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Research Article

BIOACTIVE COMPOUNDS FROM MARINE ACTINOMYCETES ISOLATED FROM THE SEDIMENTS OF BAY OF BENGAL

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ABSTRACT

Studies on the enzyme activites and antibacterial potential of actinomycetes isolated from the sediments obtained from different locations of Bay of Bengal, India has been carried out. A total of 63 isolates of actinomycetes were isolated from 20 sediment samples by using two different methods of isolation. All the isolates were tested for their ability to produce cellulase, amylase, lipase, chitinase, L-Asparaginase and protease. The results revealed that 81%, 59%, 44%, 31% and 24% of the isolates exhibited amylase, protease, chitinase, L-Asparaginase and cellulase, activites respectively. Screening for antibacterial activity revealed that 10 isolates exhibited antibacterial activity. The marine actinomycete isolates exhibited more antimicrobial activity against gram positive bacteria than the gram negative bacteria. All the 63 actinomycetes were characterized to the generic level based on the colony and microscopic morphology. Out of 63 isolates, 85% (*n*=53) isolates belong to the genus *Streptomyces,* 7 % (*n*=5) to *Micromonospora*, 4 % (*n*=3) to *Nocardia* and 3 % (*n*=2) to *Streptosporangium*. The study revealed the diversity of marine actinomycetes from Bay of Bengal and their potential as a source of novel bioactive compounds.

Keywords: Marine actinomycetes, Bioactive compounds, Streptomyces.

INTRODUCTION

Actinomycetes represent a ubiquitous group of microbes that are the most economically and biotechnologically valuable prokaryotes. several ecologically significant properties of actinomycetes were reported, which made the screening source expand into uncommon environments[1]. Actinomycetes comprise 10% of the total bacteria colonizing marine aggregates[2]. It is a boon in marine bioprospecting for the exploration and exploitation of the rich biological and chemical diversity found in marine organisms that inhabit the oceans.

For discovering novel bioactive compounds of biotechnological interest is important. Identification and study of marine microorganisms with unique physiological traits. Several factors such as: choice of screening source, pretreatment, selective medium, culture condition and recognition of actinomycetes colonies on a primary isolation plate are used for identification of unique actinomycetes[3].

In view to the significance of marine ecosystems which provide a rich source of novel actinomycetes, the present study is aimed for bioprospecting of actinomycetes from the marine sediments of Bay of Bengal, with special focus on the production of bioactive compounds by the isolates.

MATERIALS AND METHODS

1) Sample Collection and Processing

Twenty marine sediment samples were collected from different locations of Bay of Bengal along the south east coast of

India from a depth of 30-200 meters. Samples were brought to the laboratory in sterile bags and stored in refrigerator[4]. The location and depths of these sampling stations are summarized in Table 1.

2) Isolation of actinomycetes from the marine sediment

Two different methods of isolation of actinomycetes were followed-

a) Method 1

10 gm of each sample was suspended in 100 ml of sterile sea water and agitated at 150 rpm for 1 day to ensure the separation of the filamentous actinobacteria. 500µl of each supernatant was mixed with the molten agar media and poured into the petriplates.

b) Method 2

1gm of the each sample was added to the 10ml of sterile sea water in a conical flask. The flasks were agitated for about one hour. The marine sediment was filtered and the filtrate was serially diluted to obtain 10^{-1} to 10^{-7} dilutions using the sterilized sea water. An aliquot of 100µl of each dilution was spread on the media.

Different media like Starch Casein Agar (SCA)[5] Glycerol Asparagine Agar (GA Agar)[6] Humic acid-B vitamin agar (HV Agar)[7]and Glucose yeast extact malt extract agar (GYM)[8] were used for isolation of actinomycetes. The media containing 50% of sterile sea water were supplemented with rifampcin (5ug/ml) and nystatin (25ug/ml) (Himedia Mumbai) to inhibit bacterial and fungal contamination respectively[9] The petriplates were incubated upto 3 weeks at 28 °C. The isolated discrete colonies were observed and used for identification.

3) Enzymatic activities screening

The actinomycete isolates were inoculated on suitable medium by spot inoculation method to screen their enzyme activities. The plates were incubated at 28 °C for 4- 7days. The media as criteria for enzyme activites are given in Table 2.

4) Screening for Antimicrobial activity

Plate diffusion method[10]was used for the antimicrobial testing against *S.aureus* (MTCC 3160), *B.Subtilis* (MTCC 441), *B. cereus* (MTCC 430), *P. aeruginosa* (MTCC 424), *E. coli* (MTCC 443) and *P. vulgaris* (MTCC 426). The plates were incubated at 28 °C for 24 hrs and analyzed for zone formation.

