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In vitro Antimicrobial Activity of *Streptomyces* against Some Pathogenic Microorganisms

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To study the antimicrobial activity of streptomycetes isolated from marine soil sample in Tamil Nadu . The most potential isolate was identified based on its morphological, cultural, biochemical features and also confirmed by 16s rRNA partial gene sequencing. A total of 50 streptomycetes were subjected to primary screening by cross streak method against Gram-positive and Gram-negative bacteria and also by human and plant pathogenic fungi. Putative isolate was subjected to secondary screening by agar well diffusion method to further test the capabilities of primarily screened organisms. The antimicrobial substances were extracted with ethyl acetate from active strain by solvent extraction method. The active isolate showed more active against *Klebsiella pneumoniae* and *candida glabrata*. The most potential isolate was identified as *Streptomyces coelicolor Strain SU6* (JQ828940). The observations from this study suggest that *Streptomyces* isolated from marine environment may be potentially used for extracting novel antibiotics for treating both bacterial and fungal infections in human.

Key words: *Streptomyces,* marine soil, Antimicrobial activity.

Streptomyces are the largest antibiotic producing genus, producing both antibacterials and antifungals and also a wide range of other bioactive compounds such as immunosuppressant[1]. Antibiotics, because of their industrial importance, are the best known products of streptomycetes. The streptomycetes produce an enormous variety of bioactive molecules, e.g., antimicrobial compounds. One of the first antibiotics used is streptomycin produced by *Streptomycin griseus*. On the whole, the last 55 years have seen the discovery of more than 12,000 antibiotics. The streptomycetes yielded about 70 % of these, and the remaining 30 % are products of filamentous fungi and non-actinomycete bacteria. Around 11,900 antibiotics had been discovered by 1994 of which around 6600 (55%) were produced by *Streptomyces*, whereas filamentous fungi produced 2600 (22%) , bacteria produced 1400 (12%) and non-streptomyces strains of Actinomycetes produced 1300 (11%).[2]. Over recent years, it has become more and more useful to find novel bioactive compounds derived from streptomycete strains isolated from marine soil. Antibiotics continue to play a crucial role in the development of tissue culture techniques and basic screenings, primarily in biochemistry, molecular biology, microbiology and genetics. Streptomycetes are the most fruitful source for the production of bioactive secondary metabolites. The streptomycetes were originally considered an intermediate group between bacteria and fungi then were recognized as prokaryotic organisms. Antibiotics of streptomycetes origin evidence a wide variety of

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chemical structure, including amino glycosides, anthracyclines, β – lactams , nucleosides peptides, polyenes, actinomycins and tetracycline[3]. Generally antibiotics are non-growing associated secondary metabolites.

The present study is aimed at isolation and characterization of the antimicrobial activity of *Streptomyces* strains by using marine soil sediments.

MATERIALS AND METHODS

Collection and processing of soil sample

Marine soil samples were collected from muttukad region of Chennai in the depth of 5cm. The samples were air dried for 7 days to prevent bacterial and fungal contamination. The soil sample (1 g) was added to a conical flask containing 100 ml of sterile water and few drops of Tween 80. The flasks were shaken for 30 min in an orbital shaker incubator at 27 °C and their contents designated stock cultures[4,5]. A series of culture tubes containing 9 ml of sterile water was taken. From the stock culture, 1 ml suspension was transferred aseptically to the 1st tube (10-1) and mixed well. Further serial dilutions were made to produce 10^{-5} suspensions were made. Suspension (0.1 ml) from each culture tube was spread on sterile starch-casein agar medium plates aseptically in a laminar-air flow cabinet. The plates were incubated at 37°C for 7 days. The plates were observed intermittently during incubation. After 72 hrs, whitish pin-point colonies, characteristic of Streptomycetes and with a clear zone of inhibition around them were seen. The pinpoint colonies with inhibitory or clear zone of inhibition were selected and purified into ISP2 medium (International streptomyces project medium – 2 or Yeast extract - Malt extract agar medium) . The selected strains were further purified by multiple streaking method. The stock cultures of each selected strain was prepared and maintained in ISP - 2 agar slants at 4°C [4,6-7]. The streptomyces colonies isolated from the crowded plate were selected for the further studies.

