Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 4, 2013

Research Article

SELECTIVE SCREENING, ISOLATION AND CHARACTERIZATION OF ANTIMICROBIAL AGENTS FROM MARINE ACTINOMYCETES

Y.S.Y.V. JAGAN MOHAN[1*], B. SIRISHA[1], R. HARITHA[2], T. RAMANA[3]

[1]Department of Biotechnology, College of Science and Technology, Andhra University,Visakhapatnam 530003, [2]Department of Biotechnology, Visakha Government Degree College (Women), Visakhapatnam530020, [3]Dean of school of life sciences, GITAM University, Visakhapatnam 530045, India. Email: yedla.jagan87@gmail.com

Received: 14 Sep 2013, Revised and Accepted: 19 Oct 2013

ABSTRACT

Objectives: The present study was concerned with the isolation, screening and characterization of the actinomycetes from the sediments, which are collected from different locations of Bay of Bengal.

Materials: Selective enrichment and pretreatment strategies can enhance isolation and screening of novel marine actinomycetes. A total of 15 marine samples were collected from different locations of the Bay of Bengal starting from Visakhapatnam to Singarayakonda. The pre-heat treatment method and a combination of 3 enrichment media were found to be effective in selectively isolating marine actinomycetes. The top five potent isolates were subjected to detailed morphological, cultural, biochemical and physiological characterization.

Results: A total of 93 marine actinomycetes were isolated. The antimicrobial activity was studied with all the 93 isolates. The preliminary study of 93 isolates for antimicrobial activity by cross streak method indicated that 36 isolates have antagonistic properties. All these 36 isolates were subjected to submerged fermentation studies. It was observed that 16 isolates (17.2%) exhibited antibacterial activity, 9 isolates (9.6%) showed antifungal activity while 11 isolates (11.8%) showed both antibacterial and antifungal activities.

Conclusion: The present study was an attempt to use different methods to screen, select and isolate marine actinomycetes, with intrinsic antimicrobial activity against a variety of microbial pathogens, from the sediments of Bay of Bengal.

Keywords: Marine actinomycetes, Antimicrobial activity, Bioactive compounds, Characterization.

INTRODUCTION

The biodiversity of marine environment proved to be an important resource for isolation of potent microorganisms to produce biologically active secondary metabolites [1,2]. Actinomycetes occur in a wide range of environments in which they have the ability to grow on most naturally occurring substrates [3]. Actinomycetes are of prime interest, since they are known to produce chemically diverse compounds with a wide range of unique and biologically active metabolites [4], therapeutically useful compounds and enzymes having application in industry.

Microorganisms in marine environments attract a great deal of attention, due to their adaptability to extreme environments and production of novel natural compounds [5]. Marine environmental conditions show great variation and are extremely different from terrestrial ones; hence marine actinomycetes have different characteristics from those of terrestrial counterparts. This allows the organisms to produce different types of bioactive compounds with unique properties and applications [6]. For successful isolation of actinomycetes from marine environments different methods like choice of screening source, pretreatment, selective medium, culture condition and selection of potential colonies on a primary isolation plate are important.

Marine environment in Bay of Bengal is believed to have rich microbial diversity and the vast pool of indigenous marine micro flora is not fully explored. Recent focuses on marine actinomycetes show that they are being extensively explored for the discovery of drugs and other bioactive metabolites [7]. Though reports are available on antibiotic production by marine actinomycetes the demand for new antibiotics continues to grow due to the rapid emergence of multidrug resistant pathogens.

MATERIALS AND METHODS

Sampling

A total number of 15 marine sediments were collected along the South East coast of the Bay of Bengal at various depths of 30 – 200mts using grab sampler. They were maintained at ambient temperature with sea water and brought to the laboratory in sterile polypropylene bags for isolation of marine actinomycetes. The sampling locations are given in Table-1.