5) Characterization of the isolates

The isolates were characterised upto genus level as per the Bergey's Manual of Determinative Bacteriology, 9th edition6and and International Streptomyces Project (ISP)8.

RESULTS AND DISSCUSSION

1. Isolation of actinomycetes from the marine sediment

A total of 54 isolates were obtained from 20 sediments using the first method of isolation while, 36 isolates were obtained employing the second method of isolation. These results clearly indicate that the method 1 is more advantageous over method 2 for isolating actinomycetes. Among the 90 isolates obtained by both the methods, 63 isolates were found to be morphologically distinct. Hence these 63 isolates are used for further studies.

The total count of actinomycetes isolated using different media is summarized in Table 3. Out of the five media used for isolation, starch casein agar media and glycerol asparagine agar have shown more number of actinomycetes while humic acid agar and glucose yeast extract malt extract agar resulted in less favorable for actinomycetes isolates. These results are in agreement with the findings of[11]who used eight different selective media for isolation of actinomycetes and observed that starch casein agar and glycerol asparagine agar yielded good growth and more number of actinomycetes than the other types of media.

2. Screening of Enzymatic activities

Among the 63 isolates, 52 isolates (81%) exhibited amylase activity, 36 isolates (59%) exhibited proteolytic activity, 30 isolates (49%) exhibited lipase activity, 28 isolates (44%) exhibited chitinase activity, 20 isolates (31%) exhibited L-Asparaginase activity and 15 (24%) exhibited cellulase activity. Agar plates showing the hydrolytic activity of L-asparaginase, cellulase and lipase produced by actinomycete isolates are shown in Figure.1.

The findings of [12]and [13]indicated that actinomycetes possessed the potential to secrete broad range of hydrolytic enzymes. Similarly, the present work indicated significant potential of enzyme production by the marine actinomycetes.

3. Screening for Antimicrobial activity

Out of 63 isolates, 10 isolates exhibited antibacterial activity. The antibacterial activities of the isolates against the test organisms are given in Table 4. Among the 10 isolates, AUBT-206 (Fig 2), AUBT-1404 and AUBT-1501 showed high antibacterial activities. The isolates AUBT-201, AUBT-702 and AUBT-1703 did not exhibit any activity against *P. vulgaris*, while isolates AUBT-201, AUBT-504 and AUBT-1902 did not exhibit activity against *P. aeruginosa*..

According to[11]marine actinomycete isolates often show more active antimicrobial activity against gram positive bacteria than gram negative bacteria.[14]and[15]*Streptomyces* species from marine habitats showed significant antibacterial activity against *Staphylococcus aureus* and *B. subtilis*. Similarly, the present study also indicated that the marine actinomycete isolates exhibited more antimicrobial activity against gram positive bacteria.

4. Characterization of the isolates

Most of the marine actinomycete isolates produced grey and white colonies with pigmentation. The morphological characters of select actinomycete isolates are presented in Table 5. Out of the 63 isolates, 46 isolates showed growth within three days and 17 isolates showed growth after five days. 51 isolates showed brown (7), yellow (4), red (5), black (9) and grey (26) pigmentation.

All the 63 actinomycetes were identified upto the generic level based on the colony and microscopic morphology. Out of 64 isolates, 85% (*n*=53) isolates were assigned to the genus *Streptomyces*, 7 % (*n*=5) were identified as *Micromonospora*, 4 % (*n*=3) as *Nocardia* and 3 % (*n*=2) as *Streptosporangium*. In Fig.3.an isolate of Streptomycetes growing on glycerol asparagine agar plate is shown. The Distribution of the isolates studied is shown in Fig. 4.

Literature survey reveals that among the genera of marine actinobacteria, the genus *Streptomyces* is represented in largest number of species and varieties, which differ greatly in their morphology, physiology, and biochemical activities[16,10,17,12,18].

CONCLUSION

The present study also indicated that among the marine actinomycete isolates, *Streptomyces* is the dominant genera and revealed that the diversity of marine actinomycetes from Bay of Bengal and their potential as a source of novel bioactive compounds. Further studies on the molecular characterization of the isolates and purification of the bioactive compounds are in progress.

