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Growth inhibitory potential of polyene type metabolite producing *Streptomyces speibonae* ERI-01

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A Gram positive, filamentous, spore forming antagonistic *Streptomyces speibonae* ERI-01 derived from Western Ghats of Tamil Nadu, India, was studied for its antagonistic activities. Preliminary screening revealed that 38% of *Streptomyces* strains showed activity against *Bacillus subtilis* (34%), *Staphylococcus aureus* (32.4%), *Staphylococcus epidermidis* (27%), *Escherichia coli* (27%) *Enterococcus faecalis* (25%), *Pseudomonas aeruginosa* (22%) (*Klebsiella pneumonia*) and 18% (*Xanthomonas sp*), respectively. However, *Streptomyces* sp. ERI-01 exhibited significant antimicrobial activity against all the tested bacteria compared to other *Streptomyces* strains. The 16S rDNA gene based phylogenetic affiliation was determined by using bioinformatic tools identified *Streptomyces* sp. ERI-01 with 99% sequence similarity to *Streptomyces speibonae*. Fermentation conditions for antibacterial compound production were optimized. The antibacterial substances were extracted using chloroform, ethyl acetate and methanol from modified nutrient glucose medium (MNGA) in which ERI-01 had grown for 8 days at 28°C. Antimicrobial activity was assessed using the broth microdilution technique. The lowest minimum inhibitory concentration (MIC) of crude extracts and column purified fractions of ERI-01 against Gram positive and Gram negative bacterial pathogens were determined. The lowest MIC of the extract for *B. subtilis, S. aureus, S. epidermidis* and *P. aeruginosa* were 0.25 mg/ml. respectively. Extracts were evaluated for their typical polyene-like property.

Key words: *Streptomyces*, Antibacterial activity, minimum inhibitory concentration (MIC), polyene.

INTRODUCTION

Over the past 20 years there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents (Cragg et al., 1997). It has been said that researchers have so far discovered approximately over 10,000 biologically active compounds of microbial origin. Roughly, two-thirds of these are actinomycetes

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Abbreviations: MNGA, Modified nutrient glucose agar medium; **ISP,** International Streptomyces Project; **MIC,** minimum inhibitory concentrations; **MBC,** minimum bactericidal concentrations; **BLAST,** Basic Local Alignment Search Tool; **SCA,** starch casein agar.

products and more than a few have been utilized as important chemotherapeutic (Senda et al., 1996). According to many reports, bacterial resistances are spreading throughout the world. Fridkin et al. (2002) reported that the antimicrobial resistance increase in all health-care-associated pathogens. Natural products having novel structures have been observed to possess useful biological activities. Actinomycetes are distributed extensively in the soil and provide many important bioactive compounds of high commercial values including much of medical importance (Emmert and Handelsman, 1999). As an important group of actinomycetes, *Streptomyces* strains could form heat and desiccation-resistant spores and most of them are non-pathogenic to plants and animals; so *Streptomyces* strains isolated from soil have been regarded as a potential bio-control agents for controlling plant diseases (Urakawa et al., 1999). Actinomycetes can also be isolated from marine sediments, marine water, marine plants and animals. Studies on the microbial diversity by 16S rRNA gene analysis showed that a group of high-GC, Gram-positive bacteria (actionmycetes) are dominant in soil (Zheng et al., 2000). Actinomycetes also showed many interesting applications such as degradation of starch and casein and production of antimicrobial agents (Williams et al., 1983). Most *Streptomyces* is used in the production of a diverse array of antibiotics including aminoglycosides, macrolides, *β*-lactams, peptides, polyenes, polyether, tetracyclines, among others. In searching for new antibiotics, over 1,000 different bacteria, actimomycetes, fungi and algae have been investigated. Filamentous soil bacteria belonging to the genus *Streptomyces* are widely recognized as industrially important microorganisms because of their ability to produce many kinds of novel secondary metabolites including antibiotics (Watve et al., 2001). Streptomycetes are widely distributed in a variety of natural and man-made environments, constituting a significant component of the microbial population in most soils (Hwang et al., 1994). Indeed, different *Streptomyces* species produces about 75% of commercially and medically useful antibiotics (Miyadoh, 1993). The present study evaluates the cultural characteristics and antimicrobial activity of *Streptomyces* sp. (ERI-01) isolated from Western Ghats of Tamil Nadu. This study also involved in the extraction of antibacterial metabolites and its bio prospective study against various plant and animal pathogenic bacteria.