Table 1: Locations of sampling stations

Sediment no.	Sampling location	Latitude	Longitude	Depth	No of isolates
AUBT 01	Visakhapatnam	17°50.814N	84°01.422E	265	AUBT 101-109 (9)
AUBT 02	Visakhapatnam	17°50.556N	83°01.228E	52.93	AUBT 201-208 (8)
AUBT 03	Visakhapatnam	17°51.264N	83°32.060E	29.64	AUBT 301-303 (3)
AUBT 04	Kakinada	16°59.832N	82°58.065E	201.17	AUBT 401-408 (8)
AUBT 05	Kakinada	16°59.507N	82°43.923E	108.05	AUBT 501-506 (6)
AUBT 06	Kakinada	16°49.885N	82°25.665E	34.61	AUBT 601-609 (9)
AUBT 07	Divipopint	15°59.813N	81°29.045E	191	AUBT 701-708 (8)
AUBT 08	Divipopint	15°59.813N	81°24.737E	88.11	AUBT 801-805 (5)
AUBT 09	Divipopint	15°59.943N	81°20.229E	31.28	AUBT 901-908 (8)
AUBT 10	Singarayakonda	15°00.551N	80°24.985E	192.60	AUBT 1001-1003 (3)
AUBT 11	Singarayakonda	15°00.199N	80°16.943E	56.19	AUBT 1101- 1106 (6)
AUBT 12	Singarayakonda	15°00.296N	80°12.826E	34.40	AUBT 1201-1206 (6)
AUBT 13	Chennai	13°08.149N	80°35.251E	195	AUBT 1301-1303 (3)
AUBT 14	Chennai	13°08.490N	80°31.962E	99	AUBT 1401-1405 (5)
AUBT 15	Chennai	13°08.768N	80°26.478E	53.6	AUBT 1501-1506 (6)

Selective Isolation of actinomycetes

Three different pre-treatment methods were performed to selectively enhance isolation and growth of marine actinomycetes.

Serial dilution method

Isolation and enumeration of marine actinomycetes were performed by the serial dilution plate technique [8]. 1 g each of the marine sediment sample was taken in 250 ml Erlenmeyer flask containing 50 ml of sterile water. Flasks were shaken on rotary shaker for 30min for the detachment of spore chains. The particulate matter was allowed to settle down and the suspension was serially diluted up to 10^{-6} times. 1ml each of these dilutions were added to 50ml of sterile molten starch casein agar medium thoroughly mixed and poured into Petri plates and incubated at 28°C for 3 days to 3 weeks. Different media like starch casein agar, actinomycetes isolation agar, glycerol asparagine agar, oat meal agar, glucose yeast extract malt extract agar are used for isolation technique.

Heat treatment

The samples were heated by incubating at 55 °C for 15 min in a water bath [1]. 10 fold serial dilutions of the sediment samples were made using sterile 50% sea water [9]. About 0.1ml of the serially diluted samples was spread over Starch casein agar medium [10] and Actinomycetes isolation agar medium. Both the media were supplemented with 5µg/ml rifampicin and 25µg/ml of Nystatin (Himedia, Mumbai) to minimize the other bacterial and fungal growth [11].

All the plates were incubated at 28°C for 21 days. The appearance and growth of marine actinomycetes colonies were recognized by their characteristic chalky to leathery appearance. All the morphologically different actinomycete colonies were sub-cultured on yeast extract malt extract agar medium.

Pre-enrichment method

One gram of sediment was transferred to conical flasks containing 100 ml of sterile sea water, starch casein broth and glucose asparagine broth prepared with natural sea water separately for the pre-enrichment of samples. The flasks were incubated at 30 ºC for 14 days in a shaker incubator. A loop-full of inoculum from the pre-enriched starch casein broth and glucose asparagine broth was streaked on starch casein agar (SCA) and glucose asparagine agar separately and the plates were incubated at 30 ºC for 7 days. Single discrete colonies were isolated and identified. All the morphologically different actinomycete colonies were sub cultured on yeast extract malt extract agar medium (ISP No. 2) [12] by streak plate technique. After growth appeared, the actinomycetes colonies were maintained in ISP No. 2 agar slants.

Primary screening for antimicrobial activity

The antimicrobial activity of the isolates were tested by Cross-Streak method employing nutrient agar medium for bacteria and potato dextrose agar medium for fungi and yeast. The media was sterilized by autoclaving at 121°C and 15lbs pressure for 15 min and the molten sterile media was cooled to 40-45°C, poured into Petri plates (4 inch diameter) and allowed to solidify. Each plate was streaked with one isolate at the center and incubated at 28°C for 7 days. After 7 days, test organisms were streaked perpendicular to the growth of the isolate; 24 hour old cultures of bacteria, 4 day old cultures of fungi and 2 day old cultures of yeast were used to test the organisms. All the test organisms employed in the present investigation were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India.

