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Research Article

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Antimicrobial activity of bioactive compounds produced by *Streptomyces* species isolated from soil

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ABSTRACT

Streptomyces strains isolated from different agro-ecological regions of soils was taken up to evaluate their antimicrobial potentials against the target organisms S. aureus, E. coli, P aeruginosa. In primary screening out of 3 strains of Streptomyces isolated from ten different soil samples of various regions, one showed antagonistic activity against all the three targeted organisms which was secondary screened against S.aureus, Bacillus, P aeruginosa, E coli, Klebsiella sp. and it was effective against all except Klebsiella sp. The strain was found to be the yellow pigmented Streptomyces isolated from Ennore region possessed strong antagonist activity against human pathogens which was found to be efficient bioactive compound.

Keywords: *Streptomyces*, cross streak method, Bioactive compound, chromatogram, antagonistic activity,

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INTRODUCTION

Antibiotics are substances produced by micro organism that kill or inhibit other microorganism. Antibiotics are products of the earth, more specifically of soil; they are of products of cellular metabolism, and antibiotics are "all natural". The screening of microbial natural products continues to represent an important route to the discovery of novel chemicals, for development of new therapeutic agents. The screening of micro organism for the production of novel antibiotics has been intensively pursed for many years by scientists. Antibiotics have been in many fields including agriculture, veterinary and pharmaceuticals industry. It has been estimated that approximately two thirds of thousands of naturally occurring antibiotics have been isolated from *Actinomycetes*. *Streptomyces* have the capability to synthesize many different biological active secondary metabolites such as antibiotics, herbicides, pesticides, antiparasitic and enzymes like cellulose and xylanase used in waste treatment. Of these compounds antibiotics predominate in the therapeutic and commercial importance.

EXPERIMENTAL SECTION

Collection of sample – Soil samples were collected from different localities of agricultural fields from 5 -12 cm depth into sterile polypropylene bags. The soil samples were pretreated with calcium carbonate (10:1/w/w), incubated at 30°C for 4 days. This step reduced the load of non-spore forming bacteria and thus enriched the sample of desiccation resistant spore formers including *Streptomyces*, Bacilli and fungi [1].

Isolation of *streptomyces* species from soil sample
Streptomyces from soil suspensions were isolated by using *Actinomycetes* isolation agar plates. Isolated colonies of *Streptomyces* were purified by inoculating on fresh *Actinomycetes* isolation agar plates and incubated at 28°C for 7 – 8 days [2]. The microscopic characterization was done by cover slip culture method [3].

Antimicrobial activity of *streptomyces* species by cross streak method
in primary screening the activity of pure isolates were determined by cross streak method on nutrient agar [4]. The test organism used for this assay were *E. coli, S. aureus* and *P. aeruginosa.*

Production of secondary metabolite by *streptomyces* species
Shake flask cultivation was employed for production of secondary metabolite in which starch casein broth of 30 ml was used as seed medium in 250 ml Erlenmeyer flasks and inoculated aspetically with a loopful of spore mass. The flasks were incubated at 29±1°C in shaker incubator for 6 days. The Antimicrobial metabolite was recovered from the culture filtrate by solvent extraction method [5]. After six days of incubation the broth was centrifuged at 10,000 rpm for 10 mins. Ethyl acetate was added to the supernatant in the ratio of 1:1 (v/v) and shaken vigorously for 30 minutes for complete extraction. The lower aqueous layer was discarded. The upper solvent layer was retained and concentrated by keeping in water bath at 80-90°C till complete dryness was obtained.

Purification of crude extract by column chromatography
The purification of metabolite was carried out by column chromatography using silica gel as a column bed. Silica gel was then packed in the column by using ethanol: water (50:50) as solvent system. The crude extract was then loaded at the top of the column and eluted using ethanol: water (50:50) as solvent system. The fraction was collected after 15 minutes.

