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A N I N T E R N A T I O N A L J O U R N A L

Production of Antimicrobial Metabolites by *Streptomyces lavendulocolor* VHB-9 Isolated from Granite Mines

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ABSTRACT

The actinobacterial strain Streptomyces lavendulocolor VHB-9 *was isolated from granite mine soil samples of Khammam district, Telangana state, India. The strain was identified based on detailed microorphological, cultural and phylogenetic analysis. Bioactive guided isolation of the secondary metabolites of the strain was carried out by growing the strain in optimized medium (0.5% lactose, 0.5% peptone, 0.05% K_2HPO_4, 0.2% $CaCO_3$ with pH adjusted to 7.0). Separation and purification of the active fractions from the crude ethyl acetate extract was carried out by silica gel column chromatography and resulted in the isolation of two active fractions. Structural elucidation of the two (B2 and B3) active compounds was carried out by FT-IR, Mass and NMR spectroscopy and were identified as Bis (7-methyloctyl) phthalate and (Z)-3-aminoacrylic acid. The antimicrobial activity of the bioactive compounds produced by S. lavendulocolor VHB-9 was expressed in terms of minimum inhibitory concentration against opportunistic pathogenic bacteria and fungi. Both fractions exhibited good antimicrobial potential against the bacteria and fungi tested.*

Key words: *Granite, Streptomyces lavendulocolor,* spectroscopy, Bis (7-methyloctyl) phthalate, (Z)-3-aminoacrylic acid.

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INTRODUCTION

Natural products are the leading sources of novel biomolecules being used in the pharmaceutical and nutraceutical industries since their inception. Majority of the natural products currently used in the market as therapeutic agents or as health supplements are derived from the terrestrial organisms including plants, animals and microorganisms. Microbial secondary metabolites are the important sources of natural compounds with potential bioactivities being used in different fields such as medicine, agriculture and others. The search for novel natural products with useful pharmacological activities often includes the isolation of actinomycetes, such as *Streptomyces* species, from soil samples. [1-2]

A vast majority of these compounds are derived from the single actinomycete genus *Streptomyces*, raising the intriguing possibility that additional chemically prolific taxa await their discovery. They are broadly used in the human therapy, veterinary, agriculture, scientific research and in countless other areas. The diversified metabolic machinery of streptomycetes can generate an infinite variety of secondary metabolites which include dark-brown to black melanoid pigments that play important ecological roles in the immediate environment. [3-4] Some *Streptomyces* species in the rhizosphere protects plants against root pathogens [5-6], which could be important in the early stages of plant establishment.[7] In addition, Streptomycetes can facilitate mycorrhiza [8-9], which can lead to the establishment and survival of colonizing plants by aiding in nutrition. Inturn, the rhizosphere of plants influences the species composition and diversity of *Streptomyces* strains.

Given the importance of the *Streptomyces* as a source of pharmaceuticals, exploration of the natural environment with the aim of discovering novel species in this genus is important. In addition, characterization of the physiological and genotypic features of members of this genus will broaden our understanding of the behaviour of these organisms in various ecosystems. Recent progress in genome sequencing methods has led to the discovery that *Streptomyces* spp. have the potential to produce a diverse array of secondary metabolites. Furthermore, genomic data have given rise to new taxonomic parameters that can be used for species classification, such as the average nucleotide identity (ANI) of common genes and the percentage of conserved DNA. Comparison of DNA-DNA hybridization (DDH) and ANI values has shown that an ANI of 95–96% correlates well with the current bacterial species boundary of 70% DDH similarity.

In an attempt to isolate and identify the rare species of *Streptomyces* with the potential to produce bioactive compounds, we have isolated strain VHB-9 from granite mine soil samples of Kammam district, Telangana state, India. Studies on identification by polyphasic approach and antimicrobial profile against various opportunistic pathogenic bacteria are reported in the presented study.

MATERIALS AND METHODS

Site and sample collection

Soil samples used in the present study were randomly collected from granite mines of Mudhigonda, Khammam District, Telangana state, India. Three independent replicates of samples from different sites were collected and brought to the laboratory in sterilized containers. Samples were homogenized by sieving through a 5-mm screen sieve and air dried for 2-5 days at room temperature (30 ± 2°C). The air dried soil samples were pre treated with $CaC0_3$ (10:1w/w) and incubated at 37ºC for four days. [10]

Isolation and screening for antagonistic activity

Soil sample (5g) was transferred to Erlenmeyer flask containing 45 ml of sterile distilled water. Then, 10-fold serial dilutions were made and 200 µl of each dilution was spread on toYeast extract malt extract dextrose agar (YMD) medium. [11] The medium was adjusted to pH 7.0 and supplemented with 25 µg/mL Secnidazole and 25 µg/mL Streptomycin to reduce the fungal and bacterial contamination respectively and incubated at 30±2^{o}C for 7 days. Actinobacterial colonies based on their morphology were picked out and purified on YMD agar slants.[12] The actinobacterial strains were then screened for the antagonistic potential against bacteria and fungi.[13] One predominant strain, VHB-9 was selected for further studies based on its potential to exhibit high antagonistic activity among the 25 different strains screened for biological activity.