The test organisms used for the determination of antimicrobial activity are Staphylococcus aureus (MTCC 3160), Bacillus subtilis (MTCC 441), Bacillus cereus (MTCC 430), Pseudomonas aeruginosa (MTCC 424), Escherichia coli (MTCC 443), Proteus vulgaris (MTCC 426), Saccharomyces cerevisiae (MTCC 170), Candida albicans (MTCC 227), Aspergillus niger (MTCC 961), and Aspergillus flavus (MTCC 3396).

Secondary screening for antimicrobial activity

Based on the results of primary screening, 11 putative *Streptomyces* isolates namely AUBT-103, AUBT-205, AUBT-302, AUBT-506, AUBT-702, AUBT-708, AUBT-801, AUBT-902, AUBT- 1001, AUBT-1202, and AUBT-1503 were selected for the fermentation and determination of antibiotic production.

Secondary screening for antibiotic production Agar well diffusion method

Secondary screening of promising isolates was done by submerged fermentation. Slant cultures of mature actinomycete strains were inoculated in the medium containing soya bean meal 20 g, glucose 20 g, NaCl 4 g, K2HPO4 0.05 g, MgSO4 0.50 g and CaCO3 5 g for 1000 ml and maintained at pH 7.2. The cultures were incubated in a rotary shaker (180rev/min) at 27°C for 7 days and the fermented broth was centrifuged at 10,000 rpm at 4 °C for 20 min. The supernatant was filtered using 0.45µm pore size membrane filter (Millipore) [13]. The clear supernatant samples were tested for their antimicrobial activity by agar well diffusion method. To determine the antibacterial spectrum, pathogenic bacteria cultured on nutrient broth at 37°C for 24 h; the cultures were swapped on nutrient agar media. The relative activities of metabolites are determined based on the diameter of zones of inhibition formed. Secondary screening of potent actinomycetes confirmed the results of primary screening.

Characterization of actinomycetes cultures

The top five potent actinomycetes isolates selected from screening were characterized by morphological, cultural, biochemical and physiological features. Morphological and cultural characteristics such as type of aerial hyphae, growth of vegetative hyphae, diffusible pigment and spore formation was observed. Biochemical tests including melanin pigmentation, H_2S production, tyrosine reaction, starch, casein, gelatin hydrolysis, Milk coagulation & peptonization, Methyl red, Voges-Proskauer, citrate, Oxidase, Urease and Catalase tests were also performed by starch casein agar. Physiological characterization such as the effect of p^H (5-9) and temperature (10^oC-50^oC) were also tested.

Utilization of carbon sources such as Glucose, Fructose, Mannitol, Rhamnose, Raffinose, Maltose, Lactose, Sucrose, Glycerol, Starch and nitrogen sources namely L-Arginine, L-Tyrosine, L-Asparagine, L-Leucine, L-Cysteine, L-Histidine, L-Valine, and L-Glycine were tested on starch casein agar medium (Table-6&7).

Sodium chloride tolerance: Sodium chloride tolerance level [14] of the isolates was evaluated on starch casein agar supplemented with graded doses of NaCl (1, 4, 7, 10 and 13%) maximum NaCl tolerance concentration in the medium allowing any growth was recorded (Table-8).

RESULTS AND DISCUSSION

Marine sediments from South East coast of Bay of Bengal were selected as a potential source of marine actinomycetes and possible bioactivity. Various pretreatment procedures and selective media were applied to assess the optimal conditions for the isolation of marine actinomycetes from sediments. Three different pre-treatment methods were employed for maximum isolation of actinomycetes.

Serial dilution technique allowed the growth of actinomycetes, bacterial and fungal colonies (Fig 1). But the next two pre treatment methods (Fig 2 & 3) inhibited growth of bacterial and fungal colonies. Hence it has been inferred that when the sediments were cultured without pretreatment, large number of bacterial and fungal colonies were grown, the dominance of other bacterial and fungal contamination was found to inhibit the colonization of actinomycetes. Whereas when the soil was pretreated, their numbers decreased on culture plates. Previously, this type of pre-treatment methods for isolation of actinomycetes has also been suggested by several researchers [5,9,15].

The pretreatment of wet-heating for 55°C for 15min. Starch casein agar and glucose asparagine agar media were the most effective for the isolation of actinomycetes. The bacterial and fungal contamination was diminish by pre-heat treatment and allowed

selective isolation of actinomycetes. When antibacterial and antifungal agents rifampicin 5µg/ml and nystatin 25µg/ml were supplemented into the isolation medium, the number of bacteria and fungi were further decreased.

