with the potential to produce biologically active secondary metabolites. Consequently, we set out to isolate, characterize and screen actinobacteria collected from mangrove sites in Local area. The aim of our present study is to conduct intensive screening program on mangrove soil samples which is likely to yield purposeful results towards the isolation of new species of actinomycetes or new bioactive metabolites.

MATERIALS AND METHODS

Sample Collection

Sediment sample were collected from 6-10 cm depth from mangrove regions in local area of Visakhapatnam. Sample was taken in a zipped polythene bags and were carried to the laboratory under aseptic conditions for further studies.

Isolation of Actinobacteria from sediment samples

Actinobacteria were isolated by serial dilution plate technique using Glucose yeast extract malt extract agar media (International Streptomyces Project ISP 2). The media is supplemented with Rifampicin 5μg/ml and Fluconazole 25μg/ml to inhibit bacterial and fungal contamination respectively. About 1gm of soil sample was transferred to sterile 250 ml Erlenmeyer flask containing 100 ml of sterile distilled water. The flasks were shaken on rotary shaker for 30 min for the detachment of the Spore chains, if any. The flasks were kept aside for 30 min to settle down the particulate matter. The clear supernatant was diluted with sterile water. These dilutions (10-1-10-9) were used as inocula. 0.1 ml of each of these dilutions was spread over the medium by sterile bent glass rod. The plates were left for 30 minutes to dry before inverting and incubated at 28°C for 2-3 weeks. Selected colonies (rough, chalky, dry) of actinobacteria were further isolated in pure form on the solidified yeast malt extract agar by streak plate method. Colony selection was based on the color of aerial and substrate mycelia, differences in morphology and rate of growth.

Characterization of the isolates

The isolates were characterized up to genus level by observing the spore bearing hyphae, structure of spore chain, colour of the spore, aerial mass colour and colour of substrate mycelia as described by Bergy and International Streptomyces Project (ISP).

Screening of Actinobacteria for Antimicrobial activity

In preliminary screening the antimicrobial activity of pure isolates was determined by perpendicular streak method on Nutrient agar media against test organisms.
Secondary screening was performed by agar well diffusion method against the standard test organisms *S. aureus* (MTCC 3160), *B. Subtilis* (MTCC 441), *B. cereus* (MTCC 430), *E. coli*, (MTCC 443), *P. aeruginosa* (MTCC 424), *P. vulgaris* (MTCC 426) using Nutrient agar media and *S. cerevisiae* (MTCC 170), *C. albicans* (MTCC 227), *A. niger* (MTCC 961), and *A. flavus* (MTCC 3396) using potato dextrose agar.

**Fermentation process**

The isolates having the activity were cultured in about 100 ml of production media having the composition glucose 1%, soybean meal 1%, NaCl 1%, and CaCO₃ 0.1% in 250 ml Erlenmeyer flask under submerged fermentation conditions at 28°C for 96h at 180 rpm.

**Isolation of antibacterial metabolites**

Antibacterial compound was recovered from the filtrate by solvent extraction method following the process described by Westley et al. Ethyl acetate was added to the fermented broth in the ratio 3:0.1% in 250 ml Erlenmeyer flask under 24°C. The ethyl acetate extract was loaded into well bored and test plates were incubated at 37°C for 18-24 hrs. for bacteria and incubated at 28°C for 48 hrs. for fungi examined and the zone of inhibition were expressed as diameter (mm).

**RESULTS AND DISCUSSIONS**

There is no report regarding isolation of actinobacteria from mangrove soil in Visakhapatnam. In this present study, 20 actinobacteria isolates were obtained in pure form and analyzed for their antimicrobial activities. The pure cultures were maintained on the same medium that was used for isolation and were preserved at 4°C.

Out of 20 isolates, 12 active isolates were identified up to genus level and found to be belonging to genus Streptomyces based on the morphological, physiological and biochemical characteristics. The morphological and physiological characters are shown in the Table 1.

In screening for actinobacteria having antimicrobial activity, out of 20 isolates, only 12 isolates showed the activity against test microorganisms during the preliminary screening. Whereas the remaining isolates did not exhibit any activity. Out of 12 isolates that were subjected to secondary screening, all the potent strains were mass multiplied in liquid medium and the Ethyl extracts were prepared. All the extracts were analyzed for their antimicrobial activity by agar well diffusion method.