MATERIALS AND METHODS

Chemicals and enzymes

The genomic DNA isolation kit was purchased from Promega (Madison, WI, USA). The high fidelity *pfx* polymerase was purchased from Invitrogen. The mini-prep and DNA gel extraction kits were purchased from Qiagen (Mannheim, Germany). Glucose and all other chemicals were obtained from Himedia (India) and Sigma-Aldrich (St. Louis, MO, USA).

Soil sample collections

Soil samples were collected from the Western Ghats region and transported aseptically to the laboratory. The samples were shade dried and stored in refrigerator for further study. Soil pH was measured in five times volume of distilled water equilibrated with soil for 1 h.

Isolation of actinomycetes

Isolation of actinomycetes was performed by a soil dilution plate technique using starch casein agar (g/l: starch 10, casein 0.3, KNO_3 2, NaCl 2, K_2HPO_4 2, $MgSO_4$-$7H_2O$ 0.05, $CaCO_3$ 0.02, $FeSO_4$-$7H_2O$ 0.01 and agar 18) (Ellaiah et al., 1996). For long storage, it was grown in ISP-2 broth for 7 days and glycerol was added to make the final concentration of 15% and stored at -20^0C (Maniatis et al., 1989).

Primary screening of antibiotic producing strains

Antimicrobial activity was evaluated on modified nutrient glucose agar medium (MNGA) g/l, 10 g of glucose, 5 g of peptone, 3 g of beef extract, 3 g of dry yeast, 3 g of NaCl and 3 g of $CaCO_3$ by the streak method against various microorganisms. The strains were inoculated in a straight line on plates of 25 mm diameter and incubated at 30°C for 4 days. Target microorganisms were seeded in crossed streaks to actinomycete culture. The antimicrobial activity was evaluated by measuring the distance of inhibition between target microorganisms and actinomycete colony margins. The target microorganisms included Gram-positive bacteria *Bacillus subtilis (*MTCC 441), *Staphylococcus aureus (*ATCC 25923), *Staphylococcus epidermidis (*MTCC 3615) and *Enterococcus faecalis* (ATCC 29212) and Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomona aeruginosa (*ATCC 27853), *Klebsiella pneumoniae (*ATCC 15380), *Xanthomonas* sp., *Erwinia* sp. (MTCC 2760) and *Salmonella typhi* (MTCC 733). The antimicrobial activity was observed after 24 h incubation at 37°C.

Characterization of *Streptomyces* sp. ERI-01

Purified isolates of actinomycetes were identified up to the genera level by comparing the morphology of spore bearing hyphae with the entire spore chain and the structure of the spore chain with the actinomycetes morphologies as described by Bergey (1989). *Streptomyces* colonies were characterized morphologically and physiologically by following the methods given in the International Streptomyces Project (ISP) (Shirling and Gottlieb, 1966). The micro-morphology of strain was observed by light microscopy after incubation at 30°C for 2 weeks. The pigmentation of aerial mycelium and the structure of sporophores (which are highly characteristic and useful in the classification of Streptomycetes) were observed by cultivating the strains on different ISP media (ISP-2, ISP-3, ISP-4 and ISP-5).

Standardization of antimicrobial production in shake flask cultures

Fermentations were carried out in MNG broth (composition mentioned above). A seed culture was prepared with the same medium

and used to inoculate 500 ml-Erlenmeyer flask containing 100 ml of medium. Different culture conditions like incubation temperatures (20, 25, 30, 37 and 45°C), pH (6.0, 6.5, 7.0, 7.5, 8.0 and 8.5) and incubation time in hours (24, 48, 72, 96, 120 and 144) were studied to standardize the antibiotic production. Antimicrobial activities were assayed against *S. epidermidis* by the agar diffusion method (wells technique). Each well of 5 mm in diameter was filled with 0.05 ml of supernatant. Zones of inhibition were measured incubated after 24 h.