The growth of marine actinomycetes colonies was recognized by their characteristic chalky to leathery appearance. All the morphologically different actinomycete colonies were sub-cultured on yeast extract malt extract agar slants (ISP No. 2).

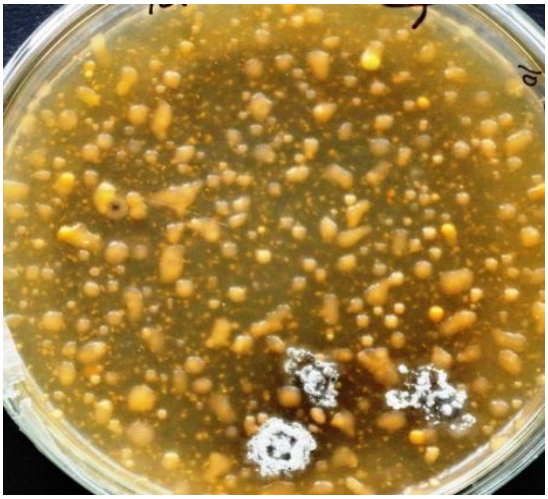

Fig. 1: Actinomycetes isolation agar plate showing growth of actinomycete colonies

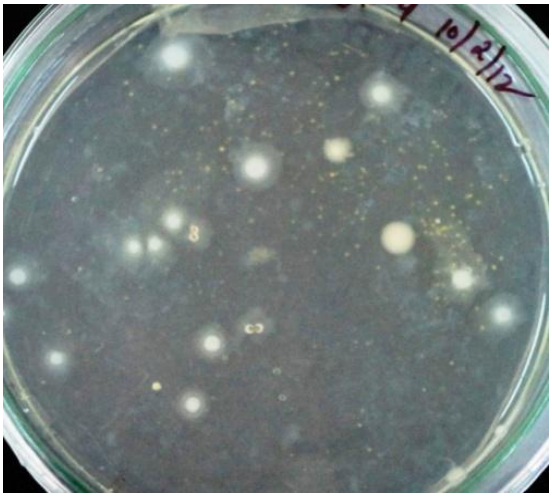

Fig. 2: Starch casein agar plate showing selective growth of actinomycete colonies.

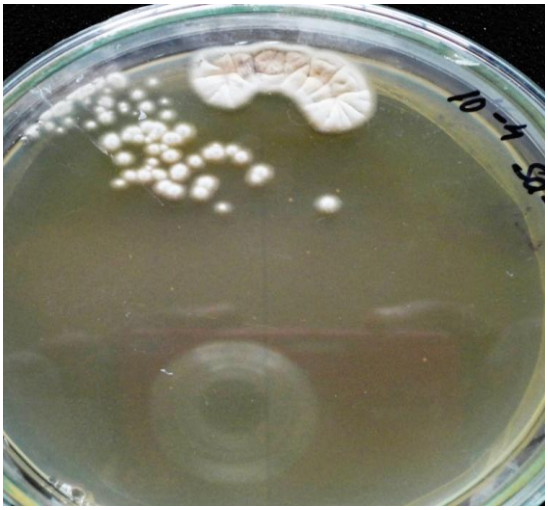

Fig. 3: Glycerol Asparagine agar plate Showing selective growth of actinomycete colonies.