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**Table 1: Morphological and Biochemical characteristics of the 12 putative Isolates**

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<th>Name of the isolates</th>
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*'+' positive, '-' negative*
BC01 32 35 29 32 35 39
BC02 30 35 28 30 35 35
BC03 27 35 23 25 35 33
BC04 30 35 30 30 35 36
BC05 17 29 18 23 30 27
BC06 10 33 17 20 32 25
BC09 28 34 21 22 31 33
BC10 10 28 14 16 27 21

Table 2: Antibacterial activities of actinobacteria isolates zone of inhibition in mm

Name of the Isolates | S. aureus MTCC 3160 | B. Subtilis MTCC 441 | B. cereus MTCC 430 | P. aeruginosa MTCC 424 | E. coli MTCC 443 | P. vulgaris MTCC 426
---|---|---|---|---|---|---
BC01 | 32 | 35 | 29 | 32 | 35 | 39
BC02 | 30 | 35 | 28 | 30 | 35 | 35
BC03 | 27 | 35 | 23 | 25 | 35 | 33
BC04 | 30 | 35 | 30 | 30 | 35 | 36
BC05 | 17 | 29 | 18 | 23 | 30 | 27
BC06 | 10 | 33 | 17 | 20 | 32 | 25
BC09 | 28 | 34 | 21 | 22 | 31 | 33
BC10 | 10 | 28 | 14 | 16 | 27 | 21

Of these, 8 isolates were found to exhibit antimicrobial activity while the remaining 4 isolates exhibited very poor activity. The diameter of the zone of inhibition against the test organisms are represented in Table 2 and 3. It was observed that most of the active isolates possessed antibacterial activities. Among these isolates BC 01, BC 02, BC 03 BC 04 and BC 05 is found to exhibit a broad spectrum of activity against bacteria and the remaining isolates BC 08 and BC 09 exhibited good antibacterial activity against the test organisms. Whereas BC 10 showed good activity against the B. Subtilis, E. coli, P. vulgaris, P. aeruginosa, B. cereus and exhibits less activity against S. aureus. Similar type of results was reported by Umasankar et al., where the actinobacteria isolated from forest soil showed good antibacterial activities. In case of the fungal studies the four isolates BC 01, BC 02, BC 03, BC 04 BC 05 exhibited highest activity. Whereas BC 05 showed intermediate activity against A. niger and C. albicans did not showed activity against S. cerevisiae and A. flavus. The isolate BC 10 showed intermediate activity against A. niger and A. flavus and did not exhibit activity against C. albicans and S. cerevisiae. Similar type of results were reported by Baskaran, et al., where Actinobacteria isolated from the mangrove sediments of Andaman and Nicobar Islands showed broad spectrum of antibacterial activity. In a similar work, Actinobacteria isolated from sediments of Parangipetai coastal water exhibited both antibacterial and antifungal activities. Mangrove ecosystem is a poorly studied area, an unexplored territory for study of their morphological, cultural, physiological, biochemical and antimicrobial activities. Detailed taxonomical studies were carried out and it was observed that new isolates were obtained. These were found to be excellent antimicrobial producers which showed broad spectrum of antibacterial and antifungal activities. In case of the fungal studies the four isolates BC 01, BC 02, BC 03, BC 04 showed good activity against C. albicans and S. cerevisiae. Similar type of results was reported by Umasankar et al., where Actinobacteria isolated from forest soil showed good antibacterial activities. In case of the fungal studies the four isolates BC 01, BC 02, BC 03, BC 04 showed good activity against C. albicans and S. cerevisiae. The four isolates BC 01, BC 02, BC 03, BC 04 exhibited good antibacterial activity. Where BC 05 showed intermediate activity. BC06 exhibited highest activity. These isolates were found to be excellent antimicrobial producers which showed broad spectrum of antibacterial and antifungal activities is been selected for detailed optimization studies. Further studies on antitumor activity and purification of the antibiotic substances and elucidation of its pathways are under progress.

ACKNOWLEDGEMENT

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Table 3: Antifungal activities of actinobacteria isolates zone of inhibition in mm

Name of the Isolates | C. albicans MTCC 227 | S. cerevisiae MTCC 170 | A. niger MTCC 961 | A. flavus MTCC 3396
---|---|---|---|---
BC01 | 19 | 14 | 28 | 18
BC02 | 17 | 13 | 24 | 15
BC03 | 14 | 11 | 15 | 12
BC04 | 18 | 12 | 20 | 16
BC05 | 10 | - | 10 | -
BC08 | - | - | 10 | -
BC09 | 13 | 10 | 14 | 12
BC10 | - | - | 11 | 10


