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Antimicrobial activity of some actinomycetes from Western Ghats of Tamil Nadu, India

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KEYWORDS

Streptomyces rimosus;
Antimicrobial activity;
Disc diffusion;
Zone of inhibition

Abstract *Introduction:* Microbial diseases are increasing year by year and they are becoming a big threat to public health. There are more than 200 known diseases transmitted by bacteria, fungi, viruses, prions, rickettsia and other microbes to humans. The emergence of drug resistance to chemical drugs is the biggest threat in controlling human pathogens. Hence novel antimicrobial agents from actinomycetes are timely needed for the control of several human pathogens.
Aim: The aim was to find some actinomycetes with antimicrobial metabolites.
Methods: Soil samples were collected from Nilgiris district in Western Ghats of Tamil Nadu, India. Actinomycetes were isolated using serial dilution and plating techniques on actinomycetes isolation agar. Streptomycin and ketoconazole (25 μg/disc) were used as reference controls. The active strains were identified by 16S *rRNA* and phylogenetic tree was constructed; the sequences were submitted in the GenBank.
Results: Totally 106 actinomycete strains were isolated and cross streaked against various human microbial pathogens. Only 44 (41.50%) exhibited good antimicrobial activity against different pathogenic microbes. Five isolates (FMS-20, TGH-30, TGH-31, TGH-31-1 and IS-4) were chosen for secondary screening using filtrate. Among them FMS-20 filtrate showed good inhibition on the 16th day against all tested microbial pathogens. Further the intracellular methanol extract of FMS-20 showed maximum zone of inhibition against *A. brasiliensis* (22 mm) at 5 mg/disc. Similarly the extracellular ethyl acetate extract of FMS-20 showed maximum zone of inhibition against *B. subtilis* (25 mm).

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Conclusions: The present work revealed that, among 106 actinomycetes screened, *Streptomyces rimosus* (FMS-20) (Accession No-KT827106) showed promising antimicrobial activity against all the tested human microbial pathogens.

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1. Introduction

Microbial diseases are increasing year by year and they are becoming a big threat to public health.[1–4] There are more than 200 known diseases transmitted by bacteria, fungi, viruses, prions, rickettsia and other microbes to humans.[5,6] Among the different microbial pathogens, viruses or prions cause 37–44% of diseases, bacteria or rickettsia cause 10–30% of diseases, protozoa cause 10.7% of diseases, fungi cause 6.3% of diseases and helminths cause 3.3% of diseases, leading to millions of death every year.[7–9] Many bacteria excrete through faeces, which can cause undesirable effects in health and environment.[10–13] The emergence of drug and multidrug-resistant pathogen is the biggest threat; consequently, novel antimicrobial agents from natural sources with novel mechanisms of action, are urgently needed in medical and pharmaceutical sectors.

Many research works have been carried out to control the pathogens and to identify new antimicrobial agents.[14–16] Microbes from soils are the most important natural sources exhibiting strong biological activity against a wide range of pathogens.[17] Generally microbes produce bioactive molecules which are unnecessary for their growth and development but useful in defence mechanism.[18] Soil microorganisms in particular are intensively exploited.[19]

Actinomycetes are Gram positive, filamentous bacteria with 55% of guanine and cytosine in their DNA.[20–23] Actinomycetes represent one of the most important classes of bacteria for their ability to produce a wide range of biologically active secondary metabolites, which are very effective against microbial pathogens.[20,23] More than 70–80% of all known antibiotics have been isolated from actinomycetes and are used in medicine and agriculture.[24] The genus *Streptomyces* is the biggest producer of antibiotics. Several microbial secondary metabolites are reported to be rich sources of therapeutic drugs.[25,26]

The present study aimed to evaluate some actinomycetes from different soils for antimicrobial activity against human pathogens.

2. Materials and methods

2.1. Isolation of actinomycetes from soil samples

Soil samples were collected from five different places in Western Ghats of Tamil Nadu India viz., Topslip greenhouse (TGH), Fishery mountain, (FMS), near dam mountain (NDM), Iduhatty (IS) and Kothagiri (KS). The soil samples were collected from 15 cm depth using sterile technique as per the method of Valan Arasu et al.[20] and transported to the laboratory. These soil samples were air-dried for 34 h at 45 °C, crushed, and sieved prior to use for isolation following established method.[27] The isolation of actinomycetes was done by standard serial dilution method. One gram of soil was suspended in 9 ml of sterile double-distilled water. The dilution was carried out up to 10^{-5} dilutions. Aliquots (0.1 ml) of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were spread on the actinomycetes isolation agar (AIA) medium containing nalidixic acid (100 mg/l) and ketoconazole (30 mg/l) and incubated at 30 °C for 7–10 days.[20,28–31] Based on the colony morphological characterization, the actinomycetes were selected and purified on ISP-2 (International Streptomyces project medium No. 2).

