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### Research Article

## ISOLATION OF THE MARINE ACTINOMYCETES FROM THE BAY OF BENGAL SEDIMENTS

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**Abstract:** Actinomycetes are a group of organisms that morphologically resemble fungi and physiologically resemble bacteria. Marine environment is an abundant source for isolation of new micro organisms. Gram positive actinomycetes bacteria are of special actinomycetes are known to produce secondary metabolites which inhibits many micro organisms. Total 2 actinomycetes were identified out of 22 isolates from marine sediment samples of different areas of Rama Krishna Beach, Visakhapatnam, Andhra Pradesh, India. For isolation of actinomycetes starch casein agar and yeast extract malt extract agar media were used. It was found that actinomycetes isolates were identified as *Actinopolyspora sp.* and *Streptomyces sp.* *Streptomyces sp.* showed higher antibacterial activity 34.2 mm inhibition diameter against *Staphylococcus aureus*. Both shows less antifungal activity compare with antibacterial activity. *Actinopolyspora sp.* showed 10 mm inhibition diameter against *Aspergillus niger*. *Streptomyces sp* showed high antifungal activity, which was 22.4mm inhibition diameter compare with *Actinopolyspora sp.*

**Keywords:** Secondary metabolites, actinomycetes, antibacterial activity, *Staphylococcus aureus*, *Actinopolyspora sp.* and *Streptomyces sp*

### INTRODUCTION:

In the human history since the days of early civilization microorganisms play a key role to produce natural products that benefit and improve our social economic lifestyle. Actinomycetes are Gram-positive bacteria belonging to the order *Actinomycetales*, characterized by the formation of substrate and aerial mycelium on solid media, presence of spores and a high GC content of the DNA (60-70 mol %)<sup>1</sup>. Actinomycetes are a group of organisms that morphologically resemble fungi and physiologically resemble bacteria and production of antibiotics is their most important contribution to mankind with the group producing half of all the known antibiotics<sup>2,3</sup>. Streptomycin, gentamicin, rifamycin are some of the antibiotics which are in use presently and erythromycin are the product of actinomycetes. The actinomycetes are important in the field of pharmaceutical industries and also the agriculture. Previous study showed that actinomycetes isolated from Malaysia soil have the potential to inhibit the growth of several plant pathogens<sup>4,5</sup>.

More than 70% of our planet's surface is covered by oceans and life on Earth originated from the sea. In some marine ecosystems, such as the deep sea floor and coral reefs, experts estimate that the biological diversity is higher than that in the tropical rainforests. As marine environmental conditions are extremely different from terrestrial ones, it is summarized that marine actinomycetes have different characteristics from those of terrestrial

counterparts and therefore might produce different types of bioactive compounds<sup>6</sup>.

A little is known about the actinomycetes diversity of marine sediments, which is an inexhaustible resource that has not been properly exploited. Many reports describe that in India, the East Coast area is a major source of actinomycetes<sup>7</sup>. Hence, the present study made an attempt to isolate actinomycetes from the sediment samples of Bay of Bengal, near visakhapatnam region, India to identify novel metabolites of marine actinomycetes.

### MATERIALS AND METHODS:

#### Collection of samples:

Four sediment samples were collected from Rama Krishna Beach, Visakhapatnam coastal, and Andhra Pradesh, India. Samples were brought to the laboratory in aseptic condition and serially named as J, K, L, M and stored at 4 °C.

#### Chemicals and Media components:

All the chemicals and media components were used in this experiment were purchased from the Hi-media Pvt. Ltd. by the Department of biotechnology, Dr. V.S.Krishna Govt. P. G. College, Visakhapatnam, and all media were prepared in double distilled water.

#### Maintenance of actinomycetes culture:

Yeast extract malt extract agar (YEME) media was used for the maintenance of pure isolated actinomycetes. YEME media composition(g/l)

Yeast extract 04.00 g  
 Malt extract 10.00 g  
 Dextrose 04.00 g  
 Agar 20.00 g  
 pH 6.7 - 7.1

**Isolation of Actinomycetes:**

Actinomycetes were isolated by spread plate technique following the serial dilution of sediment samples on starch casein agar<sup>8</sup> plates containing Rimphamycin and cyclohexamide (each at concentratin of 50µg/ml of medium) to inhibit bacterial and fungal contaminations. The petri plates were then incubated at 28±2°C and the colonies were observed from 4<sup>th</sup> day onwards for four weeks. Strains of marine actinomycetes were picked out and purified by repeated streaking on YEME medium. The pure cultures of the actinomycetes were transferred to YEME slants and preserved at 4±2°C. The isolated actinomycetes were identified based on the colony morphology and gram staining<sup>9</sup>. Identified actinomycetes colonies were incubated at 30±2°C for 10 days for the enrichment of secondary metabolitie producers. Then the culture was collected and

centrifuged at 6000 rpm for 15 min collected the supernant and filter through Millipore filter (0.45µm) to get cell free extract. Antimicrobial activity was assay by using well diffusion assay<sup>10</sup> and the test organisms were *Aspergillus niger*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**RESULTS:**

Two actinomycetes species were identified by morphological characteristics and by gram staining. Based on these characteristics the actinomycetes were identified as *Actinopolyspora sp.* and *streptomyces sp.* *Actinopolyspora* shows antifungal activity and streptomyces shows antimicrobial activity. Marine actinomycetes produce different types of antibiotics, because environmental conditions of the ocean greatly different form terrestrial conditions. Among all microorganisms, actinomycetes are note worthy as broad antibiotic spectrum producers has antibacterial, antiviral, antitumor etc. *Streptomyces sp.* covers around 80% of total antibiotic production<sup>11</sup>. Marine *streptomyces sp.* Exhibited highest antibacterial activity against *pseudomonas aeruginosa*.



**Fig 1: Isolates of *Actinopolyspora sp.***



**Fig 2: Isolates of *Streptomyces sp.***

*Aspergillus niger* is such a common contaminant, highly effective antibiotics are required. Hundred percent of the tested actinomycetes exhibited antifungal activity. Among the tested isolates *Streptomyces sp.* Showed strong antifungal activity,. Many promising antibiotics were discovered in marine actinobacteria, in particular

*Streptomyces*<sup>12</sup>. Recently several antibiotics including trioxacarcin, allophycocyanin, piperazimycin, chalcomycin, streptochlorin, phaeochromycins, marinopyrroles, essramycin, sisomicin, and T-muurolol have been reported in marine *Streptomyces*<sup>13</sup>.

**Table 1: Identification of Actinomycetes**

Organisms	Morphological characteristics	GramStaining (+ve/-ve)	Microscopical structure
Actinopolyspora sp.	Smooth, whitish, wrinkled colonies.	+ ve	Rod shaped(Chain form)
Streptomyces sp.	Initially leathery colonies later developing powedery in nature	+ ve	Coccus (Chain like)

**Table 2: Antimicrobial activity**

Organism	Zone of inhibition diameter (mm)	
	Actinopolyspora sp.	Streptomyces sp.
Staphylococcus aureus	16.8	34.2
Pseudomonas aeruginosa	-	-
Aspergillus niger	10	22.4

Hence, the results of the present investigation reveal that the marine actinomycetes from coastal environment are a potent source of novel antibiotics. It is anticipated that the isolation, characterization and the study of actinomycetes can be useful in the discovery of antibiotics and novel actinomycetes species.

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