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Antibacterial activity of some actinomycetes from Tamil Nadu, India

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PEER REVIEW

Peer reviewer

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Comments

In general, the authors have selected good fields and manuscript is well planned, organized and written followed suitable methodology. Isolation of antimicrobial compound producing actinomycetes is excellent. The results are interesting which attracts other researchers to carry out similar experiments. The manuscript is recommended for publication.

(Details on Page 942)

ABSTRACT

Objective: To isolate novel actinomycetes and to evaluate their antibacterial activity. **Methods:** Three soil samples were collected from Vengodu (village) in Kanchipuram district, Tamil Nadu, India. Actinomycetes were isolated using serial dilution and plating method on actinomycetes isolation agar. **Results:** Totally 35 isolates were obtained on the basis of colony characteristics on actinomycetes isolation agar. All the isolates were screened for antibacterial activity by cross streak method. Medium and optimization of day were done for the potent strains using Nathan's agar well diffusion method. Isolation of bioactive compounds from significant active isolates was done by using different media. The most active isolate VAS 10 was identified as *Actinobacterium* Loyola PBT VAS 10 (accession No. JF501398) using 16s rRNA sequence method. The hexane, ethyl acetate, dichloromethane and butanol extracts of VAS 10 were tested against bacteria. The maximum antibacterial activity was observed in dichloromethane and ethyl acetate; maximum zones of inhibition were observed against *Enterococcus durans*. The rRNA secondary structure and the restriction sites of *Actinobacterium* Loyola VAS 10 were predicted using Genebee and NEBCutter online tools respectively. **Conclusions:** The present study showed that among the isolated actinomycetes, *Actinobacterium* Loyola PBT VAS 10 (accession No. JF501398) showed good antibacterial activity against the tested bacteria.

KEYWORDS

VAS10, *Actinobacterium*, Antibacterial activity, Solvent extract

1. Introduction

Bioactive molecules are secondary metabolites which are non-essential for growth and reproduction but form presumably a defense mechanism to the producer microorganism to compete in nature. About 70% of all known drugs have been isolated from actinomycetes bacteria of which 75% and 60% are used in medicine and agriculture, respectively. Actinomycetes are one of the most attractive sources of new bioactive metabolites. Rare actinomycetes have been shown to be an important source of novel and useful antibiotics[1]. The actinomycetes represent the main source of secondary metabolites with anticellular activity. Organisms present in these environments are rich sources of bioactive[2]. One of the most important and well acknowledged groups of microorganisms in the soil is the actinobacteria[3,4]. Actinobacteria are Gram positive, filamentous organisms inhabiting the soil. They are wide spread in distribution[5,6]. They produce a vast array of secondary metabolites such as enzyme[3] immuno-modulators, antibiotics, antihelminthic and anticancer agents[3,7-9]. Among them *Streptomyces*, the well-known saprophytes of the soil are the major producer of antibiotics[10]. About 75-80% of the drugs available commercially are being derived from *Streptomyces*[5,6]. Reports reveal that the continuous screening of actinobacteria, especially *Streptomyces*, will lead to the discovery of 100 000 new compounds with diverse applications[11]. The aim of this study was to isolate some novel actinomycetes from different soils and evaluate their antibacterial activity.

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2. Materials and methods

2.1. Isolation of actinomycetes from soil samples

Soil samples such as ant mound soil (VAS), sugarcane rhizosphere soil (SRS), and termatorium soil (VT) were collected from Vengodu, Kanchipuram district, Tamil Nadu India. The samples were collected from 5-25 cm depth in sterile plastic bags and transported aseptically to the laboratory. The soil samples were air-dried for one week at room temperature. Isolation and enumeration of actinomycetes were performed by serial dilution and spread plate technique[12]. 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 (Himedia, Mumbai, India). To minimize the fungal and bacterial growth, actidione 20 mg/L and nalidixic acid 100 mg/L were added. The plates were incubated at 30 °C for 10 d. Based on the colony morphology, the actinomycetes cultures were selected and purified on ISP2 (International *Streptomyces* Project 2) medium.

