

Screening and Isolation of Antagonistic Actinobacteria from marine sediments of Visakhapatnam Sea Coast

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ABSTRACT

Sixty three marine actinobacterial species were isolated from ten marine sediment samples collected from visakhapatnam sea coast of bay of bengal at different depths. The isolates were screened for antimicrobial activity against 4 pathogenic fungi and 6 pathogenic bacteria. Among 63 isolates, 10 isolates showed antibacterial activity and 6 isolates showed antifungal activity. Among active isolates, isolate No. 18 showed highest antimicrobial activity against all the pathogenic bacteria and fungi studied and it was identified as *Streptomyces species*.

Keywords: Marine actinobacteria, *Streptomyces species*, Antimicrobial activity.

INTRODUCTION

Marine actinobacteria are of high commercial value because of their ability to produce new bioactive substances¹. During last two decades many novel bioactive metabolites were isolated from marine actinobacteria especially from streptomycetes. The interest in the marine microflora has been increased due to the investigation of novel bioactive compounds especially antibiotics². Adaptations of actinobacteria to unusual marine environment could potentially yield a broad spectrum of secondary metabolites³. In addition to antibiotics many commercially important bioactive compounds like antitumor agents, immunomodulators and enzymes of industrial interest have been produced from actinomycetes. It has been estimated that more than 200 naturally occurring antibiotics have been isolated from marine actinobacteria⁴. A very little is known about the microbial diversity of marine sediments, which are rich source microbial flora. One of the most successful approaches to obtain new types of useful microbial metabolites is to investigate marine sediments⁵. The visakhapatnam coast of bay of bengal, India has not been well explored for actinobacteria. The purpose of present

investigation was to screen marine sediments of Visakhapatnam coast to isolate, characterize and examine the antimicrobial activity of actinobacteria.

MATERIALS AND METHODS

Collection of sediment samples

Ten marine sediment samples were collected from Visakhapatnam coast of Bay of Bengal at different depths ranges from 5-95m using a core sediment sample collector and transferred into sterilized zip lock bags. The sediment samples were dark brown to black in color and sandy in texture. The location and depths of sediment sampling stations were shown in Table 1.

Isolation of actinobacteria from sediment samples

Different agar media like-Starch casein, Glycerol asparagine, Chitin and Glucose yeast extract malt extract^{6, 7} were used for the isolation of actinobacteria. Each medium contain 50% filtered 30 days old aged sterilized sea water. All the media were supplemented with rifampicin (5µg/ml) and nystatin (25µg/ml) to inhibit bacterial and fungal contamination respectively. The sediment samples were serially diluted with

sterilized seawater and an aliquot of 0.1 ml was spread on the media and incubated at 28°C. After incubation for 1–3 weeks, the developed actinobacteria colonies were purified by streak plate method, characterized and tested for their antimicrobial activity.

Characterization of the isolates

The isolates were characterized up to genus level by observing the spore bearing hyphae, structure of spore chain, color of the spore, aerial mass color and color of substrate mycelia as described by Bergey's manual⁸ and International Streptomyces Project (ISP)^{7,9}.

Screening of actinobacteria for antibiotic compounds

Initial screening of actinobacteria for antibiotic production was performed by cross streak method. The isolates having the activity were cultured in 50 ml of production medium containing 1% of glucose, soybean meal, NaCl, and 0.1% CaCO₃ in 250 ml conical flasks and incubated at 28° C for 96h at 180 rpm, under submerged fermentation conditions and centrifuged at 10,000 rpm for 20 minutes. The clear supernatant samples were tested for antimicrobial activities by agar well diffusion method (10). The Muller Hinton agar plates were seeded with *S.aureus*(MTCC 3160), *B.Subtilis*(MTCC 441), *B. cereus* (MTCC 430), *P. aeruginosa*(MTCC 424), *E.coli* (MTCC 443), *P. vulgaris* (MTCC 426) for antibacterial activity. Yeast extract –malt extract agar plates were seeded with *S.cerevisiae*(MTCC 170), *C. albicans*(MTCC 227), *A. niger*(MTCC 961), and *A.flavus*(MTCC 3396) for antifungal activity. Wells of 6 mm diameter were prepared in the nutrient agar plates and filled with the 50 µL of crude culture supernatant samples and the diameter of inhibition zones were measured after incubation at 37°C for 24 h for antibacterial activity and 25° C for 48 h for antifungal activity.

RESULTS AND DISCUSSION

Isolation of actinobacteria from sediment samples

As shown in Table-2, the number and species of actinobacteria varied from station to station. Highest number of marine actinobacteria was observed in sampling station No. 5, located at a depth of 45 meters. Whereas, sampling station No. 10 has only one isolate, which is located at a depth of 95 meters. A total of 63 actinomycetes were isolated from ten marine sediment samples. Out of 63 isolates 49 isolates were identified as genus

Streptomyces (spore chain was coiling, spiral and looped), 6 as *Micromonospora*, (clusters of single conidia on substrate mycelium), 5 as *Nocardia* (conidia on powdery appearance aerial hyphae, carotenoid like pigments) and 3 as *Streptoverticillium* (whorls of straight chain of conidia formed). *Streptomyces* species were predominant in all the sampling stations. Distribution and diversity of actinobacteria from marine sediments have also been reported by other scientists^{3,7}.

