

Antibacterial and Antifungal Activity of Streptomycetes Isolated from Portonova Coastal Environment

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The antibacterial and antifungal activities of 518 streptomycetes strains were tested against *Bacillus circulans*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae* and *Fusarium oxysporum*. 27.03% of the strains was found to elaborate one or more types of antibiotics. 59.27% was active on *B. circulans*, 47.01% on *S. aureus*, 30% on *E. coli*, 53.59% on *S. cerevisiae* and 39.3% on *F. oxysporum*. Majority of the isolates (46.43%) showed combined antibacterial and antifungal activities while 28.5% were only antifungal and 25%, only antibacterial. The results clearly show that the marine environment harbours many broad spectrum antibiotic-producing streptomycetes.

Since the dawn of 'Antibiotic-age' in 1939-40, an intensive search for antagonistic actinomycetes has been in progress. The antibiotics are widely used as chemotherapeutic agents in animal, plant and human diseases. Thousands of marine organisms are known to contain antibiotic substances and less than 1% have been examined for their pharmaceutical activity (Halstead, 1965). The ocean remains as an untapped source for many drugs and contemporary experimental studies indicate that pharmacologically active substances could be isolated from marine organisms (Baslow, 1969).

Among marine organisms, actinomycetes, particularly streptomycetes will prove to be as fruitful as their counterparts isolated from

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terrestrial habitat (Okami, 1986, Pisano *et al.*, 1989). However the marine streptomycetes have not received much attention. Only very few reports are available on the occurrence and distribution of antagonistic streptomycetes in the marine environment (Weyland, 1969; Okazaki & Okami, 1972; Postmaster & Frietas, 1975; Sambamurthy & Ellaiah, 1975).

The marine environment is a potent source that contain actinomycetes which are capable of producing antibiotics (Okami, 1986; Pisano, 1989). It is well known that the sediments not only harbour most of the microorganisms but also serve as the best medium for their growth and survival than the overlying water. Hence a detailed study on the occurrence and distribution of actinomycetes in the sediments of Portonovo coastal zone is made and the results on the antagonistic properties of these streptomycetes are presented in this paper.

Materials and Methods

Five hundred and eighteen streptomycetes isolates were selected at random during the counting of actinomycetes flora in sediments of estuarine, marine, backwater, freshwater and mangrove environment using Grein and Meyers agar (1958), Kuster's agar (1963) and Glucose Asparagine agar (Waksman 1961). The streptomycetes isolates were purified after repeated streaking in Kuster's agar and were maintained on the medium.

Bacillus circulans, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Fusarium oxysporum* and *Saccharomyces cerevisiae* were tested against the streptomycetes isolates.

Antibiotic assay of streptomycetes isolates against the test organisms was done by cross-streak assay method (Waksman and Lechevalier, 1962). The streptomycetes isolates were inoculated as a single streak onto the surface dried planes of Glucose-peptone agar (Rangaswami *et al.*, 1967) and incubated at room temperature ($28\pm 2^\circ\text{C}$). After good ribbon like growth, the test organisms were streaked at right angles to the original streak of streptomycetes and incubated at 37°C . The zone of inhibition was recorded at 24 and 48 h for bacteria and yeast and after 48 and 72 h for fungi. Appropriate control plates without

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streptomycetes and streaked with test organisms to assess their normal growth were maintained.

Results and Discussion

Table 1 gives the distribution of antagonistic streptomycetes in different biotopes and during various seasons. Of the 518 isolates tested, 140 (27.03%) exhibited antimicrobial activity. Maximum percent (38.71%) of antagonistic isolates were recorded in backwater sediments. Among estuarine, marine, riverine and mangrove biotopes, the percentage of occurrence varied between 27.46% and 22.41%. Higher percentage (40) of antagonistic isolates could be collected in postmonsoon period and during other seasons the percentage was 25.54% and 21.70%, the least being, during monsoon season.

Table 1. Distribution of antagonistic streptomycetes in different biotopes and seasons

Source/ Season	No. of isolates		Percentage of active isolates
	Tested	Active	
Marine	66	18	27.27
Estuarine	233	64	27.46
Riverine	58	13	22.41
Backwater	31	12	38.71
Mangrove	130	33	25.38
Postmonsoon	90	36	40.00
Summer	184	47	25.54
Premonsoon	138	34	24.64
Monsoon	106	23	21.70
Total	518	140	27.03

Totally six coloured series were recorded (Tables 2 and 3). White and grey were encountered in all the biotopes and in all the seasons. Red, yellow, green and blue were not recovered in marine, riverine and backwater biotopes. Among various coloured groups, white and grey accounted for more than 93%. 39.74% and 20% of grey and white colour series respectively exhibited antimicrobial activity. Higher percentages

Table 2. Distribution of antagonistic streptomycetes in different biotopes based on colour series