Identification of the potent strain VHB-9 by polyphasic approach

Taxonomic characterization of the strain VHB-9 was carried out by cultural, morphological, physiological and biochemical characteristics along with genomic analysis. The microscopic characterization was carried out by slide culture method [14] taking into account the nature of mycelium, color and spore arrangement. [15] The morphological characteristics were assessed using scanning electron microscopy (SEM: Model- JOELJSM 5600, Japan) of 4-day old culture grown on YMD medium at various magnifications. The strain was grown on nine different media to observe the cultural characteristics such as colour of aerial mycelium, substrate mycelium, pigment production and spore formation.[16] Hydrolysis of starch and H_2S production was performed.[17] Physiological characteristics such as the effect of pH (5-9), temperature (20-45°C) and salinity on the growth of the strain were analyzed. The susceptibility of the strain to different antibiotics was also determined by paper disc method.[18]

Molecular Identification

DNA extraction

The genomic DNA used for the Polymerase Chain Reaction (PCR) was prepared from the colonies grown on YMD agar for 3 days. The total genomic DNA from the strain was extracted by employing the DNA purification Kit (Pure Fast® Bacterial Genomic DNA purification kit, Helini Bio molecules, India) according to the manufacturer protocol.

Amplification of 16S rDNA

The 16S rDNA fragment was amplified using Actino specific forward Primer -5'-GCCTAACACATGCAAGTCGA-3' and actino specific reverse primer - 5'-CGTATTACCGCGGCTGCTGG-3'. Conditions of the PCR were standardized with initial denaturation at 94 °C for 3 min followed by 30 cycles of amplification (Denaturation at 94 °C for 60 sec, annealing temperature of 55 °C for 60 sec and extension at 72°C for 60 sec) and an addition of 5 min at 72°C as final extension. The amplification reactions were carried with a total volume of 50 µL in a Gradient PCR (Eppendorf, Germany). Each reaction mixture contained 1µL of DNA, 1 µL of 10 p mol forward 16S Actino specific primer (5´-AAATGGAGGAAGGTGGGGAT-`3), 1 µL of 10 pmol reverse 16S Actino specific primer (5´- AGGAGGTGATCCAACCGCA-`3), 25 µL of Master Mix and 22 µL of molecular grade nuclease free water. The separation was carried out at 90 Volts for 40 min in TAE buffer with 5 µL of Ethidium bromide. PCR product was analyzed using 1 % agarose gel and the fragment was purified (Helini Pure Fast PCR clean up kit, Helini Bio molecules, India) as per the manufacturer's instructions. The bands were analyzed under UV light and documented using Gel Doc.

Sequencing and phylogenetic analysis
PCR product sequencing was performed by dideoxy chain termination method using 3100-Avant Genetic Analyzer (Applied Bio systems, USA). The sequences thus obtained were analyzed for homology using BLASTN (Entrez Nucleotide database). The deduced 16S rDNA sequence was compared with the sequences in GenBank (http://www.ncbi. nlm.nih.gov/) using the Basic Local Alignment Search Tool (BLAST) then aligned with the related reference sequences retrieved from GenBank databases using the Clustal *W* method. Phylogenetic and molecular evolutionary analyses were conducted using MEGA (Melecular Evolutionary Genetic Analysis) version 6.0.[19]

Nucleotide Sequence accession number
The partial 16S rDNA sequence of the strain VHB-9 is registered in the Gen Bank database.

Growth pattern of the strain
Growth pattern of the strain was determined by inoculating the culture into 250 ml flasks containing 100 ml YMD broth and incubated at 30 °C on a rotary shaker at 180 rpm. The flasks were harvested at 24 h interval and growth was determined by taking the dry weight of biomass and expressed as mg/100mL. The culture filtrates obtained after separating the biomass were extracted with ethyl acetate and antimicrobial activity of crude solvent extract was determined by agar well diffusion method.