2.2. Morphological characterization of isolates

Morphological features of active isolates were observed with a light microscope.[18,20,30,32–34] Morphological features were observed in various media such as Actinomycetes isolation agar (AIA), Starch casein agar (SCA), Yeast peptone glucose agar (YPG), Bennet medium (BENNET), M3 medium (M3), Modified nutrient glucose agar medium (MNGA), ISP-International Streptomyces Project No. 2 (ISP-2), ISP-International Streptomyces Project No. 4 (ISP-4), ISP-International Streptomyces Project No. 6 (ISP-6), and ISP-International Streptomyces Project No. 7 (ISP-7). The results were recorded after incubation at 30 °C for 7–10 days.

2.3. Preliminary screening for antimicrobial activity

The antimicrobial activities of isolated actinomycetes were performed by cross streak method.[35] AIA plates were prepared and inoculated with isolates by single streak in the centre of petri plate and incubated at 30 °C for 10 days. The plates were then inoculated with the test organism by a single streak at 90° angles to the actinomycetes strain and incubated at 37 °C overnight. Then the antagonism of test organism was recorded. The human bacterial pathogenic organisms such as *Staphylococcus aureus* (MTCC-96), *Micrococcus luteus* (MTCC-106), *Enterococcus faecalis* (MTCC-439), *Bacillus subtilis* (MTCC-441), *Staphylococcus epidermidis* (MTCC-3615), *Klebsiella pneumonia* (MTCC-109), *Enterobacter aerogenes* (MTCC-111), *Vibrio parahaemolyticus* (MTCC-451), *Yersinia enterocolitica* (MTCC-840), *Saccharomyces cerevisiae* (MTCC-251), *Shigella flexneri* (MTCC-1457), *Proteus vulgaris* (MTCC-1771), *Pseudomonas mendocina* (MTCC-11808) and human pathogenic candidal strains like *Candida albicans* (MTCC-4748), *Candida krusei* (MTCC-9215), *Candida tropicalis* (MTCC-4370) and *Candida parapsilosis* (MTCC-1965) and human pathogenic fungal strains like *Trichophyton mentagrophytes* (MTCC-8476), *Scopulariopsis* sp. (MTCC-3553), *Aspergillus niger* (MTCC-10180), *Botyritis cinerea* (MTCC-2880), *Epidermo floccosum* (MTCC-613), *Aspergillus tubingenis* (MTCC-961) and *Aspergillus brasiliensis* (MTCC-1344) were used in the present study.

Table 1 Preliminary screening of active isolates of actinomycetes using cross streak method against different microbial pathogens.

Pathogens	Active strains				
	FMS-20	TGH-30	TGH-31	TGH-31-1	IS-4
S. aureus (MTCC-96)	+ + +	+ + +	+ + +	+	+ + +
M. luteus (MTCC-106)	+ + +	+ + +	+ + +	+ + +	+ + +
E. faecalis (MTCC-439)	+ + +	+ + +	+	+ + +	+ + +
B. subtilis (MTCC-449)	+ + +	+ + +	+ + +	+ + +	+ + +
S. epidermidis (MTCC-3615)	+ + +	+ + +	+ + +	+ + +	–
K. pneumonia (MTCC-109)	+ + +	+ + +	+ + +	+ + +	+ + +
E. aerogenes (MTCC-111)	+ + +	+ +	+ + +	–	+ +
V. parahaemolyticus (MTCC-451)	+ + +	+ + +	+ +	+ + +	+ + +
Y. enterocolitica (MTCC-840)	+ + +	+ + +	+ + +	+ + +	+ + +
S. cerevisiae (MTCC-251)	+ + +	+ + +	+ + +	+ + +	+ + +
S. flexneri (MTCC-1457)	+ + +	+ + +	+ +	+ + +	+
P. vulgaris (MTCC-1771)	+ + +	+ + +	+ + +	+ + +	+ + +
P. mendocina (MTCC-11808)	+ + +	+	+ + +	+ + +	+
C. albicans (MTCC-4748)	+ + +	+ + +	+ + +	+ + +	+ + +
C. krusei (MTCC-9215)	+ + +	+ + +	+ +	+ + +	+ + +
C. parapsilosis (MTCC-1965)	+ + +	+ + +	+ + +	+ + +	+ +
C. tropicalis (MTCC-4370)	+ + +	+ + +	+ + +	+ + +	+ + +
T. mentagrophytes (MTCC-8476)	+ + +	+	–	+ + +	+ + +
Scopulariopsis sp. (MTCC-3553)	+ + +	+ + +	+ + +	+ + +	+ +
A. niger (MTCC-10180)	+ + +	+ + +	+ + +	+ + +	+ + +
B. cinerea (MTCC-2880)	+ + +	+ + +	+ + +	+	+ + +
E. floccosum (MTCC-613)	+ + +	+	+ + +	+ + +	+ + +
A. tubingenis (MTCC-1344)	+ + +	+ + +	+	+ + +	–
A. brasiliensis (MTCC-961)	+ + +	+ + +	+ + +	+ + +	+ + +

+ + +: Good activity; + +: Moderate activity; +: Weak activity; –: No activity.