Isolation of antimicrobial products and minimum inhibitory concentrations (MIC) determinations

The 8 day's old culture broth was centrifuged to remove the biomass. The cell-free supernatant was extracted with an equal volume of (1:1) organic solvent. Chloroform, ethyl acetate and methanol were used for extraction. Organic extract was concentrated to dryness and dissolved in dimethyl sulfoxide (DMSO) and water (1:9 ratios) for bio-assay. MIC and minimum bactericidal concentrations (MBC) of extracts were tested against different test organisms by broth micro dilution method (Valan et al., 2008). The extracts were chromatographed on a silica gel column, further eluted with hexane, ethyl acetate and methanol solvent system and fractions were studied against bacteria.

Amplification of 16s rDNA and sequencing

Genomic DNA of *Streptomyces* sp. ERI-01 was extracted with the Gen Elute Bacterial Genomic DNA kit (Sigma). 27F and 1492R primers were used to amplify 16S ribosomal DNA fragments by polymerase chain reaction (PCR) (Bio-Rad I cycler) (Chenbey et al., 2000; Turner et al., 1999). The conditions for thermal cycling were as follows: initial denaturation of the target DNA at 95°C for 10 min followed by 30 cycles of amplification, denaturation at 95°C for 30 sec, primer annealing at 58°C for 1 min and primer extension at 72°C for 2 min. At the end of the cycle, the reaction mixture was held at 72°C for 10 min and cooled to 4°C. Amplified DNA was visualized at 100 V and 400 mA for 25 min on agarose gel (1% (w/v) in TAE buffer 1x, 0.1 µl ethidium bromide solution). Concentration of DNA was determined by nanodrop spectrophotometer (Nanodrop™ 1000). The amplified PCR products were purified by QIAquick® PCR purification Kit (Qiagen Ltd., Crawley, UK). The obtained sequences were subjected to Basic Local Alignment Search Tool (BLAST) at http://www.ncbinlm-nih.gov/ search in NCBI database for a phylogenetic relationship. The evolutionary history was inferred using the Neighbor-Joining method. Evolutionary analyses were conducted in MEGA5 (Felsenstein, 1985).

Detection of polyenic antibiotics

Polyene production of culture extracts was examined by disc diffusion method using *Candida albicans* as the indicator (Young-Bin et al., 2007). The sterile agar medium dispensed in a 90 mm petri dish was composed of two separate layers. In the first layer, 15 ml of the YM medium (yeast extract 0.3%, malt extract 0.3%, peptone 0.5%, glucose 1.0%) formed a base layer in the petri dish. After solidification, a 0.1 ml suspension of 0.7 $OD_{600\ nm}$ *C. albicans* mixed with 15 ml of sterile YM medium at 40-50°C was poured immediately onto the base layer to constitute the upper layer. The crude extract (5 mg) obtained from hexane and ethyl acetate were dissolved in one mili liter of respective solvents and used for the assay. Only hexane and ethyl acetate solvent containing discs were used as the negative control, the methanol extract from the *Steptomyces noursei* strain was used as the positive standard. In order to facilitate the diffusion of polyene metabolite containing extracts into the medium, the plates were incubated for 2 days at 37°C each, and the inhibitory zone was measured and charted.