2.2. Morphological characterization of isolates

The actinomycetes colonies were characterized morphologically by following methods given in the International Streptomycetes Project[13]. The micro-morphology of the strains was observed under the light microscope for Gram staining as described in Bergey's Manual. The growth, aerial mycelium color, substrate mycelium, reverse side pigmentation and spore morphology which are highly characteristic and useful in the classification of actinomycetes were observed by growing strains on different media at 30 °C for 7 d. ISP1, ISP2, ISP3, ISP4, ISP5, ISP6, ISP7, and AIA (International Streptomycetes Project) media were used for morphological analysis.

2.3. Antibacterial activity

The antibacterial activity of actinomycetes isolates was performed by using cross streak method[14]. AIA plates were prepared and inoculated with isolates by a single streak in the center of petriplate and incubated at 30 °C for 7 d. The plates were then inoculated with the test organisms by a single streak at 90° angles to the actinomycetes strains and incubated at 37 °C overnight. Antagonism was observed by the inhibition of test organism. The following test organisms were used: *Bacillus subtilis* (*B. subtilis*) (MTCC 441), *Vibrio parahaemolyticus* (*V. parahaemolyticus*) (MTCC 451), *Enterobacter aerogens* (*E. aerogens*) (MTCC 111), *Staphyloccous aureus* (*S. aureus*) (ATCC 25923), *Staphylococcus aureus* (*S. aureus*) (ATCC 29213 methiciline sensitive), MRSA clinical pathogens (15DR), *Escherichia coli* (*E. coli*) (MTCC 40), *Enterococcus durans* (*E. durans*) (MTCC 3031), and *Proteus vulgaris* (*P. vulgaris*) (MTCC 1771).

2.4. Medium and optimization of days

The five active isolates SRS2, SRS3, VAS9, VAS10, and VAS16 were grown on the following media for the production of bioactive compounds in an orbital shaker (150 r/min at lab temperature 33 °C: actinomycetes isolation medium, fermentation medium, modified nutrient glucose medium, bennet medium, M6 medium, antibiotics production medium. After the growth fermented broth was centrifuged and the supernatant was used as crude antibiotic extract. The antimicrobial activity was tested for fermented broth against microbes using Nathan's agar well diffusion method[15]. To study the influence of incubation periods the culture was maintained in the optimized production media (AIB and Bennett) for up to 15-16 d[16].

2.5. Crude extract preparation

2.5.1. Extra cellular

The total culture filtrate (3 liters) was used for serial solvent extractions using hexane, ethyl acetate, dichloromethane, and butanol. The ratio of filtrate and solvents 1:1 (v/v) was mixed and shaken vigorously. The organic layer was collected and the solvents were evaporated using fume hood.

2.5.2. Intra cellular

The mycelia were air dried and grinded well with pestle and mortar and solvents were added and kept in shaker for about one hour at 150 r/min. They were then filtered with blotting paper and the solvents were evaporated using fume hood.

2.6. Disc diffusion method

Antibacterial activity of crude extract was carried out using disc diffusion method[17]. Petri plates were prepared with 20 mL of sterile Muller Hinton agar (MHA, Himedia). The test microbes were swabbed on top of the solidified media. The tests were conducted 5 mg/disc concentrations of each crude extracts. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Streptomycin (10 mg/disc) was used as positive control. The plates were incubated over night at 37 °C and zone of inhibition was recorded.

2.7. Genomic DNA isolation

The freshly cultured cells 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. About 20 μL of the Proteinase K solution (20 mg/mL) was added to the sample; mixed and resuspended cells were transfered to 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 seconds. The lysate was transferred to new spin column and 500 μL of prewash solution was added to the spin column and centrifuged at 10 000 r/min for 1 min and supernatant was discarded. The lysate was then washed in 500 μL of wash solution and centrifuged at 10 000 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 1 min at room temperature. Finally the DNA was eluted by centrifuging the column at 10000 r/min for 1 min (Hipura *Streptomyces* DNA spin kit-MB 527-20 pr from Hi-media).