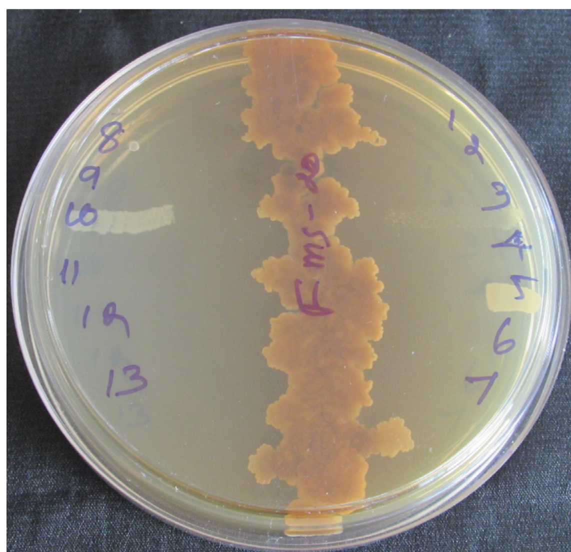

Figure 1 Preliminary screening of active isolates of *Streptomyces rimosus* (FMS-20) using cross streak method against different bacterial pathogens.

2.4. Medium optimization

The active isolates were grown in different media for the production of bioactive compounds in an orbital shaker (150 r/min) at 30 °C (M3 medium, MNGA, YPG, BENNET and ISP-2). After the growth the culture broth was centrifuged and the supernatant was used for secondary screening.

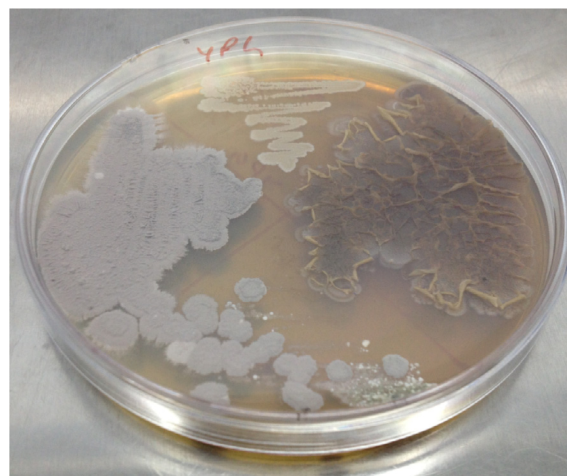

Figure 2 Morphological analysis of *Streptomyces rimosus* (FMS-20) in YPG medium.

2.5. Secondary screening for antimicrobial activity

The antimicrobial activity of selected strains was tested using filtrates against all the abovementioned human microbial pathogens using Nathans agar well diffusion method.[36]

Table 2 Morphological characterization of the active isolates of *Streptomyces rimosus* (FMS-20).

Medium	Characters					
	Aerial mycelium	Substrate mycelium	Soluble pigment	Colony margin	Growth	Gram stain
ISP-2	Brown	Yellow	–	Filaments	+ + +	+
ISP-4	Brown	White	–	Filaments	+ + +	+
ISP-6	Brown	Yellow	Brown	Filaments	+ + +	+
ISP-7	–	–	–	–	–	–
YPG	Brown	Golden yellow	–	Filaments	+ + +	+
MNGA	Brownish yellow	Yellow	–	Filaments	+ + +	+
M3	Slimy yellow	Yellow	–	Filaments	+ + +	+
AIA	Brown	Brown	–	Filaments	+ + +	+
SCA	Brown	Yellow	–	Filaments	+ + +	+
BENNET	Brown	Yellow	–	Filaments	+ + +	+

+ + +: Good growth; –: no soluble pigment, Gram stain; +: positive; –: negative.

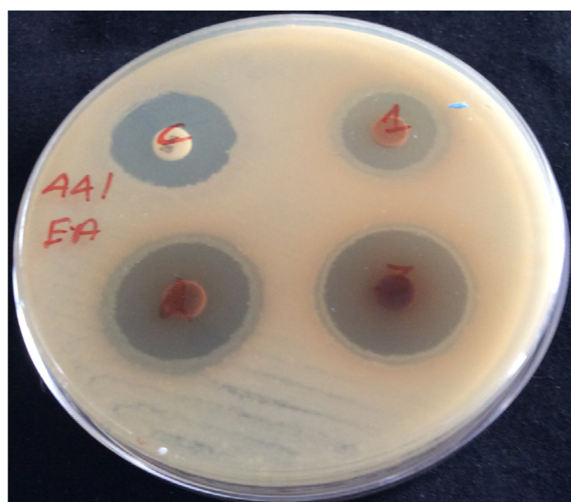

Figure 3 Antimicrobial activity of extracellular ethylacetate crude extract of *Streptomyces rimosus* (FMS-20) against *B. subtilis* (MTCC-441).