Primary Screening of isolates

All the streptomycete strains were screened for their antibacterial and antifungal activity by cross streak method[8], Using modified nutrient agar against pathogenic bacteria, human and plant pathogenic fungi. In sterile modified nutrient agar , streptomyces were streaked on one side of plates and incubate for 7 days at 37°C. After 7 days, another side of the plates were streaked by bacterial and fungal cultures and kept for incubation. After incubation, results were noted based on the zone of inhibition.

Extraction of crude compound

ISP – 2 medium was used as a production media for the extraction of crude compound. The active strain was inoculated in ISP-2 broth and incubated for 7 days in rotary flask incubator at 28°C. It was centrifuged for 15mins at 8,000 rpm and the supernatant collected was mixed with an equal volume of ethyl acetate. The crude compound were extracted by using ethyl – acetate extraction method[9]. The extracted crude compound were dried to get dry powder by using heating mantle at 40°C.

Secondary screening of isolates

The crude powder were mixed with dimethylsulfoxide DMSO solvent for secondary screening. The MIC of antimicrobial activity was carried by using agar well diffusion method for both bacterial and fungal pathogens[10].

Identification of active strain

The potential strain was identified based on cultural characteristics[11-12] microscopic spore morphology, carbon utilization test[13], growth in different temperature and pH, cultural characteristics in different medium and strains identified by 16S rRNA partial gene sequencing.

RESULTS AND DISSCUSSION

Isolation of streptomycetes

Fifty isolates were collected based on their different morphological colour variations. The colonies were purified by repeated streaking. These strains were further selected and tested for antifungal and antibacterial activity. The study of[14] low altitude sagebrush found eleven out of 153 isolates tested showed broad spectrum antifungal activity.

Primary screening

The cross streak method of fifty isolates were tested against pathogenic bacteria and fungi. Out of fifty strains , five strains active against both bacteria and fungi. The antifungal activity of active isolates showed in Fig. 1.

Table 1. Secondary screening : MIC of crude compound for Anti bacterial activity

Organism	Zone of inhibition in (mm)			
	25(µl)	50 (µl)	75 (µl)	100 (µl)
Bacillus subtilis	13	14	15	20
Staphlococcus aureus	10	12	16	23
Escherichia coli	12	15	21	26
Klebsiella pneumoniae	11	16	21	28
Psedomonas aeruginosa	14	18	20	26

Table 2. Secondary screening : MIC of crude compound for Anti fungal activity

Organism	Zone of inhibition in (mm)			
	25(µl)	50 (µl)	75 (µl)	100 (µl)
Aspergillus niger	17	20	23	26
Aspergillus flavus	9	10	11	15
Candida albicans	11	13	16	18
Candida glabrata	25	27	28	31
Fusarium oxysporum	23	23	27	30

Secondary screening

Only one active strain was selected from the primary screening. The secondary screening were done by using ethyl acetate extraction method. The MIC of crude extract was used in agar well diffusion method. As per[15] *Streptomyces* Sp BT 624 showed inhibitory activity against *C. albicans*. The MIC of Zone of inhibition were showed in Table 1 and Table 2, Fig 2-3.

Table 3.

Morphological Characteristics	Active strain
Colour of aerial mycelium	Grey
Reverse side colour	Pale Yellow
Spore chain	Flexibilis
Spore surface	Smooth
Growth	good
Test for utilization of carbon sources	
Carbon sources	
Glucose	+
Sucrose	+/-
Lactose	+
Fructose	+
Maltose	+
Galactose	+
Mannitol	+/-
Starch	+

+ : presence of growth , - : no growth , +/- : partial growth

Table 4. Biochemical Tests

Chemicals	Active strain
Citrate	-
Indole	-
MR	-
VP	-
TSI	A/A, Gas -ve, H_2S -ve
Urease	-

+ : positive , - : Negative

Table 5. Growth in different Temperature and pH

Temperature	Active strain	pH	Active strain
10°C	-	3	-
20°C	-	5	+
30°C	+	7	+
40°C	+	9	+
50°C	-	11	-