2.8. Preparation and analysis of 16S rRNA

The primer 27F (5′ AGT TTG ATC CTG GCT CAG 3′) and 1492R (5′ ACG GCT ACC TTG TTA CGA CTT 3′) were used to amplify 16S ribosomal sequence from genomic DNA in thermal cycler (ep gradient Eppendorf). The cyclic conditions were as follows: initial denaturation at 94 ℃ for 3 min, 35 cycles of 94 ℃ for 1 min, 54 ℃ for 1 min, 72 ℃ for 2 min, and final extension of 10 min, and at 10 min held at 4 ℃. The PCR products were confirmed by 1% agrose gel electrophoresis[18].

2.9. DNA sequence determination

Automated sequencing was carried out according to the dideoxy chain-termination method using Applied Biosystems automated sequencer by Synergy Scientific Services[19].

2.10. Database searching

The sequence was compared for similarity with the reference species of bacteria contained in genomic database banks, using the NCBI BLAST (Blast′n′) 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 neighbor-joining method using MEGA4 software. The 16S rRNA sequence was submitted to the GenBank, NCBI, USA.

2.11. 16S rRNA secondary structure and restriction sites analysis

The 16S rRNA secondary structure and the restriction sites on the DNA sequence were predicted using Genebee and NEBCutter version 2.0 online tools (http://tools.neb.com)[20,21].

3. Results

3.1. Isolation of actinomycetes

After 7-day incubation powdery, pigmented and smooth colony was observed in the dilution plate. Sixty-five actinomycetes were isolated and inoculated on AIA medium for purification. The pure active colonies were designated as VT 1-13, SRS 1-19, and VAS 1-17. These isolates were maintained on ISP2 medium.

3.2. Preliminary screening for antimicrobial activity

Among 65 isolates, 35 were screened against bacteria. After 24 h incubation almost all the cultures were active against bacteria. Among them only six isolates (SRS 2, 3, 6, VAS 9, 10 and 16) were taken for further studies based on their antibacterial activity. These six isolates widely inhibited most of the bacteria. Among them VAS 10 showed maximum activity (Table 1 and Figure 1).

3.3. Morphological characterization

The morphological characterization of five isolates was studied by streaking on AIA, ISP1, ISP2, ISP3, ISP4, ISP5, ISP6, and ISP7. The Gram staining showed Gram positive filamentous organism (Table 2).

3.4. Medium and day optimization

Among the six tested media for five isolates, actinomycetes isolation broth (AIB) showed maximum activity against the tested microbes and good inhibition was recorded for VAS 10. Three isolates SRS 02, VAS 10, VAS16 were grown in actinomycetes isolation broth and bennet medium; among them VAS 10 showed 38 mm zone of inhibition on the 3rd day itself. SRS 2 showed maximum 32 mm zone of inhibition on day 12 only. VAS 16 showed 19mm as the zone of inhibition on the 7th day. Among the isolates VAS 10 showed maximum zone of inhibition against tested microbes on different media (Table 3 and 4).

3.5. Mass production and extraction

The isolate VAS 10 exhibited good antibacterial activity. Hence it was chosen as unique source and was mass produced using actinomycetes isolation broth. Three litres of broth (extra cellular) was subjected to hexane, ethyl acetate, dichloromethane and butanol for extraction. For intracellular extraction hexane, dichloromethane, chloroform, ethyl acetate, acetone, butanol and methanol were used. The concentrated crude extract was used for further studies.

Table 1
Primary screening of active isolates of actinomycetes using streak method against bacteria.

Microbes	SRS 2	SRS 3	SRS 6	VAS 9	VAS 10	VAS 16
B. subtilis (MTCC 441)	+++	+++	+++	−	+++	+++
V. paraheamolyticus (MTCC 451)	+++	+++	+++	++	+++	+++
E. aerogens (MTTC 111)	+++	+++	+++	+++	+++	+++
S. aureus (ATCC 25923)	++	++	+	+++	+++	+++
S. aureus MSSA (ATCC 29213)	+	+	−	−	−	−
MRSA clinical pathogens (15DR)	+++	+++	+++	+++	+++	++
E. coli (MTCC 40)	+	+	+	−	+	++
E. durans (MTCC 3031)	+++	+++	+	−	+++	++
P. vulgaris (MTCC 1771)	+++	+++	+	++	+++	++