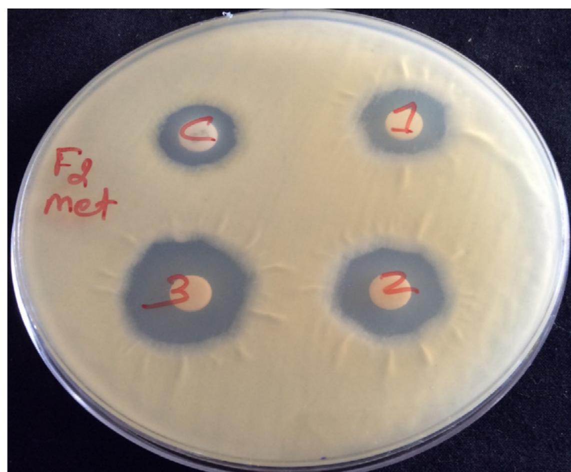

Figure 4 Antimicrobial activity of intracellular methanol crude extract of *Streptomyces rimosus* (FMS-20) against *T. mentagrophytes* (MTCC-8476).

Table 3 Biochemical characterization of the active isolates of *Streptomyces rimosus* (FMS-20).

Test	+ve/–ve
Indole	–
Voges-Proskauer test	–
Citrate test	–
Lysine test	–
Ornithine test	–
Arginine test	–
Nitrate test	+
Malonate test	–
Urease test	–
Phenylalanine-deamination test	–
H_2S production test	–
ONPG test	–
Glucose	+
Mannitol	+
Xylose	+
Inositol	+
Sorbitol	–
Rhamnose	–
Sucrose	–
Lactose	–
Arabinose	–
Adonitol	–
Raffinose	–
Salicin	–

+: Positive; –: negative.

2.6. Day optimization

For day optimization, the active strain was cultured in the selected (well grown) production media ISP-2 for up to 3–16 days following established method.[21,37]

2.7. Biochemical characterization

The active isolates were selected for biochemical studies. Biochemical characterization was done by using biochemical kit (KB014 HiAcinetobacter Identification Kit) according to the manufacturer's protocol. For biochemical tests urea hydroly-

Table 4 Antimicrobial activities (in mm) of extracellular ethyl acetate crude extract of *Streptomyces rimosus* (FMS-20).

Pathogens	*Streptomyces rimosus* (FMS-20)			
	Control	1.5 mg/disc	2.50 mg/disc	5.0 mg/disc
S. aureus (MTCC-96)	11	16	18	21
M. luteus (MTCC-106)	14	13	15	17
E. faecalis (MTCC-439)	10	11	13	17
B. subtilis (MTCC-449)	19	18	22	25
S. epidermidis (MTCC-3615)	0	10	12	14
K. pneumonia (MTCC-109)	11	14	16	18
E. aerogenes (MTCC-111)	10	12	14	16
V. parahaemolyticus (MTCC-451)	18	16	18	21
Y. enterocolitica (MTCC-840)	16	19	20	24
S. cerevisiae (MTCC-251)	18	11	12	14
S. flexneri (MTCC-1457)	10	11	12	13
P. vulgaris (MTCC-1771)	13	14	16	18
P. mendocina (MTCC-11808)	16	11	12	15
C. albicans (MTCC-4748)	13	15	17	19
C. krusei (MTCC-9215)	0	14	18	21
C. parapsilosis (MTCC-1965)	12	15	16	18
C. tropicalis (MTCC-4370)	14	10	17	20
T. mentagrophytes (MTCC-8476)	13	0	0	0
Scopulariopsis sp. (MTCC-3553)	20	0	0	0
A. niger (MTCC-10180)	14	0	0	0
B. cinerea (MTCC-2880)	23	0	0	0
E. floccosum (MTCC-613)	20	0	0	0
A. tubingenis (MTCC-1344)	25	0	0	0
A. brasiliensis (MTCC-961)	15	0	0	0

Table 5 Antimicrobial activities (in mm) of intracellular methanol crude extract of *Streptomyces rimosus* (FMS-20).

Pathogens	*Streptomyces rimosus* (FMS-20)			
	Control	1.5 mg/disc	2.50 mg/disc	5.0 mg/disc
S. aureus (MTCC-96)	12	0	0	0
M. luteus (MTCC-106)	14	0	0	0
E. faecalis (MTCC-439)	10	0	0	0
B. subtilis (MTCC-449)	20	0	0	0
S. epidermidis (MTCC-3615)	0	0	0	0
K. pneumonia (MTCC-109)	11	0	0	0
E. aerogenes (MTCC-111)	10	0	0	0
V. parahaemolyticus (MTCC-451)	20	0	0	0
Y. enterocolitica (MTCC-840)	16	0	0	0
S. cerevisiae (MTCC-251)	17	0	0	0
S. flexneri (MTCC-1457)	12	0	0	0
P. vulgaris (MTCC-1771)	18	0	0	0
P. mendocina (MTCC-11808)	15	0	0	0
C. albicans (MTCC-4748)	13	0	0	0
C. krusei (MTCC-9215)	0	0	0	0
C. parapsilosis (MTCC-1965)	12	0	0	0
C. tropicalis (MTCC-4370)	14	0	0	0
T. mentagrophytes (MTCC-8476)	12	16	17	19
Scopulariopsis sp. (MTCC-3553)	24	10	13	15
A. niger (MTCC-10180)	0	14	16	20
B. cinerea (MTCC-2880)	24	10	11	13
E. floccosum (MTCC-613)	24	10	13	14
A. tubingenis (MTCC-1344)	25	12	14	18
A. brasiliensis (MTCC-961)	18	17	19	22

sis, acid production indole, Voges-Proskauer, citrate, lysine, ornithine, arginine, nitrate, malonate, urease, Phenylalanine-deamination, H_2S production, ONPG, glucose, mannitol, xylose, inositol, sorbitol, rhamnose, sucrose, lactose, arabinose, adonitol and raffinose were carried out.