+ = growth , - = No growth

Identification of active strain

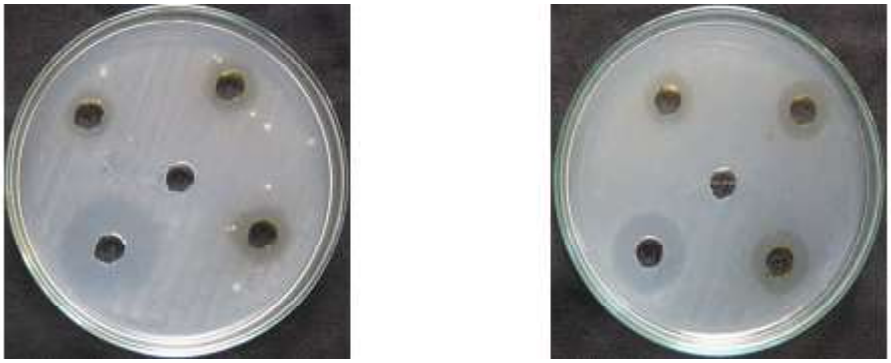
The active strain was identified by morphological characteristics, spore chain morphology by scanning electron microscopy, Fig. 4. The morphological study of streptomyces strain D332 were observed by the scanning electron microscope[16]. carbon utilization test, biochemical test , growth in different temperature and pH and 16S rRNA partial gene sequencing. Results were showed in (Table 3, Table 4 and Table 5).

16S rRNA partial gene sequencing

The active strain was identified as *Streptomyces coelicolor* Strain SU6 (JQ828940) by 16s rRNA partial gene sequencing.

Fig. 1. Primary screening : Antifungal activity of active strain against *Aspergillus niger*

Bacillus subtilis *Staphlococcus aureus* *E.coli*



Klebsiella pneumoniae *Psedomonas aeruginosa*

Fig. 2. Secondary screening : MIC of Antibacterial activity of active strain crude compound by agar well diffusion method

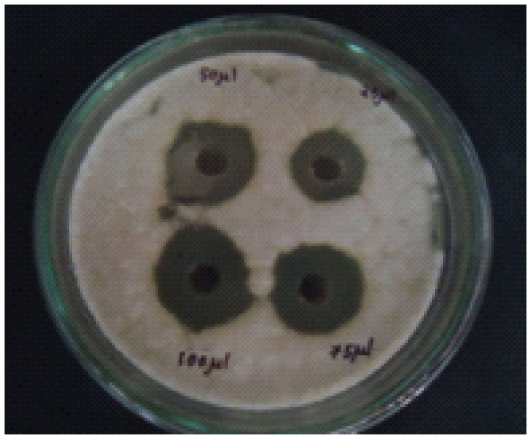


Fig. 3. Secondary Screening : MIC of antifungal activity of crude compound of active strain for *Aspergillus niger*

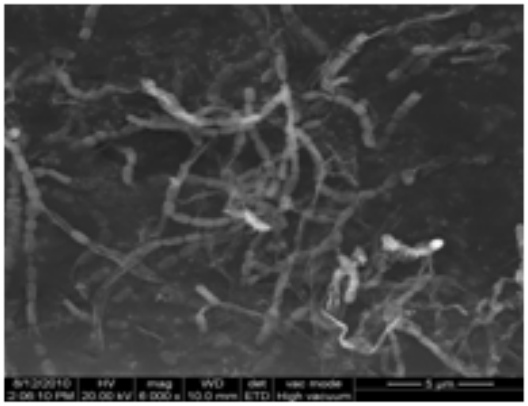


Fig. 4. Scanning Electron Microscope (SEM) photo for active strain

CONCLUSION

Findings from morphological, cultural and microscopical studies reveal that the isolated and investigated strain was designated as *Streptomyces*. Soil samples were collected from the marine region of chennai. A total of 50 isolates were collected, Of these 5 strains, were further selected and tested for antimicrobial activity. All the 5 strains, showed broad spectrum activity against all the six pathogenic fungi and bacteria . only one strain was showed more resistant to *Klebsiella pneumoniae* for antibacteria and *candida glabrata* for anti fungal. The most active strain identified as *Streptomyces coelicolor* Strain SU6 (JQ828940) based on morphological, biochemical features and 16S rRNA partial gene sequencing. The observations from this study suggest that *Streptomyces* isolated from marine environment may be potentially used for extracting novel antibiotics for treating both bacterial and fungal infections in human.

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