Determination of antibiotic sensitivity and resistance pattern

Antibiotic sensitivity and resistance of the isolated bacteria were assayed by disc diffusion method. Bacterial inoculum was prepared by growing cells in starch casein agar (SCA) medium for 48 h at 30°C. Petri plates were prepared with 25 ml of sterile SCA medium (Diffco). The test culture was swabbed on the top of the solidified media and allowed to dry for 10 min. Different antibiotics loaded discs were placed on the surface of the medium and left for 30 min at room temperature for the diffusion of the antibiotics. The plates were incubated for 48 h at 30°C. After incubation, the organisms were classified as sensitive or resistant to an antibiotic according to the diameter of inhibition zone given in the standard antibiotic disc chart

RESULTS AND DISCUSSION

Primary screening of antibiotic-producing strains

This study was conducted in Arukani, Western Ghats forest in south India, situated about 100 kilometers south-east of Kanya kumari district, Tamil Nadu, India. The temperature was about 28-32°C. Seventy three actinomycetes strains were isolated from the forest rock soil. Among the seventy three actinomycetes isolated from forest soil, ERI-01 showed activity against both Gram positive and Gram negative bacteria. Preliminary screening revealed that 38% of selected actinomycetes showed activity against *B. subtilis,* 34% against *S. aureus*, 32.4% against *S. epidermidis*, 27% against *E. coli*, 27% against *E. faecalis*, 25% against *P. aeruginosa*, 22% against *K. pneumoniae* and 18% against *Xanthomonas sp* growth (Figure 1). Among all the actinomycetes screened, ERI-01 showed best antibacterial activity against the tested bacteria. Therefore, the antagonistic isolate ERI-01 were used for further studies.

Culture characteristics of *Streptomyces* sp. ERI-01

Gram staining indicated that *Streptomyces* sp. ERI-01 was gram-positive filamentous bacteria. The morphological studies proved that the colonies were white, opaque, rough, leathery and hard to remove due to branching filaments that had grown in the media. *Streptomyces* sp. ERI-01 was non-motile, catalase positive and aerobic. *Streptomyces* sp. ERI-01 was able to produce extra-cellular enzymes like protease, amylase, catalase, cellulase and gelatinase. Other properties are shown in Table 1. Biochemical and morphological characterization indicated that the antibiotic-producing isolates belonged to *Streptomyces* species.

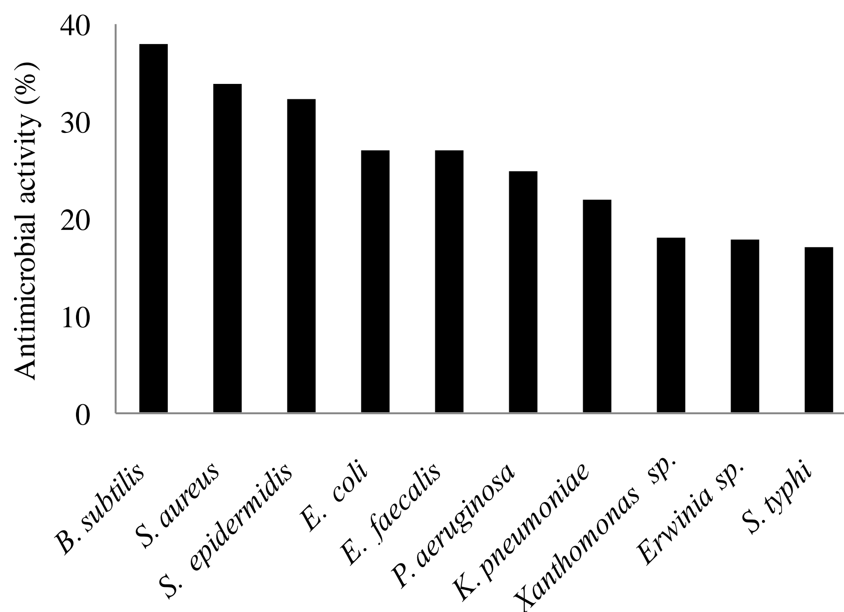

Figure1. Number of actinomycetes isolated from Western Ghats of Tamil Nadu and its percentage towards different gram positive and gram negative bacteria.

Streptomyces sp. ERI-01 showed a wide pattern of antibiotic sensitivity (Table 2). The analysis of cell wall hydrolysates showed the presence of LL-diaminopimelic acid and glycine without any characteristic sugar pattern. Taxonomic studies also indicated that it belongs to the *Streptomyces* genus. Based on these above characters, strain ERI-01 was tentatively identified as *Streptomyces*.

