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Isolation and Characterization of Antagonistic *Streptomycetes* from Marine Sediments

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A total number of 38 strains of actinomycetes were isolated from the sediments of the Mallipattinam coast. They were tested for their antagonistic activity against the potential human pathogens such as *Staphylococcus aureus, Streptococcus faecalis, Proteus vulgaris, Salmonella typhi, Candida albicans* and *Aspergillus niger.* Among them, only 3 *Streptomycetes* sp. showed good activity against the tested pathogens and they were tentatively identified upto species level.

Key words: Actinomycetes, Antagonistic Activity, Bacteria & Fungi.

The search for new antibiotics continues to be of utmost importance in research programs around the world because of the increase of resistant pathogens and toxicity of some used antibiotics. Among microorganisms, actinomycetes are one of the most investigated groups particularly members of the genus *Streptomycetes* from which, a large number of antibiotics was obtained and studied[1]. The vast majority of actiomycetes have originated from soil and their isolation method deal almost exclusively with those suitable for *Streptomycetes* species which grow rapidly on soil dilution plates.

The resistance of numerous pathogenic bacteria and fungi to commonly used bioactive secondary metabolites is presently an urgent focus of research, and new antifungal and antibacterial molecules are necessary to combat these pathogens. Filamentous soil bacteria belonging to the genus *Streptomycetes* are rich sources of a high number of bioactive natural products with biological activity which are extensively used as pharmaceuticals and agrochemicals. These bacteria produce about 75% of commercially and medically useful antibiotics[2], and approximately 60% of antibiotics which have been developed for agricultural use were isolated from *Streptomyces* species[3]

In the present work, we describe the isolation of *Streptomyces* strain, from seashore and mangrove soil sample, and its identification by conventional and molecular methods as well as the production, the isolation and the partial characterization of produced antibiotics. Therefore, we report the separation and identification of the antimicrobial components of Streptomycetes sp with the aid of GC-MS technique.

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MATERIAL AND METHODS

Sediment samples were collected from two stations (station: 1-Seashore zone; station:2-Mangrove zone) of the Mallipattinam coastal environment. Sediment samples were collected by inserting a polyvinyl cover (10 cm dia) into the sediments. The cover was sterilized with alcohol before sampling at each station. The central portion of the top 2 cm sediment sample was taken out with the help of a sterile spatula. This sample was then transferred to a sterile polythene bag and transported immediately to the laboratory. The sediment samples thus collected were air-dried aseptically. After a week, the sediment samples were incubated at 55°c for 5 min[4]. Then, 10-fold serial dilutions of the sediment samples were prepared, using filtered and sterilized 50% seawater. One ml of the serially diluted samples was plated in the Starch Casein Agar medium (SCA).

Samples were also taken from each site for analyzing physico-chemical parameters such as pH, electrical conductivity, nitrogen, phosphorous, potassium, soil texture, manganese, zinc and copper. The correlation co-efficient analysis between physico-chemical parameters of soils and actinomycetes populations was also made using SPSS package.

Starch casein agar (SCA) medium (Himedia, Mumbai, India) was used for isolation and enumeration of actinomycetes. The medium was supplemented with 10µg /ml amphotericin and 25 µg/ml streptomycin (Himedia, Mumbai, India) to inhibit fungal and bacterial contamination respectively[5]. In conventional dilution plate technique, 10 g of marine soil samples were suspended in 100 ml of sterile sea water and 0.5 ml of suspension from this was spread over 50% sea water starch casein agar medium and incubated for 7-9 days at 28°C. After incubation the actinomycete colonies were purified and sub-cultured on SCA agar plates and stored for further assay.

Antimicrobial activities of isolates were tested preliminarily by cross streak method[6]. Actinomycetes isolates were streaked across diameter on starch casein agar plates. After incubation at 28°C for 6 days, 24 hrs cultures of *Salmonella typhi, Streptococcus faecalis, Proteus vulgaris, Staphylococcus aureus, Candida albicans* and *Aspergillus niger were* streaked perpendicular to the central strip of actinomycetes culture. All plates were again incubated at 30°C for 24 hrs and zone of inhibition was measured.

