

## Studies on antagonistic marine streptomycetes

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**Abstract**—Sixty nine strains of *Streptomyces* sp. isolated from the sediments of Andaman and Nicobar islands (Bay of Bengal) were screened for their antagonistic property against a number of test cultures (*Vibrio* sp., *Klebsiella* sp., *Escherichia coli*, *Shigella* sp., *Proteus vulgaris*, *Mycobacterium smegmatis*, *Staphylococcus albus* and *Bacillus* sp.). Nearly 60% of the streptomycete cultures produced antibacterial compounds active against both Gram positive and Gram negative bacteria. Among the positive cultures three strains inhibited all the test cultures. In addition to the above test cultures marine bacteria (*Vibrio* sp., *Aeromonas* spp., *Flavobacterium* spp., *Bacillus* sp. and *Micrococcus* sp.) resistant to few known antibiotics (tetracycline, penicillin, neomycin, kanamycin and gentamycin) were also used. Thirty three streptomycete cultures were found to inhibit one or more marine bacteria tested. The ecological distribution and importance of these antagonistic marine streptomycetes are discussed.

### INTRODUCTION

A number of reports have been published on the occurrence and distribution of actinomycetes in estuarine and marine environments (WALKER and COLWELL, 1975; OKAMI and OKAZAKI, 1978; WEYLAND, 1984). The extensive survey conducted by WEYLAND (1984) has shown that while streptomycetes are most frequent in the coastal and shallow areas, members of Micromonosporae are very common in deep sea sediments. He also observed that non-sporing nocardioforms were found preponderantly in the open sea upto about 2000m. Even though many such reports describe their abundance and their activities, information on their antagonistic properties is very much limited.

It is now documented that over 55% of the antibiotics discovered between 1945 and 1978 originated from the genus *Streptomyces* representing a total of more than 5000 compounds (BERDY, 1980). Even though this genus was more frequently isolated from marine samples such as decaying materials, sediments and animals, no serious attempt was made to evaluate their antagonistic properties. However in the last decade considerable attention was paid by the Japanese workers (OKAMI, 1986) and many novel antibiotics were isolated. The detailed studies conducted by LAKSHMANAPERUMALSAMY (1978) and VANAJA-KUMAR (1979) in the coastal areas of Portonovo, east coast of India have clearly shown the potential of marine streptomycetes as antibiotic producers. While LAKSHMANAPERUMALSAMY (1978) reported that 27% of the streptomycete strains isolated from sediments were antagonistic VANAJA-KUMAR (1979) showed that this percentage could be as high as 75% in marine molluscs. Most of these studies have so far been restricted to coastal areas only. In the present study we report on the antagonistic property of *Streptomyces* spp. isolated from an oceanic island rich in corals.

### MATERIALS AND METHODS

The sediment samples were collected from the intertidal zone of Cobin's Cove and Ross island (Andaman and Nicobar islands, Bay of Bengal) and used for isolation

of streptomycete strains. The sediments were silty in nature with lots of broken shell and detritus. Serial dilutions of the samples were prepared with sterile seawater and pour plated using modified Kuster's agar (KUSTER,1963). This medium had the following composition (g/l): Glycerol 10.0, casein 0.3, KNO<sub>3</sub> 2.0, NaCl 20.0, K<sub>2</sub>HPO<sub>4</sub> 2.0, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.05, CaCO<sub>3</sub> 0.01, agar 18.0, and tapwater 1 litre. The plates were incubated at room temperature (28±2 °C) for 10 days and well isolated individual colonies were randomly picked up and subcultured on Kuster's agar slants. The cultures were checked for purity and maintained at 4 °C in a refrigerator.

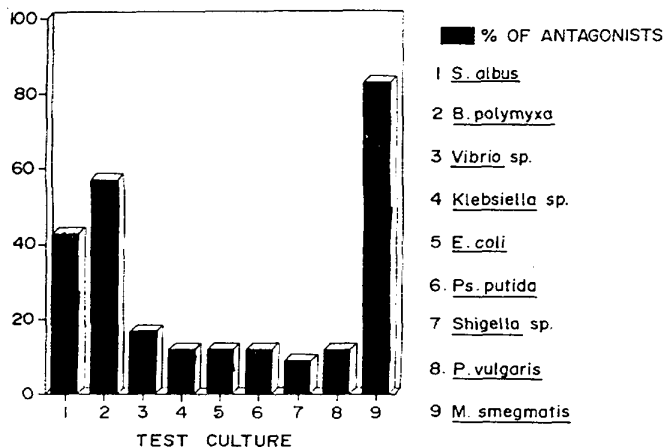


Fig.1. Percentage of antagonistic streptomycetes inhibiting various terrestrial bacterial cultures.