Table 1: Locations of sampling stations

S.No	Date	Latitude	Longitude	Depth (meters)	Location
1.	1.09.2009	17°50.814N	84°01.422E	265	Visakhapatnam
2.	1.09.2009	17°50.556N	83°01.228E	52.93	
3.	1.09.2009	17°51.264N	83°32.060E	29.64	
4.	2.09.2009	16°59.832N	82°58.065E	201.17	Kakinada
5.	2.09.2009	16°59.507N	82°43.923E	108.05	
6.	2.09.2009	16°49.885N	82°25.665E	34.61	
7.	3.09.2009	15°59.813N	81°29.045E	191	Divipoint
8.	3.09.2009	15°59.813N	81°24.737E	88.11	
9.	3.09.2009	15°59.943N	81°20.229E	31.28	
10.	4.09.2009	15°00.551N	80°24.985E	192.60	Singarayakonda
11.	4.09.2009	15°00.199N	80°16.943E	56.19	
12.	4.09.2009	15°00.296N	80°12.826E	34.40	
13.	6.09.2009	13°08.149N	80°35.251E	195	Chennai
14.	6.09.2009	13°08.490N	80°31.962E	99	
15.	6.09.2009	13°08.768N	80°26.478E	53.6	
16.	7.09.2009	12°12.519N	80°19.101E	100	Cudallore
17.	7.09.2009	12°11.718N	80°15.246E	53.26	
18.	7.09.2009	12°11.892N	80°05.725E	30	
19.	8.09.2009	11°07.639N	80°07.236E	217	Nagapatnam
20.	8.09.2009	11°07.511N	80°05.383E	107	

Table 2: Media as criteria for enzyme activities of actinomycetes

S.No.	Enzyme	Medium	Incubation days	Criteria for positive enzyme activity
1.	L-Asparaginase	M9 modified medium	7	Development of pink color
2.	Amylase	Minimal medium with starch	4	Clearing around the growth after flooding with 2% iodine
3.	Protease	Casein skim milk medium	7	Clearing around the growth
4.	Cellulase	Carboxymethyl cellulose media	7	Clear zones after flooding with 1% congred with 1M Nacl.
5.	Chitinase	Chitin agar media	5	Clearing around the growth
6.	Lipase	Tributyrin agar medium	7	Clearing around the growth

Table 3: Total count of actinomycetes in different media

S.No.	Number of actinomycetes isolated							
	Method 1				Method 2			
	SCA	GA agar	GYM agar	HV agar	SCA	GA agar	GYM agar	HV agar
AUBT-1	1	1	0	0	1	0	0	0
AUBT-2	1	2	1	0	1	1	0	0
AUBT-3	1	1	0	1	1	0	0	0
AUBT-4	0	1	1	0	1	1	0	0
AUBT-5	1	0	0	0	2	0	1	1
AUBT-6	2	1	0	2	0	1	0	0
AUBT-7	1	1	0	0	0	0	0	0
AUBT-8	1	0	0	0	1	0	0	0
AUBT-9	2	1	1	1	1	1	0	0
AUBT-10	1	0	0	0	1	1	0	0
AUBT-11	3	2	1	0	0	1	1	1
AUBT-12	2	2	0	0	1	1	0	0
AUBT-13	1	0	0	0	0	0	0	0
AUBT-14	2	1	1	0	1	0	0	0
AUBT-15	1	1	0	0	0	1	0	0
AUBT-16	1	2	0	1	1	1	0	1
AUBT-17	0	1	0	0	1	0	1	0
AUBT-18	1	1	1	0	1	1	0	1
AUBT-19	1	0	0	0	1	1	0	0
AUBT-20	0	2	0	0	1	2	0	0
Total	**24**	**19**	**6**	**5**	**16**	**13**	**4**	**3**

Table 4: Antibacterial activity of isolates against the test organisms

	Name of the test organism (Inhibition Zone diameter in mm)					
Isolate No.	***E.coli*** **(MTCC-443)**	***Proteus vulgaris*** **(MTCC-426)**	***Pseudomonas aeruginosa*** **(MTCC-424)**	***Bacillus subtilis*** **(MTCC-441)**	***Bacillus cereus*** **(MTCC-430)**	***Staphylococcus aureus*** **(MTCC-3160)**
AUBT -201	14	--	--	14	10	11
AUBT-206	20	11	16	19	16	10
AUBT-301	11	10	12	14	11	10
AUBT-504	10	12	--	11	10	11
AUBT-702	13	--	14	15	12	12
AUBT-1202	12	12	11	16	14	10
AUBT-1404	18	11	17	13	14	16
AUBT-1501	20	11	15	18	18	13
AUBT-1703	12	--	11	14	10	11
AUBT-1902	10	10	--	12	10	10