Phylogenetic studies and species identification *Streptomyces* sp. ERI-01

The complete 16S rRNA sequence of *Streptomyces* sp. ERI-01 was determined and deposited in GenBank under the accession number EU939448. *Streptomyces* sp. ERI-01 was classified to the species level based on sequence analysis of their16S rRNA. Clustering analysis obtained by the NJ method showed that the strain ERI-01 was taxonomically very close to *Streptomyces speibonae* (Figure 2).

Fermentation studies

S. speibonae ERI-01 exhibited antibacterial activity in MNGA liquid broth medium. Hence it was chosen as the production medium for the antibacterial compounds. A time course of growth and antibiotic production in MNGA broth is shown in Table 3. The antibacterial activity started at the beginning of the exponential phase of growth (2 days) and reached a maximum on the 8th day. The pH between 7.0 and 7.5 was good for antibacterial metabolite production (data not shown) and the optimum temperature for production was 30°C.

Minimum inhibitory concentration (MIC)

Preliminary screening revealed that MNGA medium was a very good base for the production of antibacterial compound(s). Crude ethyl acetate extract of ERI-01 showed MIC of 0.25 mg/ml for *B. subtilis, S. aureus, S. epidermidis* and *P. aeruginosa,* whereas methanol extract exhibited MIC of 0.125 mg/ml for *B. subtilis*. *S. typhi* and *Erwinia* sp. were inhibited at 1.0 mg/ml of methanol extract. Chloroform extracts revealed MIC of 0.75 mg/ml against *B. subtilis, P. aeruginosa* and *Xanthomonas* sp. respectively (Table 3). Among the five fractions collected from chloroform extract, fraction V showed MIC of 250 µg/ml against *S. aureus* and *B. subtilis*. MIC of *E. coli* was 500 µg/ml (Table 4). Fraction I, II, III and IV did not show activity against the tested bacteria (Table 5). Purification of ethyl acetate and methanol extracts led to the collection of seven and ten fractions, respectively; among that fractions, V and VI, of ethyl acetate showed activity against all the tested bacteria and fractions IV, V, VI, VII and VII of methanol extracts revealed significant antibacterial activity against both Gram positive and Gram negative pathogens.

Detection of poyene antibiotics in the crude extract

Each of the culture extracts was analyzed for polyene specific characteristics antibiotics by an antifungal bio-assay method using *C. albicans.* Polyene antibiotic nystatin-

Table 1. Biochemical and physiological characteristics of *Streptomyces* sp. ERI-01.

Character	***Streptomyces* sp. ERI-01**
Gram reaction	Positive
Production of diffusible pigment	-
Range of temperature for growth (^{0}C)	25 -45
Optimum temperature for growth (^{0}C)	30
Range of pH for growth	5.5- 7.5
Optimum pH for growth	7
NaCl tolerance (%)	0-5
Catalase production	+
Protease	+
Hydrolysis of	
Cellulase	-
Catalase	+
Amylase	+
Lipase	-
Gelatinase	+
H_2S production	+
Utilization of	
Arabinose	-
Fructose	+
Galactose	+
Glycerol	+
Maltose	+
Mannitol	+
Rhamnose	+
Soluble starch	+
Sucrose	-
Growth in presence of (g $^{-L}$)	
NaCl (20)	-
Sodium azide (0.04)	-
Sodium azide (0.02)	+

+, Positive (more than 90%); -, negative (more than 90%).

Table 2. Antimicrobial sensitivity pattern of *Streptomyces* sp. ERI-01 towards various antibiotics.

