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Research Article

OPTIMIZATION, ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM *STREPTOMYCES LAVENDULOCOLOR* VHB-9

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ABSTRACT

Objectives: Optimization, isolation, and characterization of bioactive compounds from S*treptomyces lavendulocolor* VHB-9 isolated from granite mines of Mudigonda village of Khammam district of Telangana state.

Methods: The potent strain was identified as *S. lavendulocolor* VHB-9 by polyphasic taxonomy. The influence of culture conditions on growth and bioactive compounds production was investigated. Purification of bioactive compounds was done using column chromatography. The structures of the compounds were elucidated on the basis of spectroscopic analysis including Fourier transform infrared, electron spray ionization mass spectrophotometry,^{1}H nuclear magnetic resonance (NMR), and^{13}C NMR. The antimicrobial activity of the compounds produced by the strain was tested against both Gram-positive and Gram-negative bacteria and fungi in terms of minimum inhibitory concentration.

Results: Isolation and identification of two compounds, namely (2*R*, 3*R*)-2, 3-Butanediol (B1A), and nonadecanoic acid (B1B). Fraction B4 was isolated partially purified fraction and identified by the gas chromatography-mass spectrometry analysis. B1B compound exhibited the highest activity against *Bacillus megaterium, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa,* and *Candida albicans* when compared to B1A and B4 compounds.

Keywords: Granite mine, *Streptomyces lavendulocolor,* Optimization, Spectroscopy, Gas chromatography-mass spectrometry analysis, Biological assay.

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INTRODUCTION

Natural products have been the largest contributors to drugs in the history of medicine. Microorganisms are attractive resources to synthesize structurally-diverse substances with various bioactivities that may be used as effective drugs or act as drug lead compounds that could be further modified and developed for higher efficacy [1]. Within the domain bacteria, actinomycetes showed unprecedented ability to produce potentially novel, clinically useful, secondary metabolites with anticancer, antioxidant, antiviral, and antibacterial compounds, the majority of these being derived from the members of the *Streptomyces* genus that include different classes of antibiotics including aminoglycosides, macrolides, and Beta-lactams [2].

Newer therapeutic agents such as daptomycin, linezolid, and streptogramin combination (quinupristin/dalfopristin) have entered the clinical area in the past few years to combat the multidrug-resistant bacteria [3]. However, certain undesirable side effects and the spread of pathogens with this new antimicrobial drug resistance emphasize the need for the development of other newer antimicrobial agents with activity against Gram-positive bacteria, Gram-negative environmental, and enteric organisms currently threaten patients in hospitals and communities with multidrug resistance [4]. The end result of this phenomenon is that many strains of bacteria have become resistant, and in many cases multi-resistant to these therapeutic agents, thus rendering these drugs ineffective as treatments of choice for severe infections caused by these pathogens [5]. Rising numbers of antibiotic unresponsive infectious disease agents confront patients worldwide [6], and consensus has emerged that it is essential that novel antibiotic classes be developed as part of the strategy to control the emerging drug-resistant pathogens.

Filamentous soil bacteria belonging to the genus *Streptomyces* are widely recognized as industrially important microorganisms and versatile producers of new secondary metabolites from different biosynthetic pathways, originate from different ecological niches that could be used to hunt for novel bioactive compounds. The great importance given to *Streptomyces* is partly because these are among the most numerous and most versatile soil microorganisms, given their large metabolite production rate and their biotransformation processes, their capability of degrading lignocellulose and chitin, and their fundamental role in biological cycles of organic matter. Indeed, different *Streptomyces* species produce about 75% of commercially and medically useful antibiotics. They have provided more than half of the naturally occurring antibiotics discovered to date and continue to be screened for useful compounds [7]. In the course of screening for new antibiotics, several studies are oriented toward isolation of Streptomycetes from different habitats.

In the view of that the strain *S. lavendulocolor* VHB-9 was isolated from a granite mine of Mudigonda village of Khammam district of Telangana state, India. An attempt was made in the present study to optimize the cultural parameters required for best yields of bioactive metabolites, and chemical characterization of the compounds was also investigated.

METHODS

Chemicals

All solvents, reagents, and media supplements used in this study were of extra pure grade and procured from Merck (Mumbai, India).

Strain isolation

The strain, *S. lavendulocolor* VHB-9, was isolated on yeast extract-malt extract-dextrose (YMD) agar medium by soil dilution technique from

a soil sample collected from granite mines of Mudigonda, Khammam District, Telangana state, India. The medium was composed of malt extract (1%), yeast extract (0.4%), dextrose (0.4%), $CaCO_3$ (0.2%), and agar (2.0%), pH 7.0±0.2. The strain was stored on YMD agar slants at 4°C.