Colour	Marine			Estuarine			Riverine			Backwater			Mangrove			Total		
	T	A	%	T	A	%	T	A	%	T	A	%	T	A	%	T	A	%
White	47	12	25.53	156	32	20.51	38	5	13.16	18	4	22.22	66	12	18.18	325	65	20.00
Grey	18	6	33.33	51	22	43.14	18	8	44.44	13	8	61.53	56	18	32.14	156	62	39.74
Red	1	-	-	6	6	100.00	1	-	-	-	-	-	2	-	-	10	6	60.00
Yellow	-	-	-	12	1	8.33	1	-	-	-	-	-	2	1	50.00	15	2	17.33
Green	-	-	-	1	1	100.00	-	-	-	-	-	-	2	1	50.00	3	2	66.67
Blue	-	-	-	7	2	28.57	-	-	-	-	-	-	2	1	50.00	9	3	33.33
Total	66	18	26.47	233	64	27.47	58	13	22.41	31	12	38.71	130	33	25.38	518	140	27.03

T : Tested
A : Active

Table 3. Distribution of antagonistic streptomycetes in different seasons based on colour series

Colour	Post-monsoon			Summer			Pre-monsoon			Monsoon			Total		
	T	A	%	T	A	%	T	A	%	T	A	%	T	A	%
White	53	15	28.30	109	24	22.02	93	17	18.28	70	9	12.86	325	65	20.00
Grey	32	18	56.25	57	18	31.58	35	13	37.14	32	13	40.63	156	62	39.74
Red	2	2	100.00	7	4	57.14	1	-	-	-	-	-	10	6	60.00
Yellow	2	1	50.00	11	1	9.09	1	-	-	1	-	-	15	2	17.33
Green	-	-	-	-	-	-	1	1	100.00	2	1	50.00	3	2	66.67
Blue	1	-	-	-	-	-	7	3	42.86	1	-	-	9	3	33.33
Total	90	36	40.00	184	47	25.54	138	34	24.64	106	23	21.70	518	140	27.03

T : Tested
A : Active

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(66.67 and 60) of green and red coloured isolates showed antimicrobial activity. 56.25% of grey colour series isolated during postmonsoon elaborated antimicrobial activity while 31.58%, 37.14% and 40.63% of the isolates collected during summer, premonsoon and monsoon respectively showed antimicrobial activity. In all the seasons percentage of antibiotic producing white coloured streptomycetes isolates were less when compared to grey colour series. Irrespective of the biotopes the grey colour series showed antagonistic properties at higher proportion than other coloured series and the maximum of 61.53% was recorded in backwater followed by mangrove, riverine, estuarine and marine environment.

Of the 140 antagonistic isolates, nearly 59.27% were active against *B.circulans*, 47.01% against *S.aureus*, 30% against *E. coli*, 53.59% against *S.cerevisiae* and 39.30% against *F.oxysporum* (Table 4). Isolates active against *P. aeruginosa* were not encountered. Majority of the isolates which were active against bacteria and yeast belonged to category II (inhibition zone of 11-30 mm) and most of the isolates showing activity against fungi were in the category I (inhibition zone of < 10 mm).

Table 4. Antibiogram of streptomycetes against test pathogens. The figures indicate percentage of antagonistic isolates

Test organisms	Category %			Total
	I	II	III	
<i>Bacillus circulans</i>	10.70	37.14	11.43	59.27
<i>Escherichia coli</i>	7.14	17.86	5.00	30.00
<i>Pseudomonas aeruginosa</i>	-	-	-	-
<i>Staphylococcus aureus</i>	14.29	26.29	6.43	47.01
<i>Saccharomyces cerevisiae</i>	16.43	32.87	4.29	53.59
<i>Fusarium oxysporum</i>	25.71	10.00	3.59	39.30

I Inhibition zone of <10 mm

II Inhibition zone of 11-30 mm

III Inhibition zone of >30 mm

All the antagonistic cultures were classified into three main groups (antibacterial, antifungal and combined antibacterial and antifungal)

and a major part fall under combined antibacterial and antifungal. Table 5 presents the antagonistic nature of streptomycetes on the basis of colour series. In total 46.43% of various colour series showed combined antibacterial and antifungal properties followed by 28.57% and 25% of antifungal and antibacterial activity. Among different colour series also, similar trend was seen. In estuarine, riverine and mangrove biotopes, isolates having combined antibacterial and antifungal activity were found at higher proportion followed by antifungal and antibacterial activity, while in marine biotopes percentage of isolates showing antibacterial activity were more followed by antifungal and combined antibacterial and antifungal (Table 6). In backwater, antifungal isolates were less than antibacterial (33.33%) and combined antibacterial and antifungal activity. Although isolates having combined antibacterial and antifungal activity were more during postmonsoon, summer and monsoon months, isolates showing antifungal activity were more in premonsoon season. During postmonsoon and summer months, isolates of antifungal activity was least while in premonsoon and monsoon months isolates of antibacterial activity was least. Isolates antagonistic to all test organisms were more common in estuarine and mangrove biotopes. Generally at monsoon period, recovery of these groups from sediments was very poor.