Antibiotic group	**Antimicrobial agent**	**Disc potency (µg)**	**Diameter of inhibition zone (mm)***
Aminoglycoside	Amikacin	30	26.83±1.26
	Gentamicin	10	15.42±0.52
	Kanamycin	30	17.17±0.76
	Streptomycin	10	0±0.0
	Tobramycin	10	21.59±0.85
Carboxypenicillin	Carbenicillin	50	25.17±0.76
β-lactamase inhibitor	Ampicillin	50	29.17±0.76
	Augmentin	30	27.87±1.86
	Imipenem	10	32.92±0.68
	Ticarcillin	75	37.33±3.06
Fluroquinolone	Ciprofloxacin	5	20.07±1.10

Table 2. Contd

	Gatifloxacin	5	17.33±1.04
	Levofloxacin	5	21.95±0.84
	Moxifloxacin	5	22.17±1.26
	Nalidixic acid	30	18.48±1.83
	Norfloxacin	10	24.19±0.74
	Ofloxacin	5	24±1.00
	Sparfloxacin	5	28.74±1.09
Cephalosporin	Cefpodoxime	10	0±0.0
	Cetriaxone	30	28.33±1.89
Polymixin	Colistin	10	0±0.0
Sulphonamide	Co-Trimoxazole	25	36.83±3.88

*Zone of inhibition was measured after incubating *Streptomyces* sp. ERI-1 at 30 °C for 48 h in starch casein agar medium (SCA).

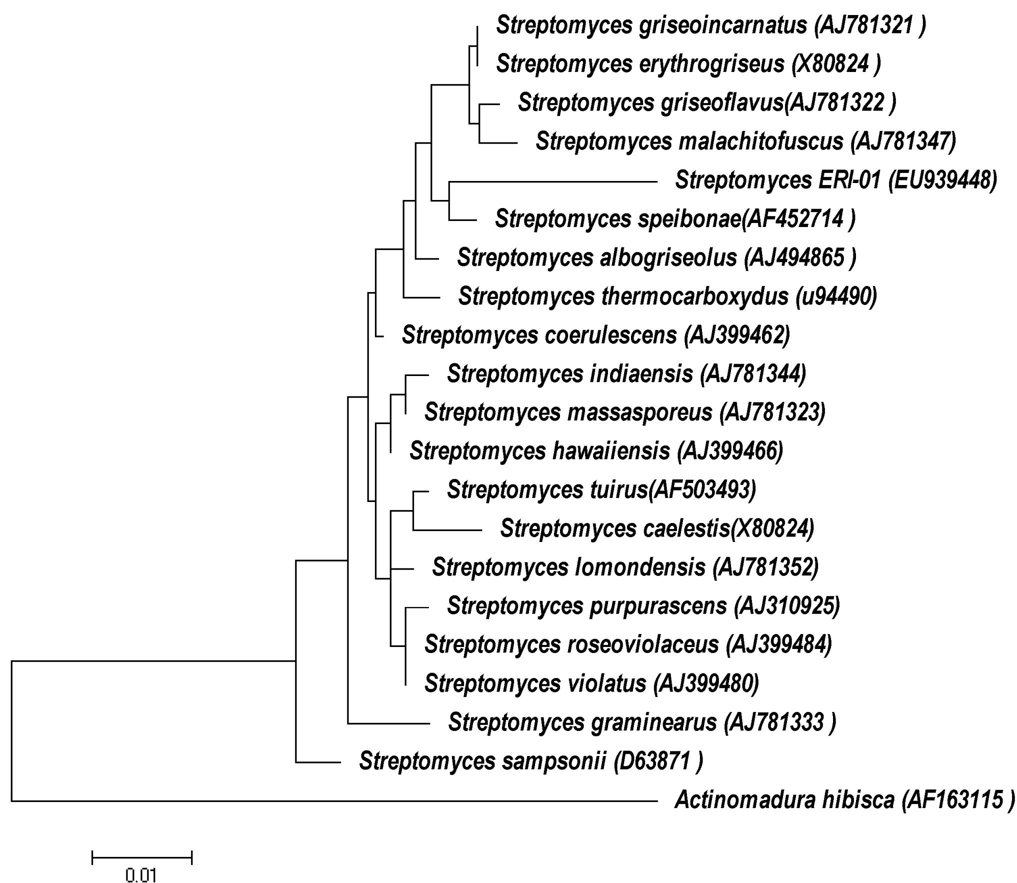

Figure 2. Phylogenetic tree based on 16S rDNA gene sequence showing the relationship between *Streptomyces speibonae* ERI-01 and species belonging to the genus *Streptomyces* was constructed using the neighbour-joining method.