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

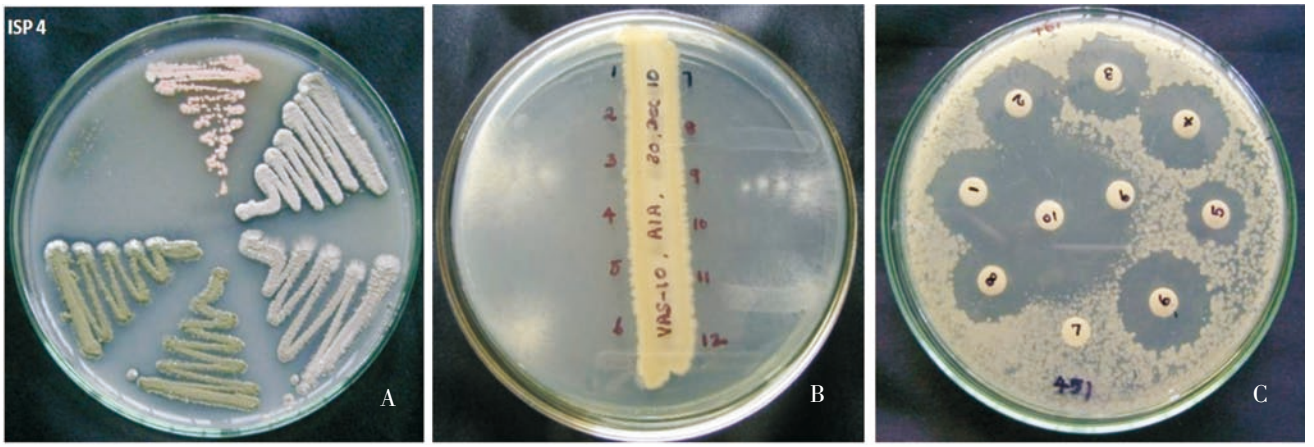

Figure 1. Characterization of the isolates.
A: Morphological analysis of actinomycetes on ISP–4 medium; B: Primary screening of *Actinobacterium* Loyola PBT VAS 10 against bacteria using cross streak method; C: Antibacterial activity of intra cellular different solvents extract of *Actinobacterium* Loyola PBT VAS 10 using disc diffusion method.

Table 2
Morphological characterization of the isolates.

Culture	Medium	Aerial mycelium	Substrate mycelium	Soluble pigment	Reverse side	Growth
SRS 2	AIA	Dark green	White	–	Whitish green	+++
SRS 3	AIA	Dark green	White	–	Whitish green	+++
VAS 9	AIA	Light grey	White	–	Light grey	+++
VAS 10	AIA	White	White	–	White	+++
VAS 16	AIA	Dark red	Creamy red	Red	Red	+++
SRS 2	ISP–1	Dark green	White	–	Creamy white	+++
SRS 3	ISP–1	Dark green	White	–	Creamy white	+++
VAS 9	ISP–1	Pink grey	Pale grey	–	Pale grey	+++
VAS 10	ISP–1	White	White	–	Creamy	+++
VAS 16	ISP–1	Light pink	White	–	Light red	+++
SRS 2	ISP–2	Dark green	Whitish grey	–	Creamy yellow	+++
SRS 3	ISP–2	Dark green	Whitish grey	–	Creamy yellow	+++
VAS 9	ISP–2	Grey	White	–	Whitish brown	+++
VAS 10	ISP–2	Whitish grey	White	–	Yellow	+++
VAS 16	ISP–2	Whitish red	White	–	Dark red	+++
SRS 2	ISP–3	Creamy white	White	–	Creamy yellow	+++
SRS 3	ISP–3	Creamy white	White	–	Creamy yellow	+++
VAS 9	ISP–3	Dark grey	White	–	Grey	+++
VAS 10	ISP–3	White	White	–	Creamy	+++
VAS 16	ISP–3	Whitish red	White	Orange	Orange	+++
SRS 2	ISP–4	Dark green	Greenish white	–	Creamy white	+++
SRS 3	ISP–4	Dark green	Greenish white	–	Creamy white	+++
VAS 9	ISP–4	Dark grey	Light grey	–	Pale grey	+++
VAS 10	ISP–4	Light grey	White	–	Creamy	+++
VAS 16	ISP–4	Whitish orange	White	–	Dark red	+++
SRS 2	ISP–5	Pale green	White	–	White	+++
SRS 3	ISP–5	Pale green	White	–	White	+++
VAS 9	ISP–5	White	White	–	Pale grey	+++
VAS 10	ISP–5	White	White	–	Creamy	+++
VAS 16	ISP–5	Creamy white	Creamy white	–	Light red	+++
SRS 2	ISP–6	White	White	–	Creamy white	+++
SRS 3	ISP–6	White	White	–	Creamy white	+++
VAS 9	ISP–6	–	Transparent yellow	–	Transparent white	+++
VAS 10	ISP–6	–	Creamy white	–	White	+++
VAS 16	ISP–6	–	–	–	Transparent yellow	+++
SRS 2	ISP–7	Dark green	White	–	Creamy white	+++
SRS 3	ISP–7	Dark green	White	–	Creamy white	+++
VAS 9	ISP–7	Pink grey	Pale grey	–	Pale grey	+++
VAS 10	ISP–7	White	White	–	Creamy	+++
VAS 16	ISP–7	Light pink	White	–	Light red	+++