Extraction of metabolites and antimicrobial assay
The antimicrobial activity of the strain was determined by agar well diffusion assay. Seed culture at the rate of 10 % was transferred to YMD broth (Fermentation medium) and fermentation was carried out at 30°C for 96 h under agitation at 180 rpm. The crude extract recovered from the filtrate by solvent extraction method using ethyl acetate was concentrated using rotavac and the residue thus obtained was used to determine antimicrobial activity. Ethyl acetate itself was used as negative control. 80µl of the crude extract and 80µl of negative control were poured in to separate wells. The standard antibiotic disc was placed on the agar surface as positive control. For each bacterial strain, controls were maintained with pure solvent. Plates were incubated at 37 °C for 48 h. and inhibition zones (in mm) were measured after 24-48 h. Experiment was carried out in triplicates for each test organism and the mean values were computed.

Test organisms
Bacteria: *Staphylococcus aureus* (MTCC 3160), *Lactobacillus casei*, *Bacillus megaterium* (NCIM 2187), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 9027).
Fungi: *Aspergillus niger*, *Fusarium solani F. oxysporum* and *Candida albicans* (MTCC 183).

Extraction purification and Identification of active compounds
For the production of bioactive compounds, seed broth was prepared by culturing *S. lavendulocolor* on ISP-2 medium and incubated on a rotary shaker (180 rpm) at 30°C. After 72h of incubation, the seed culture @ rate of 10% was transferred to the optimized fermentation medium (production medium) consisting of 0.5% lactose, 0.5% peptone, 0.05% K_2HPO_4, 0.2% $CaCO_3$ with pH adjusted to 7.0. The culture filtrates (30L) collected after 96 h of incubation were extracted twice with ethyl

acetate and concentrated to dryness in a rotavac. The crude dark brown residue (3.0g) thus obtained was applied to a silica gel G column (25X 5 cm, Silica gel, Merck, Mumbai, India) for the isolation and purification of bioactive compounds.
The separation of crude extract was conducted via gradient elution system of hexane: ethyl acetate. The eluent was run over the column and small volumes of eluent collected in test tubes were analyzed via thin-layer chromatography (TLC) using silica gel plates (Silica gel, Merck, Mumbai, India) with hexane: ethyl acetate solvent system. Compounds with identical retention factors (*R*f) were combined and assayed for antimicrobial activity against Gram positive (*B. megaterium*), Gram negative (*E. coli*) bacteria and yeast (*C. albicans*) by using agar well diffusion assay.[20] Two different fractions (B2 and B3) were collected at gradient solvent system of Hexane: Ethyl acetate (60-40v/v, 50-50v/v). These two different fractions (B2- 160 mg and B3-140 mg) were purified by re-silica gel column chromatography (22 X 2.5 cm, Silica gel 100; Merck). The chemical structures of the purified compounds were elucidated on the basis of FTIR, ESI-MS, ^{1}H NMR and ^{13}C NMR.

Biological assay

Minimum inhibitory concentration

The antimicrobial spectra of the bioactive principles produced by the strain were determined in terms of minimum inhibitory concentration (MIC) by using the agar plate diffusion assay. Muller-Hinton agar and Sabouraud dextrose agar media were prepared to grow the bacteria and fungi, respectively. Dilutions of the bioactive principles and the reference drug were prepared in DMSO at concentrations ranging from 0 to 1000 µg/ml and were added to cups. The Petri dishes were inoculated with 1.5×10^{4} colony forming units (cfu/ml) and incubated at 37 °C for 24-48 h for bacteria and 48-72 h at 28 °C for fungi.[21] The lowest concentration of the bioactive compounds exhibiting significant antimicrobial activity against the test microbes was taken as the MIC of the compound.

Test organisms

MIC of the bioactive principles produced by the strain was determined against several pathogenic bacteria and fungi.

Bacteria:

Staphylococcus aureus (MTCC 3160), *B. megaterium* (NCIM 2187), *Bacillus subtilis* (ATCC 6633), *Serratia marcesens* (MTCC 1457), *Xanthomonas campestris* (MTCC 2286), *Proteus vulgaris* (MTCC 7299), *P. aeruginosa* (ATCC 9027), *E. coli* (ATCC 35218), *Enterococcus faecalis* (MTCC 439), *Streptococcus mutans* (MTCC 497), *L. casei* (MTCC 1423) and *L. acidophilus* (MTCC 495).

Fungi:

C. albicans (ATCC 10231), *Aspergillus* niger (ATCC 1015), *A. flavus* (ATCC 9643), *Fusarium solani* (MTCC 4634), *F. oxysporum* (MTCC 3075) and *Penicillium citrinum* (MTCC 6489).

RESULTS AND DISCUSSION

A total of 25 actinomycete strains designated as VHB-1 to VHB-25 were isolated from soil samples collected from Granite mines of Mudhigonda, Khammam District. All the strains were maintained as pure cultures on YMD agar slants and preserved at 4 ºC. One potent actinobacterial strain, VHB-9 was found to be predominant with strong antimicrobial activity against Gram positive and Gram negative bacteria. The strain exhibited typical morphological characteristics of the genus *Streptomyces*.