Table 1. Antibiotic resistance of marine bacteria

Culture No.	Name	Resistant to				
		P	T	N	K	G
NIO 34	<i>Micrococcus</i> sp.	+	+	+	+	+
NIO 264	<i>Bacillus</i> sp.	+				+
NIO 106	<i>Flavobacterium</i> sp.	+		+	+	
NIO 236	<i>Flavobacterium</i> sp.	+			+	+
NIO 95	<i>Vibrio</i> sp.	+		+	+	+
NIO 96a	<i>Aeromonas</i> sp.	+	+			
NIO 96b	<i>Aeromonas</i> sp.	+			+	
NIO 261	<i>Aeromonas</i> sp.		+			

P - Penicillin G (10 units/disc), T - Tetracycline (30mcg/disc), N - Neomycin (30mcg/disc), K - Kanamycin (30mcg/disc), G - Gentamycin (10mcg/disc)

The antibiotic production of 69 streptomycete isolates was tested as follows: The cultures were streaked on the surface of Kuster's agar medium in Petridishes as ribbons and incubated at room temperature for 7 days. Agar plugs of streptomycete growth were prepared with 4mm stainless steel cork borer and placed on the surface of Nutrient agar medium inoculated with the test cultures (*Staphylococcus albus*, *Bacillus polymyxa*, *Vibrio* sp., *Klebsiella* sp., *Escherichia coli*, *Pseudomonas putida*, *Shigella* sp., *Proteus vulgaris*, and *Mycobacterium smegmatis*). The test cultures were grown in Nutrient broth for 12-18 hrs and the culture broth was applied on to the surface of Nutrient agar medium in Petridishes with sterile cotton swabs. The plates with test cultures and streptomycete

Table 2. Antibigram of streptomycete isolates with terrestrial and marine bacteria (inhibition zone in mm).

Streptomycete isolate No.	* Test cultures																	
	Terrestrial									Marine								
	T1	T2	T3	T4	T5	T6	T7	T8	T9	M1	M2	M3	M4	M5	M6	M7	M8	
S.1	-	-	-	-	-	-	-	-	10	5	-	11	-	6	6	-	-	
S.2	18	8	-	-	-	-	-	-	10	-	10	-	10	-	-	-	6	
S.4	-	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
S.5	7	10	-	-	-	-	-	-	6	-	14	20	15	-	-	-	12	
S.6	4	8	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	
S.7	-	-	-	-	-	-	-	-	9	-	-	6	-	-	-	-	-	
S.8	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	
S.9	12	24	15	15	17	11	12	11	20	-	-	-	14	-	-	-	-	
S.11	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
S.13	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
S.14	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
S.15	-	-	-	-	-	-	-	-	-	-	-	14	10	-	-	-	-	
S.16	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	
S.17	-	-	-	-	-	-	-	-	5	6	-	-	-	-	-	-	-	
S.18	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	
S.19	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	
S.20	-	-	-	-	-	-	-	-	11	-	16	11	15	-	-	-	8	
S.21	-	-	-	-	-	-	-	-	-	-	7	-	10	-	-	-	-	
S.22	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	
S.23	-	7	-	-	-	-	-	-	9	-	-	-	-	-	-	-	-	
S.24	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	-	
S.25	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	
S.26	13	16	11	13	11	10	11	8	18	-	6	-	-	-	-	-	-	
S.27	16	11	-	-	-	-	-	-	14	-	8	6	8	-	-	-	6	
S.28	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	
S.29	-	-	-	-	-	-	-	-	-	-	6	6	-	-	-	-	-	
S.30	-	10	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-	
S.31	15	17	14	14	15	8	13	15	20	-	-	6	-	-	-	-	-	
S.32	6	15	9	12	14	11	10	20	-	-	-	-	-	-	-	-	-	
S.33	16	12	-	-	-	-	-	9	-	-	-	-	-	-	-	-	-	
S.36	-	-	-	-	-	-	-	6	6	-	-	12	-	-	-	-	-	
S.37	9	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	
S.38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	6	
S.39	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	6	
S.42	6	8	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	
S.44	17	10	4	-	-	-	-	9	-	-	12	15	18	-	-	-	8	
S.52	-	-	-	-	-	-	-	15	-	-	-	-	-	-	-	-	-	
S.53	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	
S.54	-	10	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	

Contd.