Table 3. Effect of incubation time on production of antimicrobial metabolites by *streptomyces* sp. ERI-01.

Time (h)	Antimicrobial activity (mm)
0	0±0
24	0±0
48	11±0.045
72	14±0.12
96	15±0.51
120	16±0.45
144	18±0.25
168	20±0.43
192	20±0.16

Table 4. Minimum inhibitory concentration and minimum bactericidal concentration of organic solvent extracts of *streptomyces* sp. ERI-01.

Microorganism	MIC[a] mg/ml				MBC[a] mg/ml			
	C	EA	M	W	C	EA	M	W
Gram positive								
B. subtilis	0.750	0.250	0.125	0.250	1.500	0.500	0.250	0.500
S. aureus	1.500	0.250	0.250	0.250	3.000	0.500	0.500	0.500
S. epidermidis	0.500	0.250	0.250	0.250	3.000	0.500	0.500	0.500
Gram negative								
E. coli	1.500	0.500	0.250	0.500	3.000	1.000	0.500	1.000
E. faecalis	1.500	0.500	0.500	0.500	3.000	1.000	1.000	1.000
P. aeruginosa	0.750	0.250	0.500	0.250	1.500	0.500	1.000	0.500
K. pneumoniae	0.500	0.500	0.750	0.250	1.500	1.000	1.500	0.500
Xanthomonas sp.	0.750	0.750	0.750	0.750	1.500	1.500	1.500	1.500
Erwinia sp.	1.500	1.000	1.000	1.000	3.000	2.000	2.000	2.000
S. typhi	1.500	1.000	1.000	1.000	3.000	2.000	2.000	2.000

[a], Minimum inhibitory concentration; b, minimum bactericidal concentration; C, chloroform extract; EA, ethyl acetate extract; M, methanol extract; W, lyophilized water extract.

Table 5. Minimum inhibitory concentration of different fractions of *streptomyces* sp. ERI-01.

Crude extract	Fraction No	MIC[a] (µg/ml)									
		BS	SA	SE	EC	EF	PA	KP	X	E	ST
C[b]	I	-	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-	-	-
	IV	-	-	-	-	-	-	-	-	-	-
	V	250	250	125	250	500	500	250	750	750	750
EA[c]	I	-	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-	-	-
	IV	-	-	-	-	-	-	-	-	-	-
	V	50	75	75	-	-	-	-	-	-	-

Table 5. Contd

	VI	100	100	125	250	250	125	75	75	75	100
	VII	-	-	-	-	-	-	-	-	-	-
M[d]	I	-	-	-	-	-	-	-	-	-	-
	II	-	-	-	-	-	-	-	-	-	-
	III	-	-	-	-	-	-	-	-	-	-
	IV	125	-	-	-	-	-	-	-	-	-
	V	-	125	62.5	-	-	-	-	-	-	-
	VI	62.5	-	-	-	-	-	-	-	-	-
	VII	125	31.25	31.25	125	125	62.5	62.5	125	250	250
	VIII	-	125	125	250	250	-	-	-	-	-
	IX	-	-	-	-	-	-	-	-	-	-
	X	-	-	-	-	-	-	-	-	-	-

[a], Minimum inhibitory concentration;[b], chlorofom crude extract;. [c], ethyl acetate crude extract; [d], methanol crude extract; -, no activity; BS, *B. subtilis* ; SA, *S. aureus*; SE, *S. epidermidis*; EC, *E. coli*; EF, *E. faecalis*; PA, *P. aeruginosa*; KP, *K. pneumoniae*; X, *Xanthomonas* sp.; E, *Erwinia* sp.; ST, *S. typhi.*

Table 6. Detection of polyene type antibiotics production capabilities of *streptomyces* sp. ERI-01.