Morphological and micro morphological observation of the strain by SEM studies revealed that the strain exhibited spiral arrangement of the spores (Fig.1).

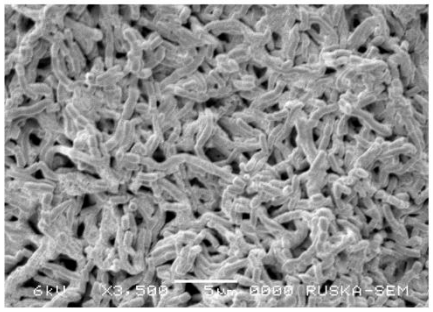

Fig.1: Scanning electron microscopic photograph of *Streptomyces lavendulocolor* VHB-9

Identification of the potent strain

The cultural characteristics of the strain grown on 9 different culture media are presented in table-1. The strain VHB-9 exhibited good growth on Tryptone yeast extract agar (ISP-1), Yeast extract malt extract dextrose agar (ISP-2), Starch-inorganic salts (ISP-4) agar, Peptone yeast extract agar (ISP-6), Nutrient agar and Czapek-Dox agar. The growth was moderate on Oat meal agar (ISP-3) and Glycerol asparagine agar (ISP-5) while it was poor on tyrosine agar (ISP-7). The color of aerial mycelium was grayish white on ISP-1, ISP-2, ISP-4 and white on ISP-3, ISP-6, ISP-7 while it was cream on ISP-5, NAM and Czapek-Dox agar. The color of substrate mycelium varied from pale yellow to yellow on ISP-1, ISP-5 and ISP-6 where as light brown to brown on ISP-4, NAM, ISP-2, ISP-3 and Czapek-Dox agar. The strain did not produce any pigment on any medium tested.

Physiological and biochemical characteristics of the strain VHB-9 are recorded in table-2. The strain could grow over a wide range of pH of 4-10 with optimum being at pH 7. The temperature range for growth was recorded between 20 and 40ºC with the optimum being 30ºC. The strain is positive for catalase production, indole, methyl red and citrate utilization but negative for Vogues-Proskauer, urease production and hydrogen sulfide production (Table-2). The enzymatic profile of the strain was interesting as it could produce a broad range of commercially important extracellular enzymes like amylase, cellulase, protease, pectinase and lipase (Table-2). The strain was resistant to streptomycin, rifampicin, furazolidone and amikacin and sensitive to Ampicillin, Penicillin, Vancomycin, Tetracycline, Ciprofloxacin, Nalidixic acid, Kanamycin and Doxycyclin (Table-2). Utilization of carbohydrate plays a crucial role in the taxonomic characterization of actinomycetes as suggested by Pridham *et al*. (1958) [15]. The strain exhibited good growth with glucose and lactose as carbon sources while growth was moderate with sucrose, maltose, galactose, fructose and sorbitol compared to starch and cellulose (Table-2).

Phylogenetic study of the strain

The taxonomic position of the strain VHB-9 was established based on the analysis of 16s rDNA. The 16S rDNA sequence of the strain was compared with the sequences in GenBank using BLAST and aligned with the sequences retrieved from NCBI Gen Bank database using the Clustal *W* method. The phylogenetic tree was constructed based on Maximum parsimony method using bootstrap analysis (Fig. 2) and deposited in the Gene Bank with Accession number KF317773. Basing on morphological, cultural, physiological, biochemical and molecular analysis, the strain VHB-9 was identified as *Streptomyces lavendulocolor* VHB-9.

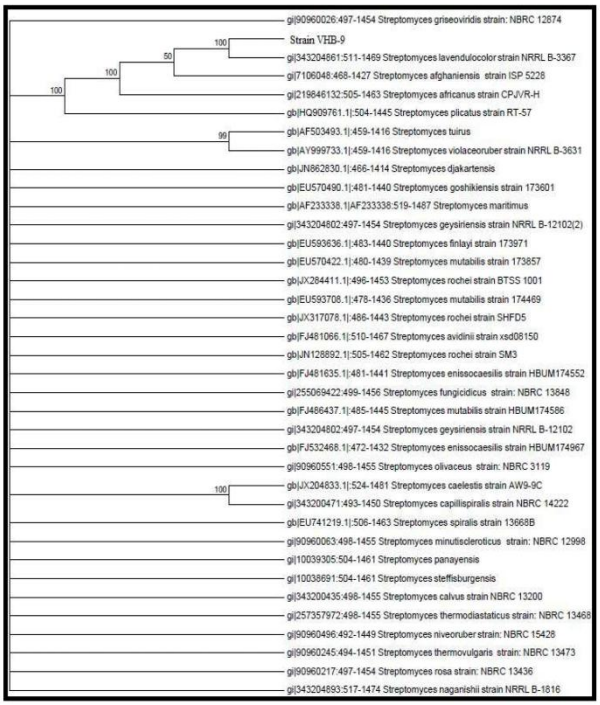

Fig. 2 Maximum Parsimony tree based on partial 16S rRNA gene sequence showing relationship between *Streptomyces* strain VHB-9 and related members of the genus *Streptomyces*. The numbers at the nodes indicate the level of bootstrap support based on Maximum parsimony analysis of 1000 resampled datasets; only values above 50% are given.