Streptomycete isolate No.	* Test cultures																
	Terrestrial								Marine								
	T1	T2	T3	T4	T5	T6	T7	T8	T9	M1	M2	M3	M4	M5	M6	M7	M8
S.55	-	5	-	-	-	-	-	-	8	-	-	-	-	4	-	-	-
S.56	9	17	10	6	9	-	9	9	16	-	6	-	10	-	-	-	-
S.57	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.58	-	4	-	-	-	-	-	-	8	-	10	4	12	8	-	-	6
S.59	-	-	-	-	-	-	-	-	7	-	-	5	-	-	-	-	-
S.60	10	-	-	-	-	-	-	-	9	-	15	6	13	7	-	-	7
S.61	9	-	-	-	-	-	-	-	8	-	14	10	15	8	-	-	8
S.62	-	-	-	-	-	-	-	-	7	-	8	-	6	-	-	-	-
S.63	-	-	-	-	-	-	-	-	10	-	-	8	-	10	-	-	-
S.64	-	7	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-
S.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.67	8	5	-	-	-	-	-	-	4	-	-	-	-	6	-	-	-
S.68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.69	5	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-

\* Isolate No. S.3, S.10, S.12, S.34, S.35, S.40, S.41, S.43, S.45-51 and S.65 did not inhibit any test cultures.

- T1 *Staphylococcus albus*
- T2 *Bacillus polymyxa*
- T3 *Vibrio* sp.
- T4 *Klebsiella* sp.
- T5 *Escherichia coli*
- T6 *Pseudomonas putida*
- T7 *Shigella* sp.
- T8 *Proteus vulgaris*
- T9 *Mycobacterium smegmatis*

- M1 *Micrococcus* sp. (NIO 34)
- M2 *Bacillus* sp. (NIO 264)
- M3 *Flavobacterium* sp. (NIO 106)
- M4 *Flavobacterium* sp. (NIO 236)
- M5 *Vibrio* sp. (NIO 95)
- M6 *Aeromonas* sp. (NIO 96a)
- M7 *Aeromonas* sp. (NIO 96b)
- M8 *Aeromonas* sp. (NIO 261)

growth plugs were incubated at room temperature and the diameter (mm) of zone of inhibition was measured after 24 hrs.

In addition to the above test cultures, 8 marine bacterial cultures which are known to be resistant to some antibiotics (Table-1) were also tested for their sensitivity to antibiotics produced by streptomycete isolates. The method of testing was essentially the same as described above except that seawater was used instead of freshwater for the preparation of Nutrient medium.

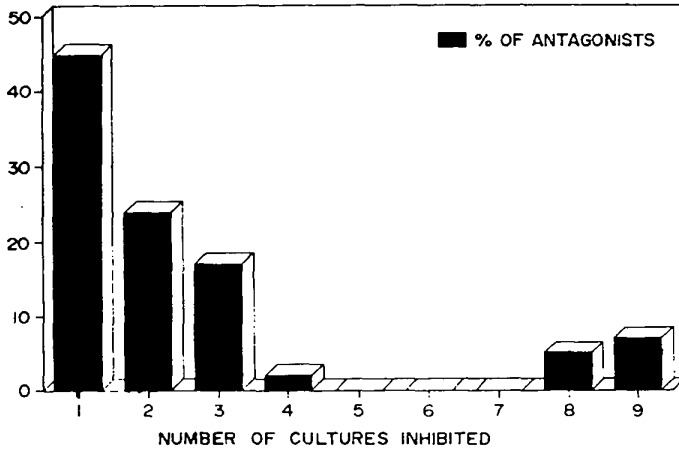


Fig.2. Percentage of antagonistic streptomycetes showing multiple inhibition of terrestrial cultures.

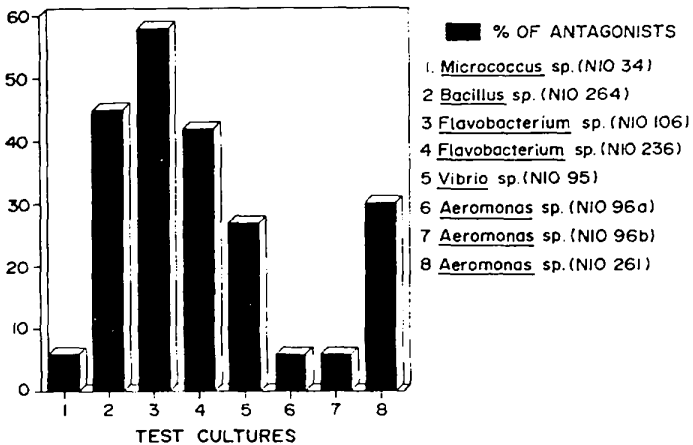


Fig.3. Percentage of antagonistic streptomycetes inhibiting various marine bacteria.