Characterization of the isolates

The cultural characteristics of actinobacteria were presented in Table 3. Out of 63 isolates, 49 isolates showed pigment production. 30 isolates produced melanin, 18 isolates showed distinctive reverse side pigment and 20 isolates produced soluble pigments. With reference to the morphology of spore-bearing hyphae, 24 isolates show (38%) flexous sporophores followed by 12 isolates show (19%) spiral sporophores, 10 isolates show (15%) retinaculum apertum sporophores, 9 isolates show rectus (14%) and 7 isolates show monoverticillus (11%).

Antimicrobial activity of Selective isolates:

The degree of antimicrobial activity of the isolates was classified depending on mean diameter of inhibition zones. In the present study, the diameter of the inhibition zone divided as follows: excellent activity (≥ 18 mm), good activity (12-15 mm) moderate activity (10-12 mm) and weak activity (≤ 9 mm). The zone of inhibitions are the mean of triplicates.

As shown in Table-4, out of 63 isolates 27 isolates were active against the pathogenic bacterial species. Out of 27 isolates, 8 isolates (03, 15, 18, 21, 28, 32, 42, and 51) showed excellent activity against all the tested gram positive bacteria and gram negative bacteria and rest of the 2 isolates (54 and 60) inhibited the growth of 5 bacterial species only and does not exhibit antagonistic activity against the *S. aureus*.

Antifungal studies showed that out of 63 isolates, three isolates (03, 18 and 23) showed various degrees of antagonistic activity against all the 4 fungal species studied and two isolates (31 and 52) showed antagonistic activity against three fungal species only. The isolate No. 48 was antagonized only single fungal species (Table-5). The antimicrobial studies of the actinobacteria revealed that isolate No, 18 showed excellent antagonistic activities against all bacterial and fungal species studied.

Table 1: Location and depths of sediment sampling stations of Visakhapatnam sea coast

Station	Depth(meters)	Latitude	Longitude
1	5	+17.46515	+83.23943
2	15	+17.30500	+83.02300
3	25	+17.30500	+83.04400
4	35	+17.30500	+83.08000
5	45	+17.29000	+83.00816
6	55	+17.29000	+83.02205
7	65	+17.29000	+83.05086
8	75	+17.29015	+83.07480
9	85	+17.27000	+83.59398
10	95	+17.27000	+83.59295

Table 2: Distribution of actinobacteria in different sampling stations of Visakhapatnam sea coast

Sampling station	Total No. of actinobacteria isolated	actinobacterial species			
		<i>Streptomyces</i>	<i>Micromonospora</i>	<i>Nocardia</i>	<i>Streptoverticillum</i>
1	6	4	1	1	-
2	8	5	1	1	1
3	6	5	1	-	-
4	10	8	-	1	1
5	14	12	1	1	-
6	9	6	1	1	1
7	4	3	1	-	-
8	4	4	-	-	-
9	1	1	-	-	-
10	1	1	-	-	-

Table 3: Cultural characteristics of actinobacteria

Character	No. of isolates	%
Spore morphology		
Flexuous	24	38
Spiral	12	19
Retinaculum apertum	10	15
Rectus	9	14
Monoverticillus	7	11
Pigment production		
Melanin	30	47
Reverse colour	18	28
Soluble colour	20	31
Isolates producing pigment	49	77

Table 4: Antagonistic activity of active marine actinobacteria against gram-positive and gram-negative bacteria

Isolate No.	Name of the test organism (Inhibition Zone diameter in mm)					
	<i>E.coli</i> (MTCC-443)	<i>P. vulgaris</i> (MTCC-426)	<i>P.aeruginosa</i> (MTCC-424)	<i>B.subtilis</i> (MTCC-441)	<i>B.cereus</i> (MTCC-430)	<i>S.aureus</i> (MTCC-3160)
MB-03	18±0.5	18±1	18±1	19±0.5	20±1	18±1
MB-15	15±1	15±1	19±0.4	12±1	12±1	18±0.6
MB-18	22±0.4	20±1	20±1	20±0.6	24±1	22±1
MB-21	18±0.5	19±0.6	18±1	18±1	19±0.5	19±1
MB-28	18±1	19±1	18±0.5	18±1	20±1	19±1
MB-32	18±1	19±0.4	18±0.6	18±0.8	18±1	18±0.6
MB-42	19±0.6	18±1	20±1	18±1	19±0.5	18±1
MB-51	18±1	18±0.6	18±0.4	19±1	19±1	19±1
MB-54	19±1	20±1	17±1	11±1	19±1	--
MB-60	22±0.4	19±1	15±0.2	16±1	20±1	--

Table 5: Antagonistic activity of active marine actinobacteria against pathogenic fungi

Isolate No.	Name of the test organism (Inhibition Zone diameter in mm)			
	<i>A.Niger</i> (MTCC-961)	<i>A.flavus</i> (MTCC-3396)	<i>C.albicans</i> (MTCC-227)	<i>S.cerevisiae</i> (MTCC-170)
MB-03	10±0.5	10±1	12±1	10±1
MB-18	19±1	19±0.4	20±1	19±0.4
MB-23	12±1	10±1	14±0.5	14±1
MB-31	10±0.6	10±1	--	12±0.6
MB-48	--	--	14±1	--
MB-52	12±1	10±1	--	12±0.4

CONCLUSION

By combining pretreatment with suitable media supplemented with specific antibiotics and by conventional dilution-plating techniques marine actinobacteria genera can now be successfully isolated. Further studies on the characterization of the isolates, purification of the antibiotic substance are underway. It is expected that the current attempt for the isolation, characterization and the study on marine actinobacteria of Visakhapatnam coast of Bay of Bengal will be useful for the identification of new antibiotics that are effective against challenging pathogens.

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