Table 1- Cultural Characteristics of the actinomycete strain VHB-9

Name of the Medium	Growth	AM*	SM**	Pigmentation
Tryptone yeast-extract agar (ISP-1)	Good	Greyish white	Pale Yellow	Nil
Yeast extract malt extract dextrose agar (ISP-2)	Good	Greyish white	Brown	Nil
Oat-meal agar (ISP-3)	Moderate	White	Brown	Nil
Inorganic salts starch agar (ISP-4)	Good	Greyish white	Light Brown	Nil
Glycerol asparagine agar (ISP-5)	Moderate	Cream	Yellow	Nil
Peptone yeast extract iron agar medium (ISP-6)	Good	White	Pale Yellow	Nil
Tyrosine agar (ISP-7)	Poor	White	Dark Brown	Nil
Nutrient agar	Good	Cream	Light Brown	Nil
Czapek-Dox agar	Good	Cream	Brown	Nil

* AM- Aerial mycelium ** SM- Substrate mycelium

Table 2- Physiological and Biochemical characteristics of strain VHB-9

Physiological and biochemical characteristics	Response		
Gram reaction	+	**Antibiotic sensitivity**	**Result**
Production of melanin pigment	-	Ampicillin (30)*	S
Range of temperature for growth	20-40^{o}C	Streptomycin (30)	R
Optimum temperature for growth	30^{o}C	Rifampicin (30)	R

Range of pH for growth	4 to 10	Penicillin (10)	S
Optimum pH for growth	7	Vancomycin (30)	S
NaCl tolerance	7%	Tetracycline (30)	S
Biochemical characters		Ciprofloxacin (5)	S
Catalase production	+	Nalidixic acid (30)	S
Urease production	-	Kanamycin (30)	S
Hydrogen sulfide production	-	Doxycyclin (30)	S
Methyl red test	+	Furazolidone (50)	R
Voges Proskauer test	-	Amikacin (30)	R
Indole production	+	*** (µg/disc)**	
Citrate utilization	+	**S – Sensitive; R – Resistant**	
Enzymatic Activity	**Result**		
Amylase	P		
Cellulase	P		
Pectinase	P		
L-Asparaginase	N		
Protease	P		
RNase	N		
DNase	N		
Lipase	P		
Utilization of carbon sources	**Growth***		
Fructose	++		
Sucrose	++		
Sorbitol	++		
Lactose	+++		
Galactose	+++		
Maltose	++		
Glucose	+++		
Cellulose	+		
Starch	+		

* Growth of the strain measured as dry weight of the mycelium '+ + +' - good growth; '+ +' - moderate growth; '+' - weak growth; '-'indicates negative/no growth;

Growth Pattern and antimicrobial profile of *Streptomyces lavendulocolor* VHB-9

For determination of growth pattern and antimicrobial potential of the strain, it was initially cultured on ISP-2 broth for 48 h to obtain seed culture. Then it was transferred aseptically into the production medium (ISP-2) at a rate of 10%. Fermentation was allowed to run for 8 days at 30 ºC as shake culture. The culture was harvested at every 24 h interval and the biomass was separated by filtration. The growth of the strain was measured in terms of dry weight of biomass (g/100 ml) to record the growth phases. The strain entered log phase after 24 h of incubation and exhibited exponential growth up to 96 h followed by stationary phase extended up to 120 h (Fig. 3). The fermentation broth collected simultaneously at 24 h interval was extracted with ethyl acetate and the solvent extract was tested for antimicrobial activity. Among the microorganisms tested, *B. megaterium* and *B.subtilis* were highly affected followed by *Pseudomonas aeruginosa* and *Shigella flexneri*. The

bioactive compounds produced by the strain VHB-9 after four days of incubation exhibited maximum antimicrobial activity against the test microorganisms (Table-3). The metabolites collected from four day old culture of *Streptomyces griseus* exhibited good antifungal activity.[22] Similarly the extracts of four day old cultures of *Streptomyces psammoticus* [23], *Streptomyces tendae* TKVL_ 333 [24] and *Nocardia levis* MK-VL_113 [25] were active against test bacteria and fungi.