2.8. Crude extract preparation

2.8.1. Extra cellular

The total culture filtrate (20 l) of selected strain (FMS-20) was used for solvent extractions using ethyl acetate. The ratio 1:1

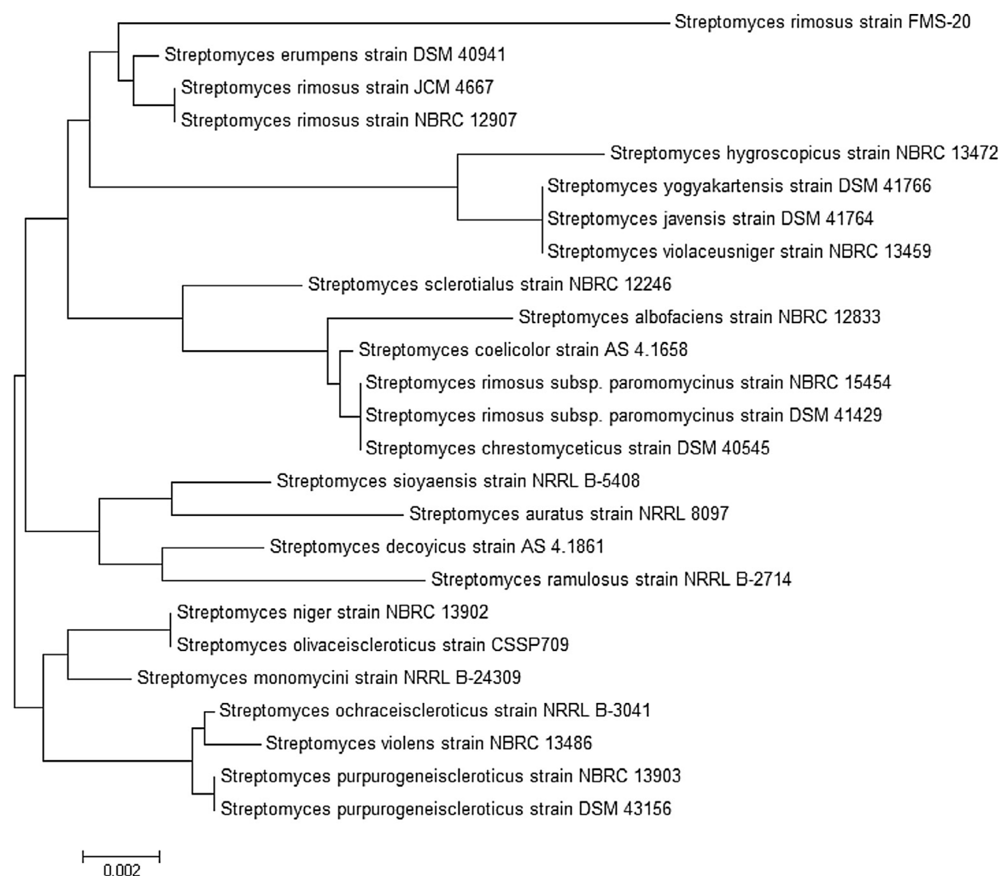

Figure 5 Phylogenetic tree indicating the taxonomic position of *Streptomyces rimosus* (FMS-20).