The selected antagonistic actinomycete isolates were inoculated into starch casein broth, and incubated at 28°C in a shaker (200-250rpm) for seven days. After incubation the broths were filtered through Whatman No.1 filter paper and then through Millipore filter (Millipore Millex-HV Hydrophilic PVDF 0.45um). The filtrate was transferred aseptically into a conical flask and stored at 4°C for further assay. To the culture filtrate, equal volume of ethyl acetate was added separately and centrifuged at 5000 rpm for 10 min to extract the antimicrobial compound[7]. The compound obtained from solvent was tested for their activity against the test pathogens (*Staphylococcus aureus, Streptococcus faecalis, Proteus vulgaris, Salmonella typhi, Aspergillus niger* and *Candida albicans*) by well diffusion method.

One microlitre sample was injected into the GC column (100% dimethyl Polysiloxane) with the dimension of 30 m x 0.25 mm ID x1 µm df. The GC column used was a non-polar column. The carrier gas used was helium gas at the rate of 1 ml per minute. The split used in the injector was 10:1. The injector temperature maintained was at 250°C throughout the experiment. In the GC programme, the oven temperature was kept at 110°C for 2 min and the temperature was raised to 280°C at the rate of 5°C per minute. The holding time after reaching the temperature of 280°C was nine minutes. The total GC programme was worked out to be 45 min. The equipment used was Perkin Elmer-Clarus 500 GC.

The software used in the above GC MS programme was Turbo mass gold ver.5.1. The library used in analysing the spectrum of unknown components was NIST (National Institute of Standards and Technology, USA) of ver. 2.1. The EI source used was 70 eV. The mass numbers (m/z) monitored was 45-450. The total programme time in the MS was 36 minutes.

RESULTS AND DISCUSSION

Of the 178 colonies (belonged to 38 isolates were isolated, maximum number of

Table 1. Cultural characteristics of actinomycetes isolates

S. No	Name of the actinomycetes Sea shore isolates	Aerial myceliumcolour	Reverse side colour	Diffusible pigment	Colony size (nm)
1	*Streptomyces sp*	Dark ash	Light ash	-	3.0
2	*Saccharopolyspora sp*	Greenish	Light ash	-	2.0
3	*Nocardiopsis sp*	Ash	Light ash	-	3.5
4	*Streptomyces sp*	Greenish ash	Dull ash	-	6.0
5	*Nocardiopsis sp*	Light green	Yellow	-	3.5
6	*Streptomyces sp*	Milky white	Light yellow	-	2.0
7	*Streptomyces sp*	White	Light brown	-	3.0
8	*Saccharopolyspora sp*	Pure white	Light yellow	-	3.0
9	*Streptomyces sp*	Greenish ash	Black	-	3.5
10	*Actinopolyspora sp*	Dark ash	Dark Bluish	-	2.5
11	*Actinopolyspora sp*	Dull white	Ash	-	7.0
12	*Streptomyces sp*	Dark ash	Light Black	-	3.0
13	*Streptomyces sp*	Light white	Dull white	-	1.0
14	*Streptomyces sp*	Sandal white	Yellowish	-	6.5
15	*Streptomyces sp*	White	Brown yellow	-	1.0
16	*Streptomyces sp*	White	Light yellow	-	4.0
	Mangrove isolates				
17	*Streptomyces sp*	White	Light yellow	-	2.0
18	*Actinopolyspora sp*	Dull white	Yellow	-	5.0
19	*Streptomyces sp*	White	Dark ash	-	3.0
20	*Actinopolyspora sp*	Dull ash	Dark blue	Greenish	2.0
21	*Streptomyces sp*	White	Yellow	-	3.6
22	*Actinomadura sp*	Blue	Pink	-	2.5
23	*Streptomyces sp*	Light green	Dull whitish	-	5.2
24	*Streptomyces sp*	Dull white	Yellowish	-	3.4
25	*Streptomyces sp*	Pure white	Light yellow	-	3.0
26	*Streptomyces sp*	White	White	-	2.0
27	*Saccharopolyspora sp*	Ash	Light black	-	2.0
28	*Micromonospora sp*	Ash	Yellowish	-	6.0
29	*Streptomyces sp*	Bluish ash	Colourless	-	4.0
30	*Streptomyces sp*	Bluish white	Yellowish	-	6.0
31	*Saccharopolyspora*	Dark ash	Dull ash	-	2.5
32	*Streptomyces sp*	Dark ash	Dark ash	-	3.0
33	*Streptomyces sp*	Pure white	Dark yellow	-	9.0
34	*Actinomadura*	Dark ash	Light ash	-	3.0
35	*Streptomyces sp*	White	Yellowish	-	5.5
36	*Saccharopolyspora sp*	Light ash	Green	-	5.0
37	*Actinomycets*	Light rose	Light orange	-	2.0
38	*Streptomyces sp*	Colour less	Light yellow	-	6.0