+++: good growth; –: no soluble pigment.

Table 3
Media optimization for most active isolate *Actinobacterium* Loyola PBT VAS 10 for production of antimicrobial metabolites (mm).

Microbes	AIB	FEM	MNGB	BENN	M6	ANTI
B. subtilis (MTCC 441)	27	26	24	–	–	–
V. paraheamolyticus (MTCC 451)	25	22	–	–	22	12
E. aerogens (MTTC 111)	20	16	–	–	18	16
S. aureus (ATCC 25923)	27	24	–	–	23	–
S. aureus MSSA (ATCC 29213)	27	25	–	–	24	–
MRSA Clinical pathogens (15DR)	30	27	–	–	25	–
E. coli (MTCC 40)	16	17	–	–	–	–
E. durans (MTCC 3031)	28	–	–	–	–	–
P. vulgaris (MTCC 1771)	25	–	–	–	–	–

AIB: Actinomycetes isolation broth; FEM: Fermentation broth; MNGB: Modified nutrient glucose broth; M6: M6 Broth; ANTI: Antibiotic production broth.

Table 4
Optimization of antimicrobial metabolites production in different day duration for *Actinobacterium* Loyola PBT VAS 10 (mm).

Microbes	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15
B. subtilis (MTCC 441)	35	35	35	30	32	33	12
V. paraheamolyticus (MTCC 451)	20	20	18	18	18	17	13
E. aerogens (MTTC 111)	21	21	20	19	20	18	17
S. aureus (ATCC 25923)	27	27	26	22	25	24	20
S. aureus MSSA (ATCC 29213)	25	24	21	21	24	22	21
MRSA Clinical pathogens (15DR)	30	30	30	28	30	25	24
E. coli (MTCC 40)	27	27	27	22	24	24	22
E. durans (MTCC 3031)	38	35	33	36	38	38	30
P. vulgaris (MTCC 1771)	25	19	21	19	23	23	22

Table 5
Antimicrobial activity of intracellular of *Actinobacterium* Loyola PBT VAS 10 different solvent extracts (mm).