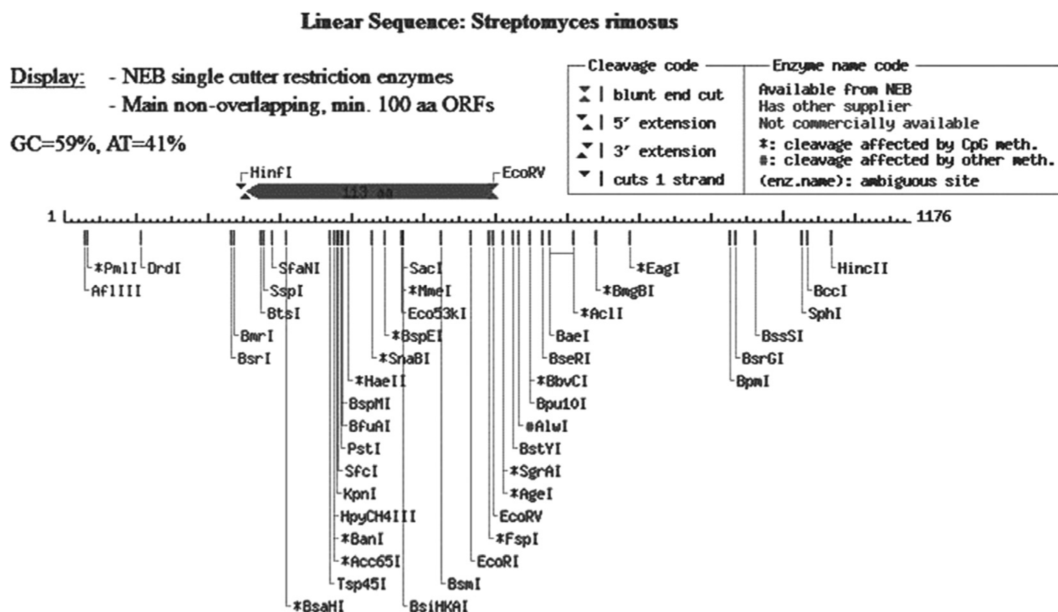

Figure 6 Restriction sites on the 16S *rRNA* sequence of *Streptomyces rimosus* (FMS-20).

(v/v) of the solvent and filtrate was shaken and mixed well. The upper layer was collected using separating funnel and solvents were evaporated using rotary evaporator.

2.8.2. Intra cellular

The mycelia of the active actinomycetes were soaked in methanol and kept in shaker for about two days at 180 r/min. Finally

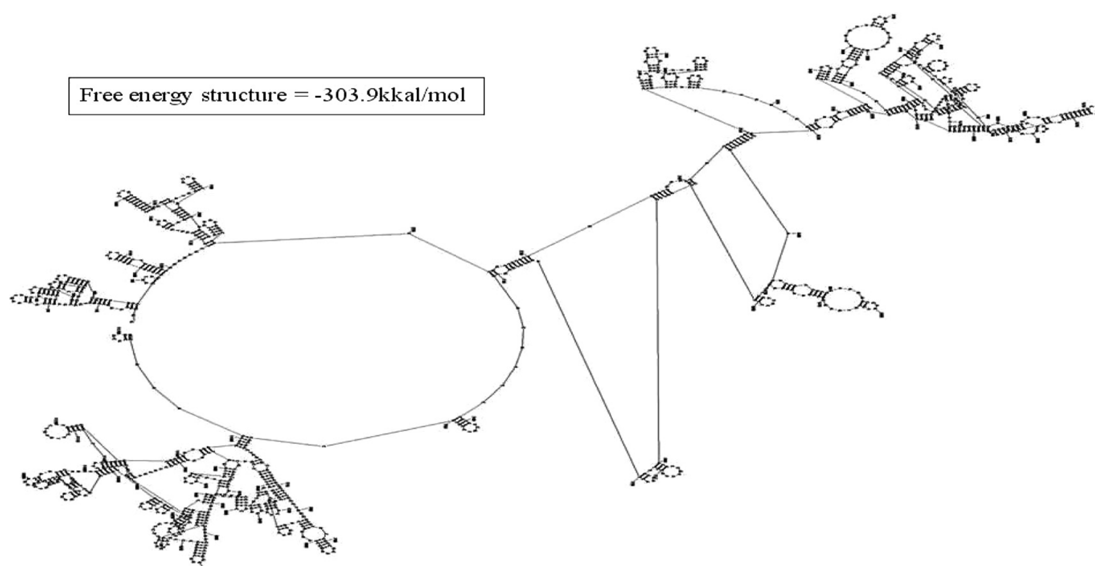

Figure 7 Secondary structure of 16S *rRNA* sequence of *Streptomyces rimosus* (FMS-20).

it was filtered with blotting paper and crude extract was separated using rotary evaporator.

2.9. Antimicrobial activity of crude extracts

Antimicrobial activity of crude extract was carried out using disc diffusion method.[38] Petri plates were prepared with 20 ml of sterile Muller Hinton agar (MHA, Himedia) for bacteria and potato dextrose agar (PDA Himedia) for fungal pathogens. The selected human pathogens were swabbed on top of the solidified media. The crude extracts were tested at 1.25 mg, 2.50 mg and 5.0 mg/disc concentrations. Control was maintained separately; streptomycin for bacteria and ketoconazole for fungi (25 μg/disc) were used as positive controls. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for diffusion. The plates were incubated overnight at 37 °C and zones of inhibition were recorded.[18]

2.10. Genomic DNA isolation

The genomic DNA of active strain (FMS-20) was isolated using the HipurA Streptomyces DNA purification kit-MB 527–50 pr (Himedia), according to the manufacturer's instructions. Briefly, the pure cultures were pelleted by centrifuging for 2 min at 12,000 r/min to obtain 10–15 mg (wet weight). The cells were resuspended thoroughly in 300 μl of lysis solution. 20 μl of RNase A solution was added, mixed and incubated for 2 min at room temperature. Then 20 μl of proteinase K solution (20 mg/ml) was added. The samples were mixed and resuspended. The cells were transferred to the Hibead Tube and incubated for 30 min at 55 °C. The mixture was vortexed for 5–7 min and incubated for 10 min at 95 °C followed by pulse vortexing. Supernatant was collected by centrifuging the tube at 10,000 r/min for 1 min at room temperature. About 200 μl of lysis solution was added, mixed thoroughly by vortexing and incubated at 55 °C for 10 min. To the lysate 200 μl of ethanol (96–100%) was added and mixed thoroughly by vortexing for 15 s. The lysate was transferred to new spin column and centrifuged at 10,000 r/min for 1 min and the supernatant was discarded. The lysate was then washed in 500 μl of prewash solution which was added to the spin column and centrifuged at 10,000 r/min for 1 min and the supernatant was discarded. The lysate was then washed in 500 μl of wash solution and centrifuged at 1000 r/min for 3 min. Two hundred microlitre of the elution buffer was pipetted out and added directly into the column without spilling, and incubated for one min at room temperature. Finally the DNA was eluted by centrifuging the column at 10,000 r/min for one min.