Extract	Polyene activity (mm)				
	1.0 (mg/ml)	0.50 (mg/ml)	0.25 (mg/ml)	0.125 (mg/ml)	0.0625 (mg/ml)
CE[a]	-	-	-	-	-
EA[b]	21.86±1.02	16.66±1.52	13.16±0.76	6.0±0.45	-
M[c]		11.26±1.16	-	-	-
Nystatin*	-	37.6±0.25	26.86±1.20	20.43±0.92	16.15±1.62

[a], Chlorofom crude extract; [b], ethyl acetate crude extract; [c], methanol crude extract; -,: no activity; *, standard polyene antibiotics. Zone of inhibition was measured after incubating the strains at 48 h at 37°C.

producing *S. noursei* used as a positive control in this study revealed inhibition zone of 37.6 mm against *C. albicans* (Table 6). Methanol extracts of the strains showed zone of inhibition at 0.50 mg/ml concentration. Ethyl acetate extracts revealed inhibition zone at concentration 1.0 and 0.50 mg/ml as well as 0.250 and 0.125 mg/ml, respectively. Chloroform extract did not exhibit activity, this indicated that the crude extract did not contain polyene antibiotic.

Actinomycetes strain ERI-01 isolated from Western Ghats of Tamil Nadu have been characterized and identified as *S. speibonae* ERI-01. Seventy three actinomycetes strains were isolated using the SCA as the base, 38% of them inhibited the growth of *B. subtilis.* Ellaiah et al. (1996) isolated 60 actinobacteria from the Bay of Bengal near Kakinada coast with distinct characteristics using SCA medium. Among them, 11 isolates exhibited antibacterial, 10 isolates showed antifungal while 2 isolates showed both antibacterial and antifungal activities. Most of the isolated actinomycetes showed activity against bacteria in the primary screening. Laksmanaperumalsamy et al. (1978) checked 518 *Streptomyces* strains for both antibacterial and antifungal activities. It was found that 27.03% of the strains showed one or more types of antibiotics; 59.27% were active against *B. circulans*, 47.01% against *S. aureus,* 30% against *E. coli,* 53.59% against *S. cerevisiae* and 39.3% against *F. oxysporum*. Majority of the isolates (46.43%) showed combined antibacterial and antifungal activity and 25% showed only antibacterial activity.

Balagurunathan et al. (1989) studied the antagonistic actinobacteria isolated from the littoral sediments of Parangipettai. Among 51 strains, only 11 strains showed good antibiotic activity and they were identified as *Streptomyces.* A broad spectrum antibiotic producing *S. speibonae* ERI-01 has been isolated and identified as *Streptomyces* species from the rocky soil of Western Ghats. By using a wide range of isolation media, culture characteristics combining physiological and biochemical characteristics, we identified the isolate which was found to produce pigment. Researchers claimed that in tropical rain forest, actinomycetes diversity is indeed very high (Wang et al., 1999).

Actinomycetes are useful biological tools producing

antimicrobials against bacteria and fungi. In the past two decades however, there has been a decline in the discovery of new lead compounds from common soil-derived actinomycetes. For this reason, the cultivation of actinomycetes taxa has become a major focus in the search for the next generation of pharmaceutical agents (Takizawa et al., 1993; Mincer et al., 2002). Our results indicate that the synthesis of antimicrobial metabolites depends on the medium constituents. In fact, it has been shown that the nature of carbon and nitrogen sources strongly affects antibiotic production in different organisms and the antibiotic production was increased by glucose rich medium (Holmalahti et al., 1998). Antimicrobial metabolite was extracted from the modified nutrient glucose medium. The production of antimicrobial metabolite by *S. speibonae* ERI-01 was increased using glucose in the fermentation medium. Actinomycete produces a wide variety of secondary metabolites with diverse biological activities, some of which have been developed for human medicine. It is urgent to explore new microbial habitats and discover novel compounds. Theoretically, novel actinomycetes possessing unique rRNA gene sequences may produce totally new biologically active compounds. Our present study identified new actinomycetes strains ERI-01 which can be useful for many applications such as control of infectious diseases in humans.

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