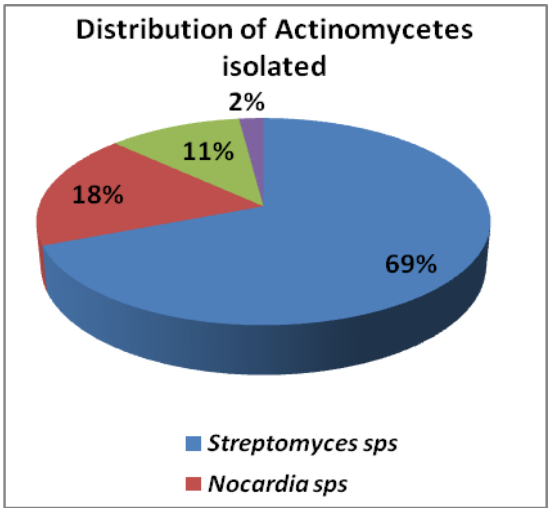

Fig. 4: Pie diagram showing percentage frequency of isolated actinomycete genera

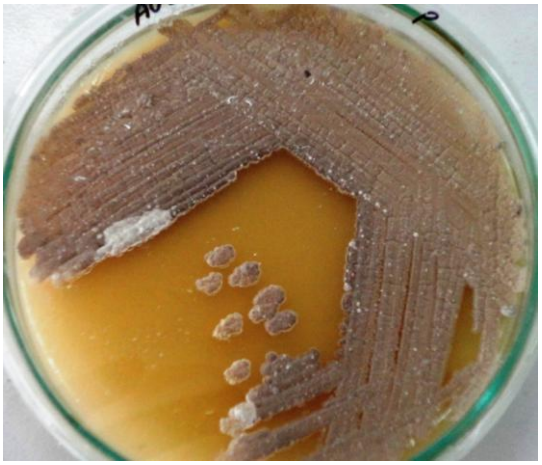

Fig. 5: Pure culture of AUBT – 205 *streptomycetes* on GYM agar plate.

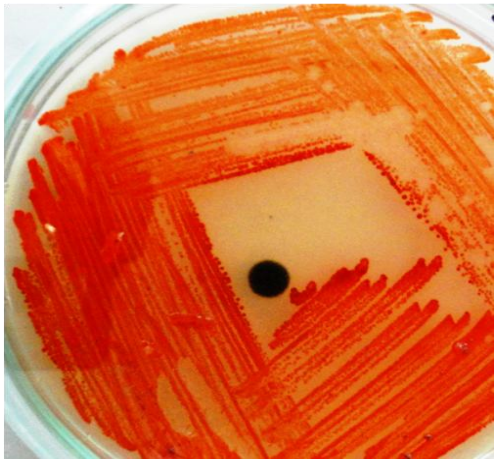

Fig. 6: Pure culture of AUBT – 113 *Nocardia* on Starch casein agar plate.

Among 15 sediments screened, 93 actinomycete colonies were isolated and 36 isolates exhibited antimicrobial activity. 11 isolates showed both antibacterial and antifungal activities (Table 2 & 3). All the 93 isolates were identified at generic level based on the colony morphology and microscopic morphology. Their distribution pattern was shown in Fig 4. 69 % of the isolates belonged to white and grey colour series and morphologically similar to *Streptomyces spp* (Fig 5), 18% belonged to family *Nocardia* (Fig 6), 11 % to *Micromonospora* and 2% to *Rhodococcus* (Fig 7).

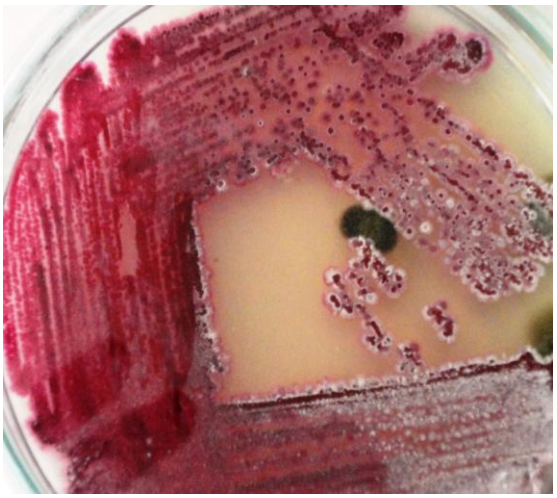

Fig. 7: Pure culture of AUBT - 1008 *Rhodococcus* on Starch casein agar plate.

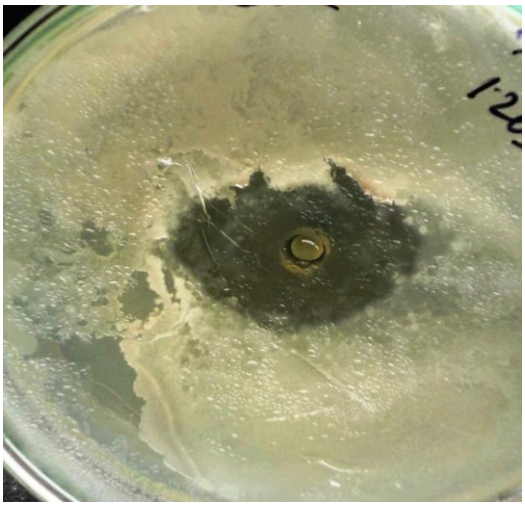

Fig. 8: Zone of inhibition against *Bacillus subtilis*by isolate AUBT – 902.

