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Research Article

ANTAGONISTIC ACTIVITIES OF ACTINOBACTERIA FROM MANGROVE SEDIMENT

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ABSTRACT

The present study aims at isolation and characterization of biologically diverse strains of Streptomyces from mangrove sediment sample for the production of bioactive secondary metabolites. A total of 20 isolates were obtained. These isolates were screened for the presence of antimicrobial substances. Out of 20 isolates, only 12 isolates showed antimicrobial activity against *S. aureus*, *B. Subtilis*, *B. cereus*, *E. coli*, *P. aeruginosa* , *P. vulgaris*, *S. cerevisiae*, *C. albicans*, *A. niger*, and *A. flavus* during preliminary screening. The active isolates were morphologically distinct on the basis of spore mass, spore colour, reverse side colour, aerial and substrate mycelia formation, production of diffusible pigment and biochemical characterization. The 12 active isolates were subjected to submerge shake flask cultures. The culture supernatant was extracted with ethyl acetate. The partially purified ethyl acetate extract were used for the determination of antimicrobial activities. Of these 12 isolates, 8 were found to be active against the test organisms used in the study. Most of the isolates showed activities against both bacteria and fungi respectively.

Keywords: Mangrove sediment, Actinobacteria, Bioactive secondary metabolites, Antimicrobial activities.

INTRODUCTION

Actinobacteria are gram positive bacteria frequently filamentous and sporulating with DNA rich in G+C from 57-75%. Some of their secondary metabolites have employed as useful antimicrobial compounds[1]. 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[2-3].

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[4]. Actinobacteria synthesize numerous natural metabolites with diverse biological activity such as antibiotics, herbicides, pesticides, enzymes and antiparastic compounds. Antibiotics from Actinobacteria origin evidence a wide variety of chemical structures including aminoglycosides, anthracyclines, Beta- lactans, nucleosides, peptides, polyenes, polyketides, actinomycins and tetracyclins[5-6]. 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[7]. Antibiotics have been used in many fields including Agricultural, Veterinary and Pharmaceutical industry[8]. 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[9].

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[10-11]. Marine environments are largely untapped source for the isolation of new microorganisms with potentiality to produce active secondary metabolites[12]. Among such Microorganisms, actinobacteria are of special interest, since they are known to produce chemically diverse compounds with a wide range of biological activities[13]. 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[14-16]. So, 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^{-3}) 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 Bergey[17] and International Streptomyces Project (ISP) [18-19].

Screening of Actinobacteria for Antimicrobial activity

In preliminary screening the antimicrobial activity of pure isolates was determined by perpendicular streak method[20-21] on Nutrient agar media against test organisms.

Secondary screening was performed by agar well diffusion method[22] 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 $CaCo_3$ 0.1% in 250 ml Erlenmeyer flask under submerged fermentation conditions at 28° C for 96h at 180 rpm[23].

Isolation of antibacterial metabolites

Antibacterial compound was recovered from the filtrate by solvent extraction method following the process described by Westley *et.al,.*[24]**.** Ethyl acetate was added to the fermented broth in the ratio of 1:1(v/v) and shaken vigorously for 1 hour for complete extraction. The ethyl acetate phase that contains bioactive compound was separated from the aqueous phase. It was evaporated to dryness under reduced vacuum 80°-90°C and the residue obtained was used to determine the antimicrobial activity.

Determination of the antimicrobial activity

The antimicrobial activity was determined by agar well diffusion method. The partially purified extract obtained by the evaporation of the ethyl acetate extract was loaded into well bored and test organisms were used for the antimicrobial assay. The 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.

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

	Name of the isolates											
Name of the test	**BC 01**	**BC 02**	**BC 03**	**BC 04**	**BC 05**	**BC 06**	**BC 07**	**BC 08**	**BC 09**	**BC 10**	**BC 11**	**BC 12**
Substrate Mycelia	White	Pale green	Creamy	Green	White creamy	White creamy	White creamy	Black	Pale green	Black	Grey	White creamy
Aerial Mycelia	Gray	Black	Grey	Dark green	White chalky over the surface on the culture	White chalky over the surface on the culture	White chalky and colonies were rough	Pale yellow	Pale yellow	Dusty brown	Pale green	Pale orange
Pigmentation	Nil	Bluish black diffusible pigment	Black diffusible pigment	Nil	Nil	Nil	Nil	Nil	Nil	Black	Black diffusible pigment	Nil
Indole Production	+	+	+	+	-	+	-	+	-	+	+	+
Methyl red	+	+	+	-	+	+	+	+	+	+	+	+
Voges proskauer	+	-	+	+	+	+	-	-	-	-	-	-
Citrate utilization	+	-	+	-	+	+	+	+	+	+	+	+
H_2S Production	+	+	+	+	+	+	+	+	-	+	-	+
Nitrate Reduction	+	-	+	+	+	-	-	+	-	+	-	+
Urease	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Melanin Production	+	-	+	+	+	-	-	-	+	+	-	-
Starch hydrolysis	+	+	+	+	+	-	+	+	-	+	-	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Casein hydrolysis	+	-	+	-	+	-	-	+	+	+	-	-
Tyrosine	+	-	+	+	+	+	+	-	-	+	+	-

'+' positive, '-' negative

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	38
BC02	30	35	28	29	35	35
BC03	27	35	23	25	35	33
BC04	30	35	30	30	35	36
BC05	17	29	18	23	30	27
BC08	10	33	17	20	32	25
BC09	28	34	21	22	31	33
BC10	10	28	14	16	27	21

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

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.*, [25] 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 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.*, [11] 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 Parangipettai coastal water exhibited both antibacterial and antifungal activities[26]. Mangrove ecosystem is a poorly studied environment of bioactive compounds[27]. Hence, the development and application of new strategies for the detection, isolation and subsequent description of novel actinobacteria, from natural mangrove habitat was an essential need for the bioactive compound discovery[28]. In this study, concentrated culture filtrates demonstrated increased activity as shown by *in vitro* tests. The present finding highlights the importance for further investigation towards the goal of obtaining novel antimicrobial agents. Further studies on the bioactive metabolites produced by the antagonistic isolates, which exhibit a broad spectrum of activity, is under progress moreover, this study gives the primary information on the antimicrobial activity of actinobacteria from the mangrove sediment.

CONCLUSION

In the present study effort was mainly directed towards the isolation of actinobacteria from mangrove sediments collected from local 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 shown broad spectrum of antibacterial and antifungal activities is been selected for detailed optimization studies. Further studies on anticancer activity and purification of the antibiotic substances and elucidation of its pathways are under progress

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