## RESULTS

The results of screening of streptomycete cultures for antibacterial activity are given in Table-2. If both terrestrial and marine cultures are considered together 53 of the 69 cultures (76.8%) screened showed antibacterial activity. On the other hand, 62.3% and 47.8% of the streptomycete cultures showed antagonistic properties against terrestrial and marine cultures respectively. In general marine streptomycetes inhibited Gram-positive cultures better than Gram-negative cultures (Fig.1). Interestingly 83% of the cultures inhibited the growth of *M.smegmatis*. When the antagonistic cultures were examined for multiple inhibition, majority of them (45%) inhibited only one bacterial test cultures (Fig.2). However, cultures with multiple inhibition were also recorded. While 7% of the antagonistic streptomycetes inhibited all the 9 test cultures 5% showed activity against 8 test cultures.

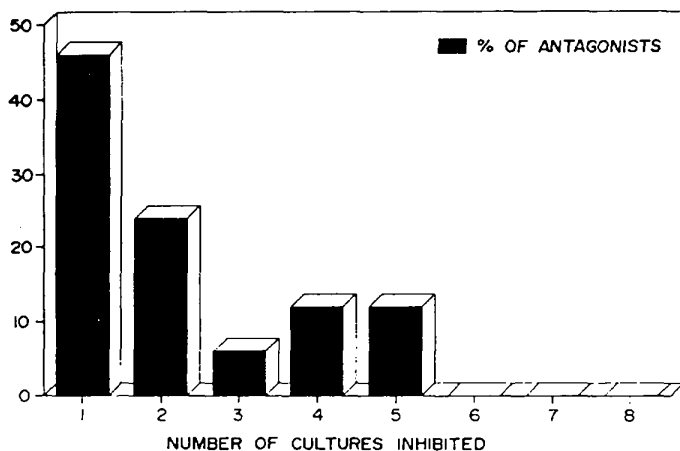


Fig.4. Percentage of antagonistic streptomycetes showing multiple inhibition of marine bacteria.

In the case of marine test cultures which showed resistance to one or more antibiotics, Gram-positive strains were found to be more susceptible to antibiotics produced by *Streptomyces* spp. except *Micrococcus* sp. (Fig.3). Both genera of Gram-negative bacteria viz. *Vibrio* and *Aeromonas* were also found to be inhibited. However, two of the three strains of *Aeromonas* tested were found to be more resistant to antibiotics produced by the streptomycete isolates. The pattern of multiple inhibition by the antagonistic cultures was almost the same for both terrestrial and marine bacteria (Fig.4). However antagonists capable of inhibiting all the marine test cultures were not encountered.

## DISCUSSION

*Streptomyces* is known to be the most common genus in the marine environment especially in the shallow organic rich coastal areas. Eventhough genera such as *Micromonospora*, *Nocardia*, *Streptosporangium*, *Micropolyspora* and *Streptoverticillium* in addition to the *Streptomyces* have been recorded in marine sediments (ELLAIAH and REDDY,1987), *Streptomyces*, *Micromonospora* and *Nocardia* are the three genera which

seem to be most common in the world seas ( WEYLAND,1986; BARCINA,1987; PISANO,1987). Eventhough many of these strains were examined for their ecological and physiological properties, studies on their antagonistic properties are very much limited ( LAKSHMANAPERUMALSAMY, 1978; VANAJA-KUMAR, 1979; OKAMI, 1986; ELLAIAH and REDDY,1987; TAKAHASHI *et al.*,1989). In the last decade few novel bioactive compounds (antibiotic, antimalarial, anticancer, antitumour and acaricidal) have been isolated from marine strains of *Streptomyces* (OKAMI,1986;TAKAHASHI *et al.*,1989). Almost all of these screening studies were carried out with terrestrial, especially clinical, bacteria and fungi and not with marine strains. In the present study in addition to the routine test cultures, 8 cultures of marine bacteria which are known to be resistant to one or more antibiotics (Table-1) were included. The idea of inclusion of such antibiotic resistant strains in the screening programme was to examine their sensitivity to antibiotics produced by other marine microbes. As it can be seen from the results, many of the marine streptomycete isolates inhibited marine bacteria. In general, Gram-positive bacteria were found to be more susceptible to antibiotics produced by marine *Streptomyces* spp.(Table-3). However Gram-negative marine bacteria such as *Vibrio* and *Aeromonas* were also found to be sensitive to *Streptomyces* spp.