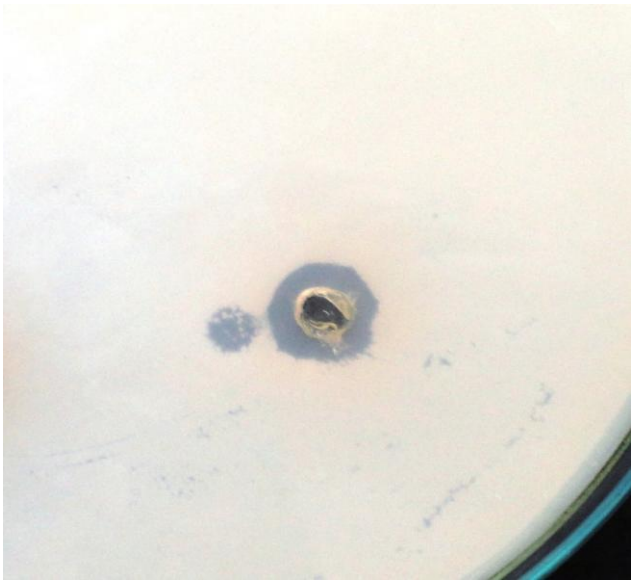

Fig. 9: Zone of inhibition against *C. albicans* by isolate AUBT – 902.

Frequency and dominance of *Streptomyces* among actinomycetes in various soil types was reported by several workers [16,17]. The present study correlates with earlier reports that among the isolates *Streptomyces* was the dominant genera. Out of 93 strains, 36 isolates (38.7%) had antimicrobial activity, of which 16 isolates (17.2 %) showed antibacterial activity, 9 isolates (9.6 %) showed antifungal activity, 11 isolates (11.8%) showed both antibacterial and antifungal activity.

On both nutrient agar and PDA media, notably the strains (AUBT 103, AUBT 205, AUBT 302, AUBT 506, AUBT 702, AUBT 708, AUBT 801, AUBT 902, AUBT1001, AUBT1202, AUBT 1503) have supreme activity against the organisms tested (Table 2 & Table 3). On nutrient agar, the strain AUBT 902 produced maximum zone of inhibition against *S. aureus* (24 mm), against *B. subtilis* (22mm) (Fig 8) and showing highest anti-fungal activity against *C. albicans* (16mm) (Fig 9) and against *S. cereviseae* (14mm). Hence, the culture was selected as a promising isolate and for further identification.

Morphological characterization of the broad spectral antagonistic isolates revealed dark grey coloured aerial mycelia, and dark grey to white coloured spore mass. However, the strain AUBT- 302 developed yellow coloured substrate mycelium, and AUBT - 506 developed brick red coloured substrate mycelium. Further, the strain AUBT - 708 developed spirally nature spore chain in its aerial mycelium, whereas the strain AUBT – 1503 developed hooked spore chain (Table-4).

The strain AUBT-902 developed grey aerial mycelium and spiral spores. The details of biochemical and physiological characteristics (Table-5), utilization of carbon and nitrogen sources of the isolates are given in table 6 & 7. It is also evident that different physiological characteristics are influencing the growth rate of the actinomycetes [18].

Table 2: List of isolates showing antibacterial activity Inhibition Zone diameter in mm

Isolate code	*E. coli*	*B. subtilis*	*P. vulgaris*	*P. aeruginosa*	*B. cereus*	*S. aureus*
	(MTCC-443)	(MTCC-441)	(MTCC-426)	(MTCC-424)	(MTCC-430)	(MTCC-3160)
AUBT-103	11	12	--	11	12	10
AUBT-205	--	11	12	10	--	14
AUBT-302	11	10	11	10	11	12
AUBT-506	14	16	12	--	14	10
AUBT-702	12	11	--	12	11	12
AUBT-708	10	11	10	10	11	10
AUBT-801	11	12	11	09	12	09
AUBT-902	18	22	16	14	18	24
AUBT-1001	--	11	12	11	12	09
AUBT-1202	10	12	11	--	11	14
AUBT-1503	12	11	--	--	11	10

Table 3: List of isolates showing antifungal activity

	Inhibition Zone diameter in mm			
Isolate code	*C. albicans*	*A. Niger*	*A. flavus*	*S. cereviseae*
	(MTCC-227)	(MTCC-961)	(MTCC-3396)	(MTCC-170)
AUBT-103	10	--	--	12
AUBT-205	08	08	10	09
AUBT-302	12	10	--	08
AUBT-506	09	--	14	10
AUBT-702	08	--	--	12
AUBT-708	12	10	12	08
AUBT-801	08	--	10	09
AUBT-902	16	12	10	14
AUBT-1001	06	10	--	09
AUBT-1202	10	08	--	12
AUBT-1503	10	08	12	10