Microbes	H 5 mg/disc	DCM 5 mg/disc	CHL 5 mg/disc	EA 5 mg/disc	ACE 5 mg/disc	BUT 5 mg/disc	ME 5 mg/disc	Strep 10 mg/disc
B. subtilis (MTCC 441)	–	–	–	10	10	–	15	39
V. paraheamolyticus (MTCC 451)	–	16	22	24	22	22	19	40
E. aerogens (MTTC 111)	–	10	18	19	17	16	16	40
S. aureus (ATCC 25923)	–	14	22	24	22	21	21	29
S. aureus MSSA (ATCC 29213)	–	15	24	25	21	22	21	40
MRSA Clinical pathogens (15DR)	–	–	13	14	22	10	20	22
E. coli (MTCC 40)	–	–	16	17	16	14	10	25
E. durans (MTCC 3031)	–	24	32	33	30	30	30	40
P. vulgaris (MTCC 1771)	–	10	21	23	21	21	21	40

H: Hexane, DCM: Dichloro methane, CHL: Chloroform, EA: Ethyl acetate, ACE: Acetone, BUT: Butanol, ME: Methanol, Sterp: Streptomycin.

Table 6
Antibacterial activity of extracellular of *Actinobacterium* Loyola PBT VAS10 different solvent extracts (mm).

Microbes	H 5 mg/disc	EA 5 mg/disc	DCM 5 mg/disc	BUT 5 mg/disc
B. subtilis (MTCC 441)	–	12	13	–
V. paraheamolyticus (MTCC 451)	–	24	21	–
E. aerogens (MTTC 111)	10	20	18	–
S. aureus (ATCC 25923)	–	24	22	–
S. aureus MSSA(ATCC 29213)	10	25	22	–
MRSA Clinical pathogens (15DR)	13	17	25	–
E. coli (MTCC 40)	10	20	19	–
E. durans (MTCC 3031)	12	32	30	–
P. vulgaris (MTCC 1771)	10	24	22	–

H: Hexane; EA: Ethyl acetate; DCM: Dichloro methane; BUT: Butanol.

3.6. Antibacterial activity of crude extracts using disc method

Ethyl acetate and dichloromethane extracts of VAS 10 were found to be active against tested pathogenic bacteria when compared to positive control streptomycin (10 mg). The intracellular crude extracts such as acetone, benzene, chloroform, ethyl acetate and methanol showed good activity against tested microbes. The results are summarized in Table 5 and 6.

3.7. PCR amplification of genomic DNA from actinomycetes cultures targeting 16S rRNA

Using Hipura *Streptomyces* DNA spin kit-MB527-20pr from Himedia, the genomic DNA was isolated and electrophoresed on 1% agarose stained with EtBr. The genomic DNA was observed under UV transilluminator. The isolates showed good yield of DNA. The PCR reaction mix was analyzed by agarose gel electrophoresis and the DNA of the expected size was purified and sequenced. An amplicon of 1 400 bp was obtained on PCR amplification of the DNA with specific forward and reverse primers.

3.8. Phylogenetic studies and species identification

The partial 16S rRNA sequence of isolate VAS 10 (840 base pair) was determined. Using BLAST search in the NCBI data bank, sequence homologous to our isolate was collected. The isolate VAS 10 showed 95% homology to *Actinobacterium* C13 16S ribosomal RNA gene, partial sequence (HM209317) NCBI BLAST available at http://www.ncbi-nlm-nih.gov/. The DNA sequences were aligned and phylogenetic tree was constructed by using MEGA4 software (bootstrap method[22] (Figure 2).

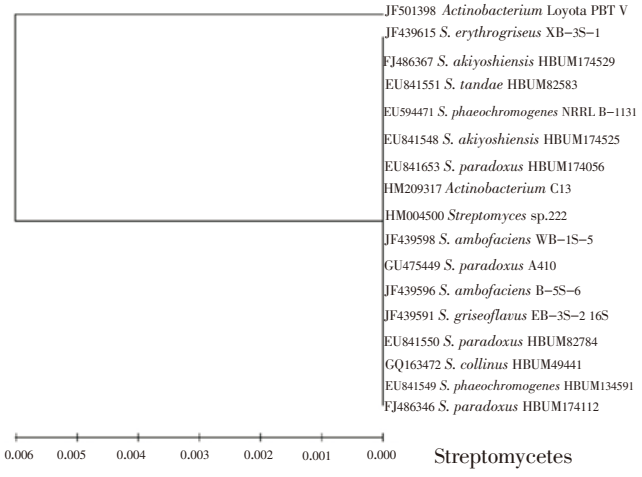

Figure 2. Phylogram indicating the taxonomic position of *Actinobacterium* Loyola PBT VAS 10.