Table 3. Percentage of antagonistic streptomycete isolates active against various test cultures.

Bacteria	References		
	Lakshmana- perumalsamy, (1978) (n=140) *	Vanaja kumar, (1979) (n=290)	Present study (n=42)
<b>TERRESTRIAL STRAINS:</b>			
<i>Bacillus circulans</i>	59	87	-**
<i>Bacillus polymyxa</i>	-	-	57
<i>Staphylococcus aureus</i>	47	-	-
<i>Staphylococcus albus</i>	-	-	43
<i>Streptococcus faecalis</i>	-	52	-
<i>Escherichia coli</i>	30	27	12
<i>Pseudomonas aeruginosa</i>	0	20	-
<i>Pseudomonas putida</i>	-	-	12
<i>Klebsiella</i> sp.	-	-	12
<i>Shigella</i> sp.	-	-	9
<i>Proteus vulgaris</i>	-	-	12
<i>Vibrio</i> sp.	-	-	17
<i>Mycobacterium smegmatis</i>	-	-	83
<b>MARINE STRAINS:</b>			
(antibiotic resistant)			(n=33)
<i>Micrococcus</i> sp. (NIO 34)	-	-	6
<i>Bacillus</i> sp. (NIO 264)	-	-	45
<i>Flavobacterium</i> sp. (NIO 106)	-	-	58
<i>Flavobacterium</i> sp. (NIO 236)	-	-	42
<i>Vibrio</i> sp. (NIO 95)	-	-	27
<i>Aeromonas</i> sp. (NIO 96a)	-	-	6
<i>Aeromonas</i> sp. (NIO 96b)	-	-	6
<i>Aeromonas</i> sp. (NIO 261)	-	-	30

\* Total number of antagonistic cultures, \*\* Not Tested

When we compare the antibiograms of marine streptomycetes obtained with terrestrial and marine cultures, a different picture emerges. The antibiogram developed

with terrestrial cultures differ considerably from that of marine cultures. For example, culture S.1 which inhibited only one of the 9 terrestrial cultures could inhibit as many as 5 of the 8 marine cultures (Table-2). Similarly culture S.9 or S.26 which showed inhibitory property against all the terrestrial cultures was found effective against only one of the 8 marine cultures tested. Obviously, this indicates that when we use marine microbes for antibiotic screening programmes, the marine test cultures should also be included, if susceptibility of marine microbes to antibiotics is considered in marine environment.

The most resistant *Micrococcus* sp. (NIO 34) was also inhibited by 6% of the antagonistic streptomycetes. These observations give clear indication that such antagonistic streptomycetes which are known to inhibit the most common genera of marine bacteria that are seen in the primary film-forming microbial communities, may prove useful in antifouling studies. For example some of the marine animals rich in antagonistic forms, in addition to other benefits, may derive protection from both micro- and macro-fouling organisms. It is true that evidence of the natural roles of antibiotics is still equivocal in terrestrial environment and more so in the marine environment. The main reason is our limited knowledge of the geographical and ecological distribution of these microbes. The problems in understanding of the ecology of antibiotic producing microbes have recently been summarised by WILLIAMS and VICKERS (1986). The most interesting observation made by VANAJA-KUMAR (1979) that as much as 75% of the streptomycete strains associated with marine molluscs were showing antimicrobial activity suggest the possible existence of a strong animal-microbe interaction. This is further supported by the observation made in the present study that nearly 60% of the streptomycete isolates obtained from the sediments colonized by corals are antagonistic to many bacteria. It is possible that animals may derive some useful metabolites associated with them. For example compounds like 'renierone' and their dimeric metabolites called 'renieramycins' isolated from sponges (*Reneira* sp.) have striking structural similarities with the bacterial metabolites 'mimocin' and 'saframycins' respectively produced by *Streptomyces lewendulae* (KARUSO, 1987). In an earlier study FRINCKE and FAULKNER (1982) had also reported the isolation of 'mimosamycin' a known bacterial metabolite from the same sponge. Subsequently STIERIE *et al.* (1988) also demonstrated that *Micrococcus* sp. associated with the sponge, *Tedania ignis*, produced secondary metabolites (diketopiperazines) ascribed to the sponge host. One of the recent studies has clearly shown that tetrodotoxin present in 'Puffer fish' is actually from the associated bacteria and not from the animal itself (KOGURE *et al.*, 1989). The microbial origin of such bioactive compounds in marine animals seems to be more common than what was thought earlier. However more detailed studies are warranted to elucidate the role of such antagonists in animal-microbe associations.

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