Table 4: Morphological characteristics of selective actinomycete isolates

isolate code	sAerial mycelium	Substrate mycelium	Diffusible pigment	Spore morphology	Spore mass Colour
AUBT-302	Grey	Yellow	--	Spiral	Yellow
AUBT-506	--	Orange	Pink	Retinaculum	White
AUBT-708	Brown	White	--	Spiral	Brown
AUBT-902	Grey	Yellow	Black	Spiral	Grey
AUBT-1503	Pink	Yellow	Black	Spiral	Grey

Table 5: Biochemical and Physiological Characteristics

	Isolates				
Reaction	AUBT-302	AUBT-506	AUBT-708	AUBT-902	AUBT-1503
Melanin reaction					
a. ISP-1	+	+	-	+	-
b. ISP-6	+	+	+	+	+
c. ISP-7	+	+	+	+	+
H2S production					
a. ISP-6	+	+	+	+	+
Tyrosine reaction					
a. ISP-7	+	+	+	+	+
Starch hydrolysis	+	+	+	-	-
Casein hydrolysis	+	-	+	-	-
Gelatin hydrolysis	+	+	+	-	+
Milk coagulation & peptonization	-	-	+	+	+
Nitrate reduction	+	+	+	-	+
Methyl red	-	+	+	-	+
Voges-Proskauer	-	-	-	-	-
Citrate	+	+	+	+	+
Oxidase	-	-	-	-	-
Urease	+	+	+	+	+
Catalase	-	-	-	-	-
Growth temperature					
a. 10°C	+	+	+	+	+
b. 20°C	+	+	+	+	+
c. 28°C	+	+	+	+	+
d. 37°C	+	+	-	+	+
e. 42°C	+	-	-	-	+
P^H tolerance	5-9	5-9	6-8	5-8	5-9

Table 6: Utilization of Carbon source

	Isolates				
Carbon source	**AUBT-302**	**AUBT-506**	**AUBT-708**	**AUBT-902**	**AUBT-1503**
D-Glucose	Good	Good	Good	Good	Good
D-Fructose	Good	Good	Good	Good	Good
D-Mannitol	Good	Good	Good	Good	Good
Raffinose	No growth	No growth	No growth	No growth	No growth
Maltose	Good	No growth	Moderate	Good	No growth
Lactose	Moderate	No growth	Moderate	Good	Moderate
Sucrose	Good	Moderate	Moderate	Good	Moderate
Glycerol	Good	Moderate	Moderate	Good	Good
Starch	Good	Moderate	Moderate	Good	Good

Table 7: Utilization of Nitrogen source

Isolates	**Nitrogen source**							
	KNO_3	**L-Arginine**	**L-Tyrosine**	**L-Asparagine**	**L-Leucine**	**L-Histidine**	**L-Valine**	**L-Glycine**
AUBT-302	+	+	-	+	-	+	+	-
AUBT-506	+	+	-	+	-	+	+	+
AUBT-708	+	+	+	+	+	+	+	+
AUBT-902	+	+	+	+	+	+	+	+
AUBT-1503	+	+	+	+	+	-	-	-

Table 8: Sodium chloride tolerance

Sodium chloride Tolerance					
Isolates	**1%**	**4%**	**7%**	**10%**	**13%**
AUBT-302	Good	Good	Good	Moderate	No growth
AUBT-506	Good	Good	Good	No growth	No growth
AUBT-708	Good	Good	Good	No growth	No growth
AUBT-902	Good	Good	Good	Moderate	No growth
AUBT-1503	Good	Good	Good	No growth	No growth

In general, biochemical and physiological characteristics and antimicrobial susceptibility patterns of the actinomycetes vary from isolate to isolate depending on the growth conditions. The present investigation concluded that the physiological characteristics of actinomycetes varied depending on the available nutrients in the medium and the physical conditions. Thus, it was concluded on the basis of the present and previous studies that the nutrient compositions of the medium greatly influence the growth and morphology of organisms [19]. The cultural characteristics and spore morphology place the organism under the family *Streptomycetaceae* and genus *Streptomyces.* Further study is in progress to evaluate the potential of the organism for production of anti microbial compounds

CONCLUSION

The search for novel metabolites especially from actinomycetes requires screening large number of isolates (over thousands) in order to discover actinomycete population with novel compound of pharmaceutical interest. The present study was an attempt to use pretreatment methods to screen, select and isolate marine actinomycetes, with intrinsic antimicrobial activity against a variety of microbial pathogens, from the sediments of Bay of Bengal.