Table 2. Activity of three strains of actinomycetes against human bacterial and fungal pathogens

S. No	Strain No.	Activity against human pathogens inhibition zone in (mm)					
		Sa	Sfa	Pv	St	Ca	An
1	ST-5	22	21	18	20	20	14
2	ST-20	20	20	17	18	20	15
3	ST-22	18	19	20	20	18	14

Sa – *Salmonella typhi*; Sfa – *Streptococcus faecalis*; Pv – *Proteus vulgaris*;
St – *Staphylococcus aureus*; Ca – *Candida albicans*; An – *Aspergillus niger.*

actinomycetes isolates was high in mangrove sediment[8] followed by seashore[9]. The actiomycetes isolates of mangroves and seashore sediments are of halotolerant forms (Table 1).

Out of 38 isolates of actinomycetes, 24 isolates were identified as genus Streptomycetes (spore chain with coiling, spiral and looped), 6 as *Saccharopolyspora* (very long chains of conidia on the aerial mycelium), 2 as *Nocardiopsis* (aerial mycelium totally sporulated), 4 as *Actinomadura* (spore chains are straight and open hooked) and 1 as *Actinomyces*[9] (branching vegetative mycelium) Actinomycetes, especially *Streptomycetes*, have been reported from the marine sub habitats such as marine sediments[10,11]; marine soil[12] and also from almost all parts of the world. Thus they have worldwide distribution, which indicate their plasticity and adaptability to extremely varied environments.

38 strains were isolated from the littoral sediments of Mallipattinam coastal water. Out of these only 3 strains showed very promising antibiotic activity against bacteria and fungi. The antibiotic activity of the crude extracts of the