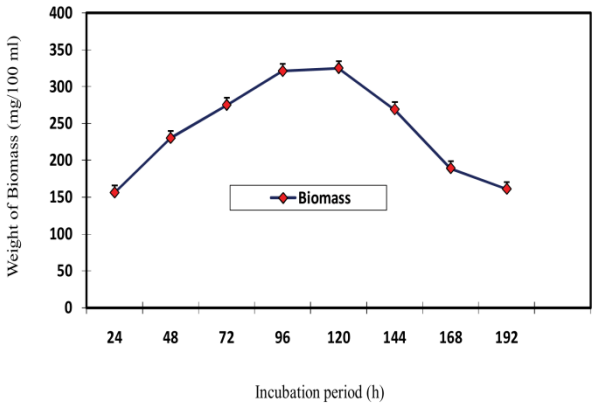

Fig. 3: Growth pattern of the strain *Streptomyces lavendulocolor* VHB-9

Isolation, purification and structural elucidation of the active metabolites

The separation of crude extract was conducted via gradient elution with hexane: ethyl acetate. The eluent was run over the column and small volumes of eluent collected in test tubes were analyzed with thin-layer chromatography (TLC) using silica gel plates (Silica gel, Merck, Mumbai, India) with hexane: ethyl acetate solvent system. Compounds with identical retention factors (*R*f) were combined and assayed for antimicrobial activities. The flowchart showing the isolation and purification of bioactive fractions is illustrated in Figure-6.

Among the different fractions collected, fractions (B2-160 mg and B3-140 mg) collected at gradient solvent system of Hexane: Ethyl acetate (60-40v/v, 50-50v/v) were purified by re-silica gel column chromatography (22 × 2.5 cm, Silica gel 100; Merck). The chemical structures of the purified compounds were elucidated on the basis of FTIR, ESI-MS, ^{1}H NMR and ^{13}C NMR. Compound B2 appeared as white solid, which was soluble in $CHCl_3$, MeOH, DCM and DMSO. The IR absorption maxima V*max* at 2929, 2860, 2860, 1730 and 1666 cm^{-1} suggested the presence of functional groups like carbonyl group (Fig.S1). In ESIMS, the compound showed molecular ions at *m/z* = 419 [M+1] inferring the molecular weight of $C_{26}H_{42}O_4$ [M+1]+ (Fig.S2). The proton NMR of the compound displayed proton signals at δ 7.71 (2H, dd, *J*= 7.39, 2.26 Hz), 7.53 (2H, dd, *J*= 7.39, 2.26 Hz), 4.22 (4H, Q, *J*=7.17 Hz), 1.72-1.65 (2H, m), 1.48-1.28 (10H, m), 0.93(6H, d, *J*= 7.4Hz), 0.90 (6H, d, *J*= 7.5Hz) (Fig.S3). ^{13}C NMR depicted peaks at δ 167.7, 132.4, 130.8, 128.7, 68.1, 38.7, 30.3, 28.8, 23.7, 22.9, 14.0, 10.9 (Fig.S4). Based on these spectral data, the active fraction B2 was identified as Bis (7-methyloctyl) phthalate (Fig. 4). This is the first report of this compound from the strain VHB-9.

B3 fraction was eluted with gradient eluent system of hexane: ethyl acetate 60-40v/v. It appeared as pale yellow solid, which was completely soluble in MeOH, and DMSO. The IR absorption maxima V*max* at 3380, 2974, 2931 and 1715 suggested the presence of functional groups like carboxylic acid and amine (Fig.S5). In ESIMS, the compound showed molecular ions at *m/z* =86 [M-1] inferring the molecular weight of C3H5NO2 [M-1] (Fig.S6). The proton NMR of the compound displayed signals at (DMSO) δ ppm: 10.17 (br S, 1H), 10 (br S, 1H), 6.48 (d, J= 7.5 Hz, 1H), 4.79 (d, J= 7.5 Hz, 1H) (Fig.S7). ^{13}C NMR depicted peaks at δ ppm: 170.4,

164.5, 140.6. (Fig.S8). Based on these spectral data, the active compound B3 was identified as (Z)-3-aminoacrylic acid (Fig. 5). This is the first report of this compound from the genus *Streptomyces*.