2.11. Analysis of 16S rRNA

The primers 27F (5′AGAGTTTGATCMTGGCTCAG3′) and 1492R (5′TACGGYTACCTTGTTACGACTT 3′) were used to amplify 16S ribosomal sequence from genomic DNA in thermal cycler (ep gradient Eppendorf). The cyclic conditions are as follows: initial denaturation at 94 °C for 3 min, 35 cycles of denaturation 94 °C for 1 min, annealing 54 °C for 1 min, extension 72 °C for 2 min, and final extension 72 °C for 7 min and finally hold at 4 °C. The PCR products were confirmed by 1% agarose gel electrophoresis.[18,20,39]

2.12. DNA sequence determination

Automated sequencing was carried out according to the dideoxy chain–termination method using applied Biosystems automated sequencer by Synergy Scientific Services.

2.13. Phylogenetic studies and species identification

The sequences were compared for similarity with the reference species of bacteria contained in genomic database, using the NCBI BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST). The DNA sequences were aligned and phylogenetic tree was constructed based on bootstrap test of phylogeny with neighbour-joining method using MEGA4 software. The 16S

rRNA sequence was submitted to the GenBank, NCBI, USA.[40]

2.14. 16S rRNA secondary structure and restriction sites analysis

The 16S *rRNA* secondary structure of the DNA sequence was predicted using RNA structure version 5.7 software (Mathews Lab, University of Rochester Medical Centre) and restriction site was identified using NEB cutter online tool version 2.0 (nc2.neb.com/nebcutter2/).[41,42]

3. Results

3.1. Isolation and selection of actinomycetes

The isolated strains were identified morphologically based on the colony, sporulation and pigment in the dilution plate. A total of 106 actinomycetes strains was selected and inoculated into the AIA medium for purification. The pure strains were named as FMS-20 to FMS-35, TGH-20 to TGH-35, NDM-20 to NDM-59, IS-1 to IS-8, and KS-1 to KS-26. These isolates were maintained in ISP2 medium.

3.2. Preliminary and secondary screening

All the 106 isolates were screened against human bacterial, fungal, and candida pathogens. Only 44 (41.50%) isolates showed good antimicrobial activity against pathogens. The percentage of activity to each pathogen is listed below: MTCC 96-19.8%, MTCC 106-20.7%, MTCC 439-17.9%, MTCC 441-10.3%, MTCC 3615-8.4%, MTCC MTCC 109-4.7%, MTCC 111-6.6%, MTCC 451-23.4%, MTCC 840-12.2%, MTCC 251-10.3%, MTCC 1457-22.6%, MTCC 1771-25.4%, MTCC 11808-10.3% MTCC 9215-15%, MTCC 4370-14.1%, MTCC 1965-14.1%, MTCC 4748-14.1%, MTCC 8476-14.1%, MTCC 3553-14.1%, MTCC 2030-14.1%, MTCC 2880-14.1%, MTCC 613-14.1%, MTCC 1344-14.1%, and MTCC 961-14.1%. Among them, 5 strains (TGH-30, TGH-31, TGH-31-1, FMS-20, and IS-4) showed very good activity against different human pathogens. These cultures were chosen for secondary screening (Table 1 and Fig. 1). The results revealed that FMS-20 alone showed highest antimicrobial activity against all the pathogens.

3.3. Medium and day optimization

Among the five active strains (FMS-20, TGH-30, TGH-31, TGH-31-1, and IS-4), only FMS-20 which grew in ISP-2 medium inhibited highly all the human pathogens. The day optimization results showed that FMS-20 recorded highest zone of inhibition on 16th day.

3.4. Morphological characterization

The colony morphology of active strain (FMS-20) was noted with respect to colour, aerial and substrate mycelium, soluble pigment, colony margin, Gram staining, and growth of colony. The results are given in Table 2 and Fig. 2.

3.5. Biochemical characterization

The active strain (FMS-20) was selected for biochemical studies. The strain showed positive results for nitrate, glucose, mannitol, xylose, and inositol. The results are summarized in Table 3.

3.6. Antibacterial activity of crude extracts using disc diffusion method

Extra cellular ethyl acetate crude extract of active strain FMS-20 showed good activity against all the tested human pathogenic bacteria and candida. They did not show any activity against fungal pathogens (Table 4 and Fig. 3). Intracellular methanol extract of FMS-20 also showed good activity against human fungal pathogens and it did not show any activity against bacterial and candidal pathogens (Table 5 and Fig. 4).