Thin layer chromatography
Thin layer Chromatography was done to determine the purity of the compound using Silica gel strip. Then ten microlitres of the ethyl acetate fractions was applied on the plate and the chromatogram was developed using chloroform: methanol (4:1) as solvent system. The spot in the chromatogram was visualized in the iodine vapour chamber and Rf value was determined.

Determination of antimicrobial activity of secondary metabolite produced by *streptomyces* species
Antimicrobial activity was determined by using the purified extract which was eluted in ethyl acetate by agar diffusion method using 3 hours broth culture which was compared with MacFarland standard 0.5 [6]. 100µl of the crude extract was loaded in the sterile disc which was placed over the lawn culture. The plates were incubated at 37°C for 18-24 hours and examined. The diameter of the zones of complete inhibition was measured.

RESULTS AND DISCUSSION

Out of ten samples collected a total of three strains of *Streptomyces* species were isolated from soil regions of Tamil Nadu. The strains were found to be a white pigmented and yellow pigmented *Streptomyces* grown on *Actinomycetes* isolation agar (Table 1). All the strains isolated are identified up to genus level based on macroscopic and microscopic analysis by cover slip culture of the strains when observed under microscope revealed smooth walled spores with long, branched filaments. Macroscopic and microscopic characters suggested that the strains belong to Genus *Streptomyces* (Table 2).

Table 1: The distribution of *Streptomyces* strains isolated from soil sample

Location	No. of sample	Number of isolates	Total
Ennore	3	1	1
Ambattur	3	0	0
Thirunindravur	2	1	1
Puzhal	2	1	1

Table 2: The macroscopic morphology of isolated strains

Location	Isolated strains	Morphology
Ennore	Strain I	Yellow pigmented, smooth circular colonies
Thirunindravur	Strain II	White, flat colonies
Puzhal	Strain III	White Pigmented, colonies

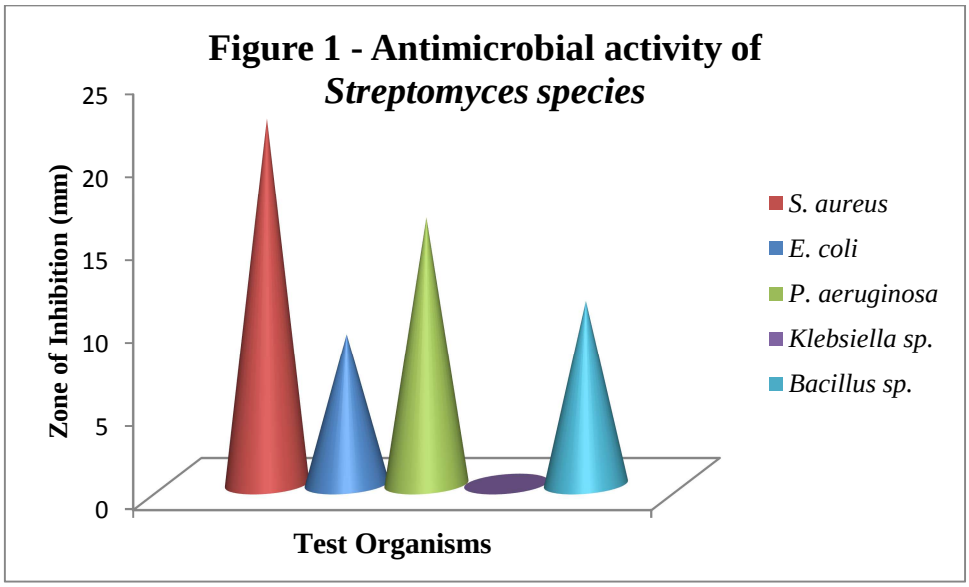
Antimicrobial activity by preliminary screening method
Out of 3 strains of *Streptomyces* species isolated, one strain showed strong antagonistic activity that is strain I (yellow pigmented) *Streptomyces* obtained from soil region of Ennore was effective against three test organism in primary screening by Cross streak method such as *S.aureus, E.coli, P. aeruginosa.*