Antimicrobial profile of bioactive metabolites produced by the strain

The antimicrobial profile of the strain *S. lavendulocolor* VHB-9 was studied by cultivating the strain in YMD broth at 30°C for 8 days. The antimicrobial activity of bioactive metabolites against *Staphylococcus aureus* (MTCC 3160), *Lactobacillus casei, Bacillus megaterium* (NCIM 2187), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 9027), *Aspergillus niger, Fusarium solani, Fusarium oxysporum,* and *Candida albicans* (MTCC 183) was determined by agar well diffusion assay, and inhibition zones against test microbes were determined [8].

Media optimization

Attempts were made to enhance the antimicrobial activity of *S. lavendulocolor* VHB-9 by optimizing the culture conditions such as pH, temperature, carbon sources, nitrogen sources, and minerals. The bioactive metabolite production of the strain was determined after 4 days of incubation. Fermentation was carried out in 250-mL Erlenmeyer flasks with constant shaking at 180 rpm. The effect of initial pH on the bioactive metabolite production was determined by adjusting pH of the production medium from 4 to 10. The optimal pH achieved at this step was fixed for further study [9]. Similarly, the optimum temperature for antimicrobial metabolite production was determined by incubating the strain at temperatures ranging from 20 to 40°C, while maintaining all other conditions at optimum levels [10]. The effect of carbon sources on bioactive metabolite production was determined by supplementing the production medium (YMD) with different carbon sources such as maltose, sucrose, mannitol, lactose, starch, cellulose, galactose, sorbitol, and fructose each at a concentration of 0.4% (w/v) replacing dextrose by keeping the other ingredients constant [11]. Influence of varying concentrations of the best carbon source (0.5–4% w/v) on bioactive metabolite production was also investigated.

Similarly, the influence of various nitrogen sources such as sodium nitrate, ammonium oxalate, ammonium sulfate, peptone, tryptophan, L-proline, tyrosine, urea, and yeast extract was studied by adding nitrogen source (0.4%) to the medium with an optimized carbon source. Further, the optimal levels of the suitable nitrogen source (0.1–1.5% w/v) for good yields of bioactive metabolites were also recorded [12]. To evaluate the effect of mineral salts, the optimized medium containing the superior carbon and nitrogen source was supplemented separately with mineral supplements such as KH_2PO_4, K_2HPO_4, NaCl, KCl, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, and $MnCl_2$ at a concentration of 0.05% (w/v) [13].

Extraction of the metabolite and antimicrobial activity assay

The strain *S. lavendulocolor* VHB-9 grown under optimized cultural conditions for 4 days was extracted with ethyl acetate, and concentrated in a rotary evaporator to obtain a crude extract. The antimicrobial metabolites produced were tested by agar well diffusion assay against the following test microorganisms:

Bacteria

Overnight grown cultures of *S. aureus* (MTCC 3160), *Bacillus subtilis* (ATCC 6633), *B. megaterium* (NCIM 2187), *Shigella flexneri* (MTCC 1457), *L. casei* (MTCC 1423), *Lactobacillus acidophilus* (MTCC 495), *Proteus vulgaris* (MTCC 7299), *P. aeruginosa* (ATCC 9027), *E. coli* (ATCC 35218), *Vibrio cholerae,* and *Streptococcus mutans* (MTCC 497) were used.

Fungi

C. albicans (ATCC 10231), *A. niger, Aspergillus flavus, F. solani, F. oxysporum* (MTCC 3075), *Penicillium citrinum,* and *Alternaria sp.* were used as test fungi for testing antifungal activity.

Extraction, purification, and characterization of antimicrobial compounds

Fermentation

A seed culture was prepared by culturing *S. lavendulocolor* VHB-9 in YMD broth and incubated on a rotary shaker (180 rpm) at 30°C for 48 h. The seed culture was then transferred to fermentation broth containing malt extract - 1%, lactose - 0.5%, peptone - 0.5%, and K_2HPO_4 - 0.05% with pH adjusted to 7 and incubated on rotary shaker (180 rpm) at 30°C for 120 h. The bioactive compounds from the fermented broth were harvested by filtration of biomass through Whatman Filter Paper No. 42 (Merck, Mumbai, India). The culture filtrate (30 L) was extracted twice with an equal volume of ethyl acetate, pooled and the organic layer was concentrated in a Rotavac. The deep brown semi-solid compound (3.0 g) obtained was applied to a silica gel G column (25 cm×5 cm, Silica gel, Merck, Mumbai, India).