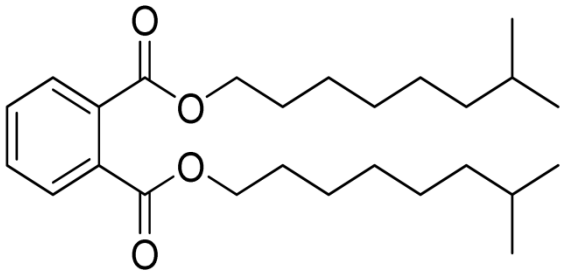

Fig. 4 Molecular structure of bis (7-methyloctyl) phthalate (B2)

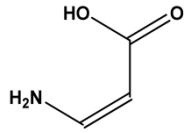

Fig. 5 Molecular structure of (Z)-3-aminoacrylic acid (B3)

Biological assay

Minimum inhibitory concentration

Minimum inhibitory concentrations of compounds B2 and B3 obtained from the strain against different microorganisms including bacteria and fungi in terms of MIC are shown in table-4. Compound B2 is more effective than B3. *B. megaterium* is highly sensitive to the compounds followed by *S. aureus, B. subtilis, L. acidophilus* and *S. mutans* among the Gram positive bacteria. *P. aeruginosa* is highly sensitive to the compounds followed by *S. marcescens, E. faecalis, X. campestris, E. coli* and *P. vulgaris* among the Gram negative bacteria. Among the fungi tested, *A. niger* was found to be sensitive when compared to others. MIC values of bis (7-methyloctyl) phthalate, (Z) 3-aminoacrylic acid and tetracycline against the test bacteria varied from 35-70 µg/ml, 50-90 µg/ml, and 25-50 µg/ml respectively. For fungi, these values ranged from 35-65 µg/ml for bis (7-methyloctyl) phthalate, 70-85 µg/ml for (Z) 3-aminoacrylic acid, 5-10 µg/ml for Carbendazim and 50 µg/ml for Griseofulvin. B2 and B3 showed good activity against *C. albicans*. Bis (7-methyloctyl) phthalate is good at inhibiting *C. albicans* than Griseofulvin.

Table 3- Antibacterial and antifungal activity of *Streptomyces lavendulocolor* VHB-9

Test organism	Zone of inhibition (mm)
Bacteria:	
Staphylococcus aureus	21
Lactobacillus casei	18
Bacillus megaterium	23
Escherichia coli	13
Pseudomonas aeruginosa	17
Proteus vulgaris	14
Fungi:	

Aspergillus niger	16
Fusarium solani	14
F. oxysporum	15
Candida albicans	17

Table 4- MIC values of the bioactive compounds produced by *Streptomyces lavendulocolor* VHB-9

	MIC (µg/ml)		
Test organism	**B2**	**B3**	**Positive control #**
Bacteria	**MIC* (µg/ml)**		
Bacillus megaterium	35	50	30
Bacillus subtilis	40	65	40
Serratia marcescens	50	75	25
Xanthomonas campestris	55	60	40
Proteus vulgaris	70	90	50
Pseudomonas aeruginosa	45	60	40
Escherichia coli	55	70	25
Enterococcus faecalis	55	85	25
Streptococcus mutans	50	60	30
Lactobacillus casei	45	70	25
Lactobacillus acidophilus	35	70	25
Staphylococcus aureus	45	55	25
Yeast			
Candida albicans	45	70	50
Fungi			
Aspergillus niger	35	70	5
A. flavus	50	75	10
Fusarium oxysporum	60	80	10
Fusarium solani	50	70	10
Penicillium citrinum	65	85	10

* **MIC:** Minimum Inhibitory Concentration
#Positive control: Tetracycline against bacteria, Griseofulvin against yeast and Carbendazim against fungi.
B2: bis (7-methyloctyl) phthalate; **B3:** (Z) 3-aminoacrylic acid

CONCLUSIONS

In the present study, a potent *Streptomyces* strain, VHB-9 was isolated on ISP-2 agar medium as a predominant strain from soil collected at granite mines. Screening of the strain for antagonistic activity revealed its potential to inhibit several opportunistic pathogens *in vitro*. The strain was identified as *S. lavendulocolor*

by polyphasic taxonomy. An attempt was also made to characterize antimicrobial metabolites by culturing the strain in a large volume of the medium. Extraction, followed by bioactivity- guided isolation and purification of a crude ethylacetate extract, revealed the presence of Bis (7-methyloctyl) phthalate and (Z)-3-aminoacrylic acid. This is the first report on these compounds isolated from *S. lavendulocolor* VHB-9.