ACKNOWLEDGEMENT

We thank the Department of Biotechnology, Andhra University for providing the facilities used in the work.

REFERENCES

1. Bhaskaran R, Vijayakumar R and Mohan PM. Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands, India. Malaysian Journal of Microbiology 2011; 7(1), pp. 26-32.
2. Ramesh S, Mathivanan N. Screening of marine actinomycetes isolated from the Bay of Bengal, India for antimicrobial activity and industrial enzymes. World J. Microbiol. Biotechnol 2009; 25:2103-2011.
3. Goodfellow M and ST Williams. Ecology of actinomycetes. Annu. Rev. Microbiol 1983; 37: 189-216.
4. Bredholt H, Fjaervik E, Jhonsen G and Zotechev SB. Actinomycetes from sediments in the TrondheinFjrod, Norway: Diversity and biological activity. Journal of Marine Drugs 2008; 6: 12-24.
5. Solingen VP, Meijer D, Kleij WA, Branett C, Bolle R, Power SD, Jones BE. Cloning and expression of an endocellulase gene from a novel Streptomyces isolated from an East African soda lake. Extremophiles 2001; 5: 333-341.
6. Goshev I, Gousterova A, Vasileva Tonkova E, Nedkov P. Characterization of the enzyme complexed produced by two newly isolated thermophilic actinomycete strains during growth on collagen rich materials. Process Biochem 2005; 40:1627–1631.
7. Prabavathy VR, Mathivanan N, Murugesan K. Control of blast and sheath blight diseases of rice using antifungal metabolites produced by Streptomyces sp. PM5. Biol Control 2006; 39:313–319.
8. Haritha R, Sivakumar K, Jagan Mohan YSYV and Ramana T. Amylolytic and Proteolytic Actinobacteria Isolated from Marine Sediments of Bay of Bengal. International Journal of Microbiological Research 2010; 1(2): 37-44.
9. Kim CM, Lec KH, Kwon OS, Shimazu A and Yoo ID. Selective isolation of Actinomycetes by physical pre-treatment of soil sample. Journal of Applied Environmental Microbial Biotechnology 1994; 22: 222-225.
10. Wellington EMH and Cross T. Taxonomy of antibiotic producing Actinomycetes and new approaches to their selective isolation. In: "Progress in industrial microbiology?" Bushell, M. E. (Eds.). Elsevier, Amsterdam 1983; pp: 36.
11. Sivakumar K, Haritha R, Jagan Mohan YSYV and Ramana T. Screening of marine actinobacteria for antimicrobial

compounds. Research journal of Microbiology 2011; 6(4):385-393.

12. Shirling EB and Gottlieb D. Methods for characterization of Streptomyces species. International Journal of Systematic Bacteriology1966; 16: 312-340.
13. Ruan JS. The basis of taxonomy of actinomycetes. The Chinese Academic Press, Beijing 1977; pp: 139-146.
14. Tresner, HD, JA Hayes and EJ Backns. Differential tolerance of Streptomyces to sodium chloride as a taxonomic acid aid. Applied Microbiol 1968; 16: 1134-1136.
15. Jensen P, R Dwight and W Fenical. Distribution of actinomycetes in near-shore tropical marine sediments. Applied Environ. Microbiol 1991; 57: 1102-1108.
16. Kim CJ, Lee KH, Shimazu A, Kwon OS, Park DJ. Isolation of rare actinomycetes on various types of soil. J. Appl. Microbiol. Biotechnol 1995; 23:36-42.
17. Jensen PR and C Mafnas. Biogeography of the actinomycete genus Salinispora. Environ. Microbiol 2006; 8:1881–1888.
18. Shimizu M, Nakagawa Y, Sato Y, Furumai T, Igarashi Y, Onaka H, Yoshida R, Kunch H. Studies on endophytic actinomycetes (1) Streptomyces sp. Isolated from Rhododendron and its antimicrobial activity. J Gen Pl Pathol 2000; 66: 360-366.
19. Gesheva V, Gesheva R. Structure of the Streptomyces hygroscopicus 111-81 population and characteristics of its variants. Actinomycetes 1993; 4: 65-72.