3.7. PCR amplification of FMS-20 genomic DNA

The genomic DNA was isolated using the HipurA Streptomyces DNA purification kit-MB 527-50 pr (Himedia) and the isolated genomic DNA was confirmed in 1% agarose gel stained with ethidium bromide. DNA was observed under UV Transilluminator and it showed good yield of DNA. The PCR product was analysed in 1% agarose gel electrophoresis and the size (1500 bp) was confirmed, sequenced and submitted in the GenBank (KT827106).

3.8. Molecular identification

The isolated active strain FMS-20 (KT827106) showed 98% homology to *Streptomyces rimosus* strain NBRC 12907 16S ribosomal RNA gene partial sequence (NR_112332). The DNA sequence was aligned and phylogenetic tree was constructed by using MEGA4 software and the results revealed that FMS-20 indicated 98% similarity to *S. rimosus* (Fig. 5).

3.9. 16S rRNA secondary structure and restriction sites analysis

The restriction analysis of 16S *rRNA* sequence of FMS-20 indicated the presence of GCAT content to 59% & 41% respectively (Fig. 6). The energy of the predicted structure was −303.9 kkal/mol (Fig. 7).

4. Discussion

In the present work totally 106 actinomycete strains were isolated from five different soil samples. These strains were cross streaked against microbial pathogens. The preliminary and secondary screening results clearly showed that FMS-20 alone possessed very high antimicrobial activity against all the tested human pathogens. Oskay et al.[35] screened 50 actinomycetes isolated from Cyprus soil against several human pathogens. It was found that 34% of strains produced antibiotics. Valan Arasu et al.[43] isolated a strain of *Streptomyces* sp. from Western Ghats soil sample and reported that it was very effective against *S. aureus*, *S. epidermidis*, *Xanthomonas* sp. and *C. albicans* at 0.25 mg/ml concentration.

Further, in the present study, the intracellular methanol extract of FMS-20 showed maximum zone of inhibition of 22 mm against *A. brasiliensis* and lowest zone of inhibition of 14 mm against *E. floccosum* at 5 mg/disc. The extracellular ethylacetate extract of FMS-20 showed maximum zone of inhibition of 25 mm against *B. subtilis* and lowest zone of inhibition of 13 mm against *S. flexneri* at 5 mg/disc. These results were comparable with the previous report of Vijayakumar et al.[44] who reported that ethyl acetate extract of *Streptomyces* sp. was more active by producing highest zone of inhibition of 23 mm against *P. vulgaris* and lowest zone of inhibition of 15 mm against *S. aureus*. In another study Balachandran et al.[45] reported extra cellular ethyl acetate extract of *Methylobacterium* sp. with highest zone of inhibition of 13 mm against *K. pneumonia* and lowest zone of inhibition of 9 mm against *B. subtilis*. Saravanakumar et al.[18] reported that intracellular methanol extract of *Actinobacterium* showed maximum zone of inhibition of 21 mm against *P. vulgaris* and lowest zone inhibition of 15 mm against *B. subtilis*.

In several reports, ethyl acetate was mostly used as an extraction solvent to isolate the crude extracts from actinomycetes.[46,47] Earlier several studies reported that most of the antimicrobial secondary metabolites were from extracellular actinomycetes.[48–50] The active strain FMS-20 is taxonomically very close to *S. rimosus* (98% similarity). This was confirmed by blast and phylogenetic analysis. *S. rimosus* was Gram positive and filamentous. It showed brown colour in appearance. This strain is notably the most identified producer of oxytetracycline and other tetracycline class of antibiotics such as Polypeptides.[51–54] Earlier, some researchers studied the crude extracts of actinomycetes on animal model. In a study, Suganthi et al.[55] isolated *Streptomyces* sp. from marine environment and checked for toxicity changes in Wistar albino rats. The extract showed no haematological, biochemical and histopathological changes in long time treatment. Similarly, Taweel et al.[56] tested the crude extract of actinomycetes viz., *Streptomyces fradiae*, *Streptomyces clavus* and *Streptomyces roseoflavus* against Ehrlich Ascites Carcinoma (EAC) on animal model using swiss albino mice. The result showed 67%, 64.2% and 75% of anticancer activity against *S. fradiae*, *S. clavus* and *S. roseoflavus*, respectively.

5. Conclusion

In conclusion, the extra cellular ethyl acetate crude extract of *S. rimosus* showed good activity against all the tested human pathogenic bacteria and candida. Similarly, intracellular methanol extract of *S. rimosus* also showed good activity against human fungal pathogens. The present results suggest that the isolated actinomycete *S. rimosus* (FMS-20) could be used as antibacterial and antifungal agent against the tested microbial pathogens.

Recommendation

The active ethyl acetate crude extract of *S. rimosus* may possibly be taken up further to identify the active principle and the efficacy of such active compound may also be tested with animal model.

Conflict of interest

We declare that we have no conflict of interest.

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