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REFERENCES

1. Ritacco FV, Haltli B, Janso JE, Greenstein M, Bernan VS. Dereplication of *Streptomyces* soil isolates and detection of specific biosynthetic genes using an automated ribotyping instrument. *J Indian Microbiol Biotechnol.* 2003; 30:472-479.
2. Sembiring L, Goodfellow M. Ecological Approach to Unravel Streptomycete Diversity as an Unsurpassed Source of Natural Bioactive Products. *Microbiol Indones.* 2008; 2:49-56.
3. Challis GL, Hopwood DA. Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. *Proc Natl Acad Sci*. 2003; 100:14555-61.
4. Fajardo A, Martınez JL. Antibiotics as signals that trigger specific bacterial responses. *Curr Opin Microbiol.* 2008; 11:161-7.
5. Madigan, M.T., Martinko, J.M. In: Brock biology of microorganisms. 11th ed. New Jersey:Pearson Education, Upper Saddle River; 2006.
6. Schrey SD, Tarkka MT. Friends and foes: *Streptomycetes* as modulators of plant disease and symbiosis. *Antonie Van Leeuwenhoek*. 2008; 94:11-9.
7. Kurtboke DI, Neller RJ, Bellgard SE. Mesophilic actinomycetes in natural and reconstructed sand dune vegetation zones of Fraser Island. Australia. *Microb Ecol*. 2007; 54:332-40.
8. Ames BN. Mycorrhiza development in onion in response to inoculation with chitin decomposing actinomycetes. *New Phytol.* 1989; 112:423-427.
9. Riedlinger J, Schrey SD, Tarkka MT, Hampp R, Kapur M, Fiedler HP. Auxofuran, a novel metabolite that stimulates the growth of fly agaric, is produced by the mycorrhiza helper bacterium *Streptomyces* strain AcH 505. *Appl Environmental Microbiol*. 2006; 72:3550-3557.
10. Munaganti RK, Naragani K, Muvva VL. Antimicrobial Profile of *Rhodococcus erythropolis* VL-RK_05 isolated from Mango Orchards. *Int J Pharm Sci Res.* 2015; 6(4):1805-12.
11. Munaganti RK, Muvva VL, Indupalli MD. Studies on optimization of L-asparaginase production by *Arthrobacter kerguelensis* VL-RK_09 isolated from Mango orchards. *Int J Pharm Pharm Sci.* 2015; 7:112-115.
12. Naragani K, Rajesh Kumar M, Vijayalakshmi M. Antimicrobial Potential of *Streptomyces violaceoruber* VLK-4 Isolated from South Coast of Andhra Pradesh, India. *Int J Pharm Sci Rev Res.* 2014; 25:125-9.
13. Atta HM, Bayoumi R, El-Sehrawi M, Aboshady A, Al-Huminay A. Biotechnological application for producing some antimicrobial agents by actinomycetes isolates from Al-Khurmah Governorate. *Eur J Appl Sci.* 2010; 2:98-107.
14. Kavitha A, Vijayalakshmi M, Sudhakar P, Narasimha G. Screening of Actinomycete strains for the production of antifungal metabolites. *Afr J Microbiol Res.* 2010; 4:27-32.
15. Pridham TG, Anderson P, Foley C, Lindenfelser L, Hesseltine CW, Benedict RGA. Selection of media for maintenance and taxonomic study of *Streptomyces*. *Antibiot Annu.* 1957; 57:947-53.

16. Pridham TG, Lyons. Methodologies for Actinomyctetales with reference to *Streptomycetes*. In: Actinomycete Taxonomy. Diatz A, Thayer DW. Eds. Sim special publication No.6, Arligton, VA; 1980. p. 153-224.
17. Gordon RE. Some criteria for the recognition of *Nocardia madura* (Vincent) Blanchord. *J Gen Microbiol*. 1966; 45:355-64.
18. Cowan ST. Cowan and Steel's manual for the identification of medical bacteria. Cambridge, Univ. Press: 2 nd. Edition; 1974
19. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, Evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*. 2011; 28:2731-9.
20. Cappuccino JG, Sherman N. Microbiology, Laboratory manual. Person education, INC, New Delhi. 2004; 282-283.
21. Khan ZK. *In vitro* and *In vivo* Screening Techniques for Bioactivity Screening and Evaluation. In: Proceedings of the International Workshop UNIDO-CDRI, 1997, 210.
22. Otani T, Sugimoto, Y, Aoyagi Y, Igarashi Y, Furumai T, Saito N, Yamada Y, Asao T, Oki T. New Cdc 25B tyrosine phosphate inhibitors, nocardiones A and B, produced by *Nocardia* sp. TP-A0248: Taxonomy, fermentation, isolation, structural elucidation and biological properties. *J Antibiot*. 2000; 53: 337-344.
23. Sujatha P, Bapiraju KV, Ramana T. Studies on a new marine Streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant *Staphylococcus aureus. Microbiol Research*. 2005; 160: 119-126.
24. Kavitha A, Vijayalakshmi, M. Production of Amylases by *Streptomyces tendae* TK-VL_333. *Int J Curr Res*. 2010; 10:110-114.
25. Kavitha A, Vijayalakshmi M. Cultural parameters affecting the production of bioactive metabolites by *Nocardia Levis* MK-VL-113. *J Appl Sci Res*. 2009;5:2138-2147.

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