3.9. 16S rRNA secondary structure and restriction sites analysis

The 16S rRNA secondary structure of *Actinobacterium* Loyola PBT VAS 10 was determined; it showed the free energy of the predicted structure to be -204.9 kkal/mol, threshold energy to be -4.0 by using Genebee (Figure 3). The restriction sites of *Actinobacterium* Loyola VAS 10 were predicted by using NEBCutter (version 2.0) online tools respectively. Also the restriction site analysis showed GC 58% and AT 42% content (Figure 4).

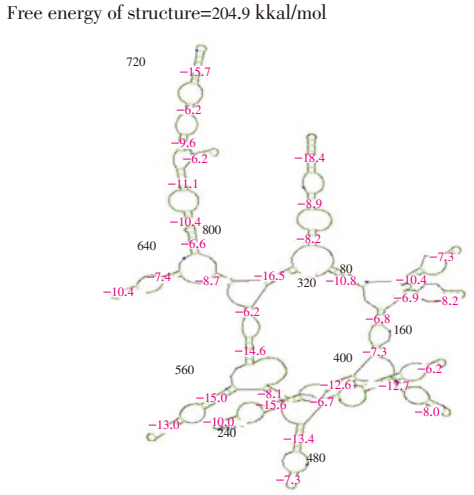

Figure 3. Secondary structure of 16S rRNA sequence of *Actinobacterium* Loyola PBT VAS 10.

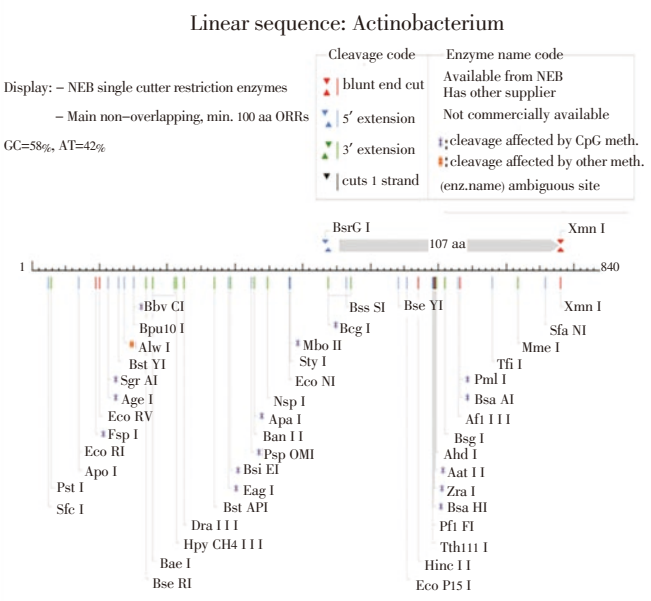

Figure 4. Restriction sites on the 16S rRNA sequence of *Actinobacterium* Loyola PBT VAS 10.

4. Discussion

In the present study 35 isolates were collected from the soil samples with the aid of actinomycetes isolation agar[12]. Out of 35 isolates, 18 isolates were found to show good antibacterial activity, 10 isolates showed moderate activity and 7 isolates showed least activity. Only five isolates (SRS 2, SRS 3, VAS 9, VAS 10, and VAS 16) were selected for further studies based on their high activity against the tested microbes. Duraipandiyan *et al.* reported that twelve actinomycetes strains were isolated from the soil samples of the Himalaya and were screened for their antimicrobial activity[23]. Thakur *et al.* reported that a total of 110 actinomycetes strains were isolated from the soil samples collected from the protected forest soil

from two states in Northeast India[24]. These were then characterized by conventional methods and assessed for their antagonistic activity against test microorganisms.

The morphology of the isolates SRS 2 and SRS 3 looked similar in all the ISP media, so they were considered as the same. SRS 2, VAS 9, VAS 10 isolates were standardized with actinomycetes isolation broth whereas VAS 16 was standardized with bennet medium. VAS 9 isolate was ruled out since it showed no activity. Similar results had been reported earlier by Valan Arasu *et al.* and Pickup *et al*[25,26].

