

Isolation of Actinomycetes from Marine Sediments Off Visakhapatnam, East Coast of India

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Actinomycetes (140) were isolated from sediment samples and identified to generic level. The genera encountered were *Streptomyces*, *Micromonospora*, *Nocardia*, *Streptosporangium*, *Micropolyspora* and *Streptoverticillium*. Of the total isolates 18% exhibited antimicrobial activity. The salt tolerance of the isolates was also tested.

Very little work has been done on marine actinomycetes¹⁻⁴. Since the environmental conditions of the sea are extremely different from terrestrial conditions, it is felt that marine actinomycetes have different characteristics from terrestrial actinomycetes and might produce different types of antibiotics. The marine actinomycete populations of Bay of Bengal near Visakhapatnam have not been studied so far. The reports of Okami and Okazaki¹ indicated the significance of marine actinomycetes and their antimicrobial principles. This communication deals with the isolation of actinomycetes from marine sediments, their identification up to genus level and their antimicrobial activities.

Ten sediment samples were collected with a core sampler from different places in Bay of Bengal. A profile was run up to 50 m bathymetric line, at a distance of 12 km away from the coast. Samples were collected at 2 km intervals starting from Visakhapatnam to Bhimunipatnam (18 km long, in N-E direction). The central portions of sediments were aseptically transferred into sterile bottles.

One gram of the sediment (dry) was suspended in 5 ml of sterile seawater. Aliquots of the suspension (0.5 ml) were spread over the surface of agar media and the plates incubated at 24°C for 2-4 weeks. Five different media¹ (glucose-asparagine agar, maltose-yeast extract agar, starch-casein agar, peptone yeast extract agar and potassium tellurite agar) were employed for the isolation, as there is a wide diversity in physiological types among the marine actinomycetes. The plates were observed from 5th day onwards for 4 weeks and the selected actinomycetes were transferred to agar slants of the same isolation medium and incubated for 2-4 weeks at 24°C. Morphological, cultural and physiological characteristics were studied on media recommended by International Streptomyces Project

(ISP)⁵ and Waksman⁶. Cell wall analyses were performed by the method of Becker *et al.*⁷. Based on the above characteristics, the isolates were grouped into different genera^{8,9}. NaCl tolerance of the isolates was also tested with various salt concentrations (0, 3, 7, 10 and 14%) according to Tresner *et al.*¹⁰.

The isolates were tested for their antimicrobial activity in two liquid media. Medium I contained (g.l⁻¹): glucose 10, starch 10, soybean meal 15, yeast extract 5, K₂HPO₄ 1, MgSO₄.7H₂O 1, and NaCl 10. Medium II contained (g.l⁻¹), glucose 10, malt extract 5, peptone 5, NaCl 5, FeSO₄.7H₂O 0.001, MnCl₂.4H₂O 0.001 and ZnSO₄.7H₂O 0.001. The flasks were incubated at 28°C on rotary shaker (220 rpm) for 5 d. The antibiotic activity in broth was tested by the standard cylinder plate method. The test organisms used were — *Bacillus subtilis*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. The isolates exhibiting an inhibition zone of 15 mm dia. were recorded.

The actinomycetes (140) isolated were grouped into 6 genera, viz. *Streptomyces*, *Micromonospora*, *Nocardia*, *Streptosporangium*, *Micropolyspora* and *Streptoverticillium* (Table 1). Great variety of actinomycetes were present in good numbers in these marine sediments. The most common genera were *Streptomyces* and *Micromonospora*.

About 18% of the isolates exhibited antimicrobial activity (Table 1). Some isolates showed both antibacterial and antifungal activities whereas others exhibited either antibacterial or antifungal activity. While several isolates possessed activity against gram-positive bacteria, only a few isolates showed antifungal activity.

In the absence of NaCl (or seawater) 34 isolates showed no or scanty growth while the rest of the isolates showed poor to moderate growth. But all the iso-

Table 1 — Actinomycete Genera Isolated and Their Antimicrobial Activity

Genera	Strains		Isolates active against			
	Tested	Active	<i>B. subtilis</i>	<i>E. coli</i>	<i>A. niger</i>	<i>C. albicans</i>
<i>Streptomyces</i>	102	19	12	9	7	5
<i>Micromonospora</i>	21	3	3	3	—	—
<i>Nocardia</i>	8	2	1	1	1	1
<i>Streptosporangium</i>	5	1	1	1	—	—
<i>Micropolyspora</i>	3	—	—	—	—	—
<i>Streptoverticillium</i>	1	—	—	—	—	—

lates were able to grow in the presence of 3% NaCl. A few isolates (19) were able to grow at 10% NaCl but none were able to grow at 14% level. The salt requirement or tolerance data suggested that some actinomycetes might be terrestrial forms that have adopted to the salinity of seawater and sediments.

The data indicate that marine sediments are also potent sources for the isolation of actinomycetes with good antimicrobial activities.

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