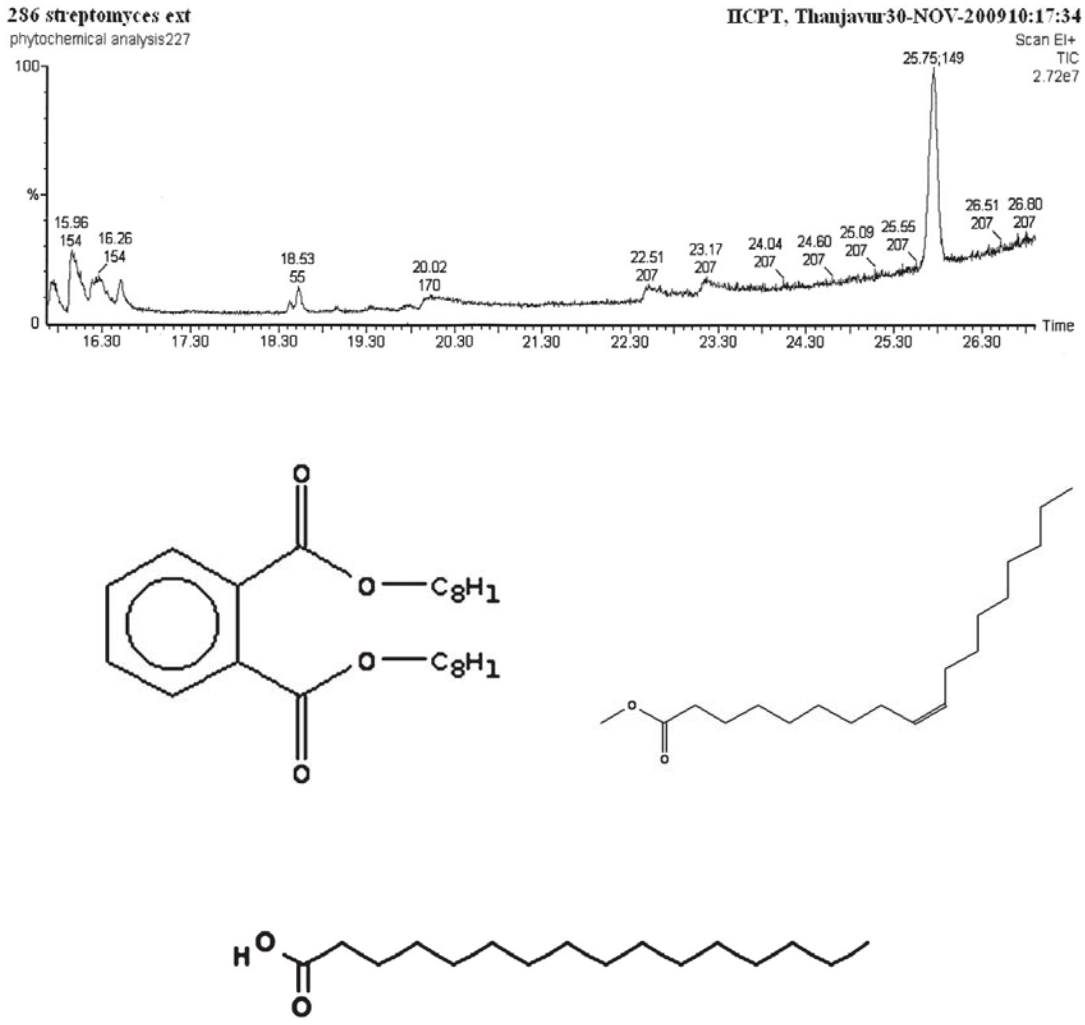

Fig. 1. GC-MS Graph

isolates is shown in Table2. The strains St-5, St-20 and St-22 exhibited high antibacterial activity against gram positive than the gram negative. All these strains showed marked activity against *Candida albicans* and *Aspergillus niger*. The selective antagonistic action against gram positive bacteria of marine actinomycetes might be the reason for the low population of gram positive bacteria in the sea. Eventhough all the 8 strains isolated from marine *Streptomycetes kanomyceticus* environment have been reported earlier in the terrestrial environment, their antibiotic spectrum showed marked difference. Therefore, the antibiotic production is not species specific[13]. Hence the specificity of antimicrobial action is a significant tool in identification and taxonomy of actinomycetes.

The results of the characterization of antagonistic Streptomycetes are shown in Table-2. All the isolates contain LL-2, 6 diaminopimelic acid and glycine in their cell wall. The chemical composition of the strain showed that they belong to the cell wall type-I. The strains were identified as *Streptomycetes xantholiticus* (strain No. ST-5), *Streptomycetes alboniger* (strain No. ST-20) and *Streptomycetes albidoflavus* (strain No. ST-22).

38 strains were isolated, out of them, only 3 (7.89%) showed good antibacterial activity against human pathogens (Table -1). The previous reports on marine antagonistic actinomycetes from various marine antagonistic actiomycetes from various marine sources exhibited prominent antagonistic activity (i.e., the inhibition zone is above 25mm). Isolated 91 strains of actinomycetes from Pitchavaram mangrove environment and 41.67% of them showed prominent antagonistic activity (But in the present study, only 8.67%) [14, 15] isolated 40 strains of actinomycetes from Vellar estuary and 12.5% of them showed prominent antagonistic activity. But in the present study, only 8.67% of the actinomycetes, isolated from the sediments of the Mallipattinam coast showed antagonistic actinomycetes may be due to the continuous fluctuations of physico-chemical parameters.

Four chemical compounds were identified in the GC-MS study with sample extract of *Streptomycetes* sp. Among these compounds, 9-Octadecenoic acids (z) - methyl ester and 1, 2-benzenedicarboxylic acid, diisoocyl ester are known antimicrobial compounds. The compound n-Hexadecanoic acid is a good antioxidant (Table 3; Fig . 1 & 2).

From the present study, the sediment samples harboured highest number of actinomycetes population. Hence, the sediment samples are meant to be the good source for enumeration of the actinomycetes population from marine environment

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