Among the tested isolates only VAS 10 isolate showed good zone of inhibition to 38 mm on the 3rd and 13th day. The active isolate VAS 10 was identified as *Actinobacterium* Loyola PBT VAS 10 using 16S rRNA studies. In contrast to the traditional methods of actinomycetes identification, which are slow, difficult and very expensive, the molecular approach for identification is often used for their speed and efficiency[27]. Highest similarity (98%) was obtained with the 16S rRNA gene of *Streptomyces* roseoflavus which produced the aminoglycoside antibiotic flavomycin[28].

Intracellular ethyl acetate extract of *Actinobacterium* Loyola PBT VAS 10 showed maximum zone of inhibition of 33 mm against *E. durans* followed by acetone, butanol and methanol extracts which showed moderate activity of 30 mm against *E. durans*. Methanol and acetone extracts showed activity against methicillin resistant *S. aureus* MSSA (20 mm) at 5 mg/disc concentration.

Under extracellular activity ethyl acetate extract showed high activity of 32 mm against *E. durans* followed by dichloromethane extract against *E. durans*. A moderate activity of 24 mm was shown by ethyl acetate and dichloromethane extracts against *V. parahaemolyticus, S. auerus*, *P. Vulgaris* and 21 mm zone against *V. parahaemolyticus, S. aureus*, and MSSA. Hexane extract showed least activity of 10 mm zone against MSSA, *P. vulgaris, E. aerogens* whereas butanol extract showed no activity against all tested microbes.

Balagurunathan *et al.* studied the antagonistic actinobacteria isolated from the littoral sediments of Parangipettai[29]. Among 51 strains, only 11 strains showed good antibiotic activity and they were identified as *Streptomyces*. TLC was carried out for the extracts both intracellular and extracellular using different concentration of solvents for standardization. Gentamycin and streptomycin were used as control. To identify the number of bands present on TLC plates the plates were subjected to normal light, UV visible light and vanillin spray. Similar results were reported earlier by Valan Arasu *et al*[25]. Many researchers have reported related to antimicrobial activity of actinomycetes[30-43]. The present study showed that among the isolated actinomycetes, *Actinobacterium* Loyola PBT VAS 10 showed good antibacterial activity against the tested bacteria.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Number of antibiotics has been isolated from a variety of microorganisms; however, studies are still being conducted to identify novel antibiotics effective against pathogenic fungi and bacteria. Filamentous acitomycetes are known to have the ability to produce a wide variety of secondary metabolites. Interestingly, the majority of these antibiotic-producing actinomycetes are found among these *Streptomyces* which lead to a growing economic importance of this group of organisms. In the present study authors was investigated antibacterial activity of some actinomycetes from Tamil Nadu, India.

Research frontiers

The present studies are highly important for the researchers working in the field of microbial diversity and natural product isolation from microorganisms. It is also important for scientist working in pharmaceutical field. This manuscript gives a clear idea for the isolation of highly recommended microorganisms and it's a bioassay activity against plant and animal pathogens.

Related reports

The authors used and followed standard scientific research reports as their template for carrying out the research work with slight modifications, especially they followed few methodology from Valan *et al*., 2008. The results reported were significant and they compared with the recent reports.

Innovations and breakthroughs

Microorganisms play an important role in production of antibiotics. There are thousands of actinomycetes has to be identified from nature with better antimicrobial activities and other industrial applications. In regards to this the authors has chosen good microbe rich place for isolation of these antimicrobial compound producing actinomycetes, therefore rare actinomycetes may identified.

Applications

The present study is useful for the development of novel antimicrobial compounds or strains can be further used for enhancing their production ability by genetic engineering tools for higher production of therapeutic compounds.

Peer review

In general, the authors have selected good fields and manuscript is well planned, organized and written followed suitable methodology. Isolation of antimicrobial compound producing actinomycetes is excellent. The results are interesting which attracts other researchers to carry out similar experiments. The manuscript is recommended for publication.

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