Table 5. Antagonistic grouping of streptomycetes isolates on the basis of colour series

Groups	White	Grey	Red	Yellow	Green	Blue	Total
AB	18 (27.69)	15 (24.2)	1 (16.67)	1 (50.00)	-	-	35 (25.0)
AF	22 (33.85)	15 (24.2)	2	1	40 (100.00)	(33.33)	(28.57)
ABF	25 (38.46)	32 (51.6)	5 (83.3)	1 (50.00)	2	65 (66.67)	(46.43)

AB : Antibacterial

AF : Antifungal

ABF : Antibacterial and antifungal

Figures give number of antagonistic isolates, percentage of antagonistic isolates to total isolates in parenthesis.

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Actinomycetes have yielded hundreds of biologically active compounds and many of them have been used successfully as chemotherapeutic agents (Umezawa, 1967). There have been a few reports on the antimicrobial activity of marine actinomycetes (Okami, 1984; Pisano *et al.*, 1989). In the present investigation 144 sediment samples from marine, estuarine, riverine, backwater and mangrove biotope of Portonovo coastal zone were analysed for the presence of actinomycetes. 518 isolates of the genus *Streptomyces* alone were selected and screened microbiologically for antimicrobial activity using the cross streak assay method.

Table 6. Grouping of antagonistic *Streptomyces* in different biotopes *

Biotope/ season	Anti- bacterial	Anti- fungal	Antibacterial & antifungal	Total
Marine	7 (38.90)	6 (33.33)	5 (27.77)	18 (27.27)
Estuarine	14 (21.88)	17 (26.56)	33 (51.56)	64 (27.46)
Riverine	2 (15.38)	5 (38.46)	6 (46.15)	13 (22.41)
Backwater	4 (33.33)	3 (25.00)	5 (41.67)	12 (38.71)
Mangrove	8 (24.24)	9 (27.27)	16 (48.49)	33 (25.38)
Postmonsoon	15 (41.67)	2 (5.55)	19 (52.78)	36 (40.00)
Summer	15 (31.91)	11 (23.40)	21 (44.68)	47 (25.54)
Premonsoon	4 (11.76)	17 (50.00)	13 (35.13)	34 (24.64)
Monsoon	1 (4.35)	10 (43.48)	12 (52.17)	23 (21.70)

*The figures indicate number of antagonistic isolates, percentage of antagonistic isolates given in parenthesis.

Of the 518 isolates tested only 140 (27.03%) isolates exhibited antimicrobial properties. Nearly 59% were active against gram-positive bacterium *B.circulans* and 47% on *S.aureus*. The Gram-negative bacterium *E.coli* was inhibited by 30% of the total isolates. As much as 54% exhibited antiyeast (*S.cerevisiae*) properties while 39% were active against the filamentous fungus, *F.oxysporum*. Gram-positive group seems to be highly susceptible to various antagonists. Similar observations have already been made on the actinomycetes strains isolated from marine environment (Okazaki & Okami, 1972; Postmaster & Frietas, 1975).

It has been reported that among antibacterial actinomycetes, those antagonistic to both Gram-positive and Gram-negative bacteria were

more (Rangaswami *et al.*, 1967). However in the present study isolates exhibiting combined gram-positive and gram-negative inhibiting properties were less in number compared to those active against Gram-positive bacteria. Okazaki and Okami (1972) and Pisano *et al.*, (1989) also reported a similar finding while screening actinomycetes isolated from marine sediments. These observations indicate that the antibacterial component of streptomycetes population in the marine environment may be different from those of terrestrial origin.

About 75% of the isolates exhibited antifungal activity, acting on both filamentous and non-filamentous fungi. Similarly as much as 83% of actinomycetes isolated from Sagamy Bay were found to be antifungal (Okazaki & Okami, 1972). Among the antifungal streptomycetes, those active against yeast, *S.cerevisiae* were more in number than those among filamentous fungi, *F.oxysproum*. However Pisano *et al.*, (1989) reported that 28% of actinomycetes exhibit antifungal activity and that filamentous and non-filamentous fungi displayed equal susceptibilities.

When the cultures were grouped on the basis of antagonistic quality, those exhibiting combined antibacterial and antifungal properties occurred in higher percentages than those active against either bacteria or fungi. The occurrence of such higher percentages of streptomycetes with combined antibacterial and antifungal property in soil has been reported by Rangaswami *et al.*, (1967). However Okazaki & Okami (1972) recorded only 32.43% of the actinomycetes isolates exhibiting the combined antibacterial and antifungal properties.

It is commonly recognized that actinomycetes of marine ecosystem have been relatively neglected (Goodfellow and Haynes, 1984) and several recent studies have demonstrated that actinomy-cetes of marine origin produce novel bioactive substances (Okami, 1986). Accordingly additional investigations appear warranted. The results of the present investigation reveal that the actino-mycetes from marine and coastal environment is a potent source for newer antibiotics.

Acknowledgement

The authors thank the authorities of Annamalai University for providing facilities. Financial support to PL by the Council of Scientific and Industrial Research, New Delhi is gratefully acknowledged.

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