Production and screening of secondary metabolite

Out of 3 strains, strain I was found to be very effective against pathogenic organism. The strain I was further subjected to production of secondary metabolite by inoculating in production media and by solvent extraction method. A crude compound was obtained which was further purified by column chromatography in which a single fraction was obtained. TLC was performed by using silica gel to check the purity and Rf value of the fraction produced by *Streptomyces* species. One spot was seen after subjecting the TLC plate to iodine vapors. The spot given by the fraction of *Streptomyces* species showed a RF value of 0.63 and Yellow color spot was observed. The antimicrobial activity of the fraction against the test organisms was determined by disc diffusion method and shown in Table 3 and Figure 1.

Table 3: Antimicrobial activity of fraction against target organism

Antimicrobial activity of fraction					
	1	2	3	4	5
Test Organisms	*S aureus*	*E coli*	*P aeruginosa*	*Klebsiella spp.*	*Bacillus spp.*
Zone (mm)	22	9	16	0	11



The search for novel metabolites would be more promising if diverse *Actinomycetes* were sampled and screened. High proportions of antimicrobial activities were produced by *Streptomyces* spp. isolated from soil and aquatic regions [7]. In the present study, 10 soil samples were collected from which 30% of the soil sample possesses *Actinomycetes.* The Genus *Streptomyces* was known as the one of the major source of producing bioactive natural product metabolites which has the antagonistic activity against target organisms. Microscopic and macroscopic morphology of the organisms was identified in the present study and characterized the isolate at Genus level as *Streptomyces* (spore chain with coiling and branching) which is in accordance with observation [3]. The author reported that the isolates producing antibiotics showed antimicrobial activity against gram positive and gram negative organisms. In the present study the antibiotic showed similar activity against selected gram positive and gram negative organisms. According to the results [8] the antibacterial activity, against gram negative bacteria were less frequent than gram positive bacteria. The result of primary and secondary screening reveals that the most active isolate from Ennore was active against gram positive than negative organisms. *S aureus* - 22mm, *Bacillus spp.*- 11mm, *P. aeruginosa*-16mm, *E. coli*-9mm, *Klebsiella spp.* -0mm which correlated the work [9,10]. For the complete characterization of antibiotic it should be isolated in a uniform and single component, which was done by Column chromatography in which during elution single fraction was obtained which reveals that one fraction is present in the crude extract. Further the fraction which was obtained from column chromatography was subjected to TLC to determine the Rf value and it was found to be 0.63 which was co-relating the work [1, 11]. The results of this study strongly support this; the isolated metabolite may be used in the management of microbial infection. In recent years the pathogenic organisms are giving resistance to existing antimicrobial agents hence the search for new, safe and more effective antimicrobial agent is required. Although the antimicrobial agent obtained in this study could not be declared as new antibiotic, there is a possibility of finding new antibiotics in Tamil Nadu because of its wide biodiversity. For proper identification of the antimicrobial extracts it is necessary to obtain further pure forms of antimicrobial metabolite which requires series of purification process and different chemical analysis said NMR spectroscopy, HPLC and other sophisticated techniques.

CONCLUSION

Screening the *Streptomyces* strains from the different agro-ecological soil regions was taken up to evaluate their antimicrobial potential against *S.aureus, Bacillus, P aeruginosa, E coli, Klebsiella spp.*. Out of three strains of *Streptomyces* isolated from ten different soil samples, one showed antagonistic activity against four test organisms (*S.aureus, Bacillus, P aeruginosa, E coli)* and *Klebsiella spp* was found to be resistant. Although the antimicrobial agents obtained in this study cannot confirmed as a new antibiotic, there is a possibility of finding new antibiotics because of its great demand in the field of agriculture and other industries and its great economic importance in the therapeutic, medicine and pharmaceutical industries.

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