Table 5: Morphological characteristics of selective actinomycete isolates

Isolate no	Aerial Mycelium	Substrate Mycelium	Diffusible pigment	Spore morphology	Spore mass colour
AUBT101	Brown grey	Black	Black	Rectiflexibles	Grey
AUBT502	White	Pink	--	Flexious	Cream or buff
AUBT802	Grey	Yellow	--	Spiral	Yellow
AUBT1002	--	orange	pink	Retinaculum	White
AUBT1203	Grey violet	Black	Brown	Hook like	Grey brown
AUBT1404	Grey	White	--	spiral	grey
AUBT1601	Lightgrey	ReddishBrown	black	Retinaculum	Brown
AUBT1803	--	yellow	--	Spiral	Grey
AUBT1902	Creamypinkish	Brown	Yellow	Rectiflexibles	pinkish-tan
AUBT2002	Brown grey	Brick red	green	Straight	bluish-green

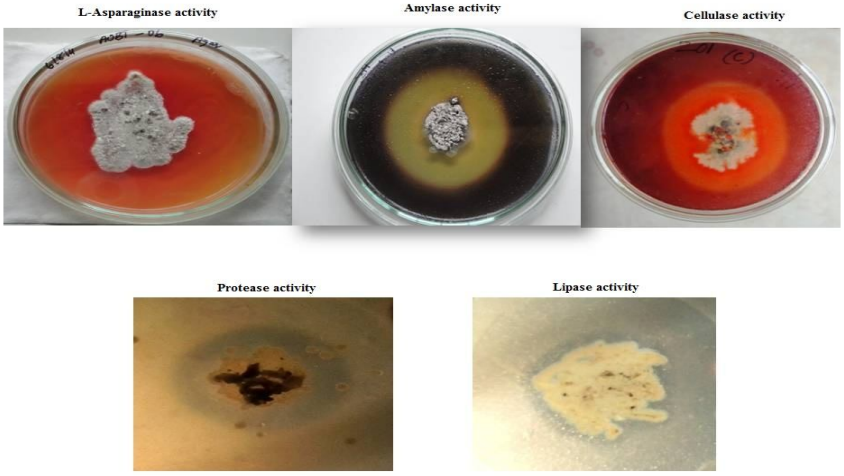

Fig. 1: Agar plates showing the activity of L-Asparaginase, lipase and cellulase produced by actinomycete isolates

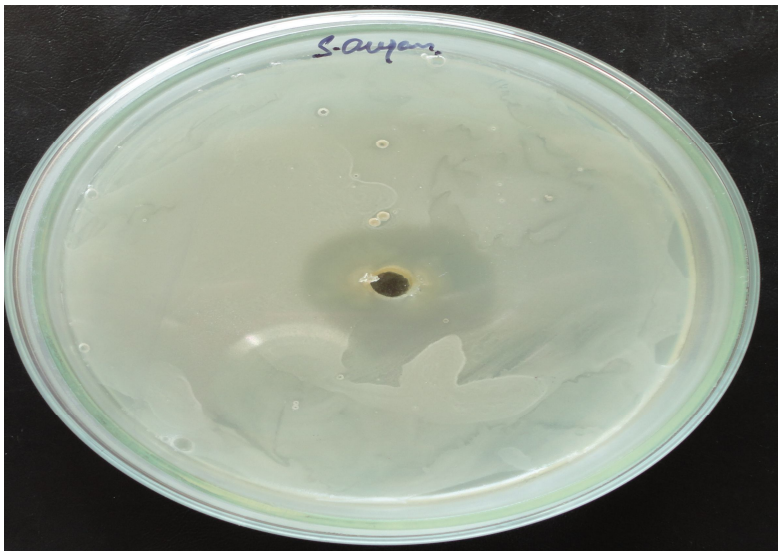

Fig. 2: Antibacterial activity of the isolate AUBT-206 by well diffusion technique

Fig. 3: Isolate of Streptomycetes growing on glycerol asparagine agar plate

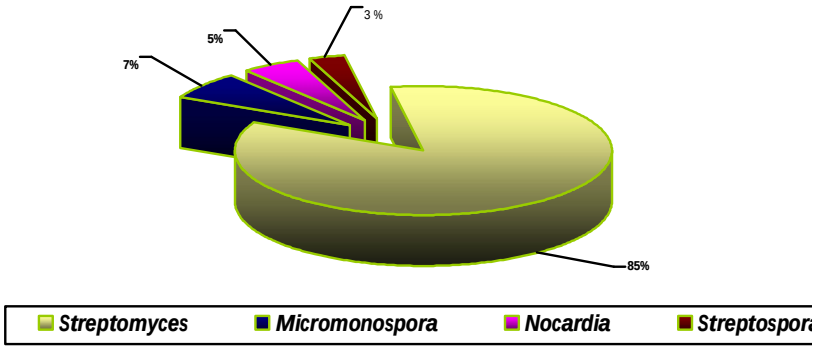

Fig. 4: Distribution of isolated actinomycetes genera

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