The separation of the crude extract was carried out through 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 through thin-layer chromatography (TLC) using silica gel plates (Silica gel, Merck, Mumbai, India) with hexane: ethyl acetate solvent system [14]. 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 [15].

Among the different fractions, two fractions B1 (polar) and B4 (nonpolar) were collected at gradient solvent system of Hexane: ethyl acetate (70-30v/v and 90-10v/v). The B1 fraction was rechromatographed (22 X 2.5 cm, Silica gel 100; Merck) to get two pure compounds ((2*R*, 3*R*)-2, 3-Butanediol (B1A), and nonadecanoic acid [B1B]). The structures of these active fractions were analyzed on the basis of Fourier transform infrared (FTIR); model: Thermo Nicolet Nexus 670 spectrophotometer with NaCl optics and electron ionization mass/electron spray ionization mass spectrophotometry (EIMS/ESIMS); model: Micromass VG - 7070H, 70eV spectrophotometer and nuclear magnetic resonance (NMR) (^{1}H NMR and ^{13}C NMR) model: Varian Gemini 200 and samples were made in $CDCl_3$ with trimethyl saline as standard.

Fractions B4 obtained as a mixture of compounds analyzed on Agilent gas chromatography-mass spectroscopy (GC-MS) system. The fused silica HP-5 capillary column (30 m×0.25 mm, ID, film thickness of 0.25 µm) was directly coupled to the MS. The carrier gas was helium with a flow rate of 1.2 ml/min. Oven temperature was programmed (50°C/min), then 50–280°C (at rate of 5°C/min) and subsequently held isothermally for 20 min. The temperature of the injector port was maintained at 250°C and that of the detector at 280°C [16]. The peaks of components in gas chromatography were subjected to mass spectral analysis. The spectra were analyzed from the available library data NIST MS search (ver. 2.0) (Included with NIST'02 mass spectral library, Agilent p/n G 1033 A).

Biological assays

Minimum inhibitory concentration (MIC)

The MIC of antimicrobial metabolites produced by the strain was determined against Gram-positive as well as Gram-negative bacteria and fungi using agar plate well-diffusion assay [15]. Nutrient agar and Czapek-Dox agar media were used for culturing bacteria and fungi, respectively. Sterilized agar medium seeded with the test bacterial suspension was transferred to Petri plates under aseptic conditions. After the solidification of agar medium, wells about 6mm diameter were cut into it with a sterilized cork borer. In case of the antifungal assay, test fungus (10^5 spores/ml) was plated on to the solidified agar medium. Metabolites dissolved in dimethyl sulfoxide (DMSO) at concentrations ranging from 0 to 1000 µg/ml were added to the wells.

After inoculation the plates were incubated at 30°C and examined after 24–48 h of incubation for bacteria and 48–72 h for yeast and filamentous fungi. The experiment was carried out in triplicates, and the solvent (DMSO) alone was kept as a negative control. Tetracycline and Carbendazim were employed as positive controls for bacteria and fungi, respectively. The lowest concentration of the bioactive compound exhibiting antimicrobial activity against the test microbes was taken as MIC of the compound.

The MICs of the bioactive compounds (B1A, B1B, and B4) produced by the strain were determined against several opportunistic pathogenic bacteria and fungi.

Test organisms employed

The cultures of *S. aureus* (MTCC 3160), *B. megaterium* (NCIM 2187), *B. subtilis* (ATCC 6633), *Serratia marcesens* (MTCC 1457), *Xanthomonas campestris* (MTCC 2286), *P. vulgaris* (MTCC 7299), *P. aeruginosa* (ATCC 9027), *E. coli* (ATCC 35218), *Enterococcus faecalis* (MTCC 439), *S. mutans* (MTCC 497), *L. casei* (MTCC 1423), and *L. acidophilus* (MTCC 495) were employed for antibacterial assay. *C. albicans* (ATCC 10231), *A. niger* (ATCC 1015), *A. flavus* (ATCC 9643), *F. solani* (MTCC 4634), *F. oxysporum* (MTCC 3075), and *Penicillium citrinum* (MTCC 6489) were used for testing antifungal activity.

Statistical analysis

The results of bioactive metabolite production by *S. lavendulocolor* VHB-9 under different cultural conditions were statistically analyzed with one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Antimicrobial profile of bioactive metabolites produced by the strain

The growth pattern of *S. lavendulocolor* VHB-9 cultured in YMD broth was reported on our earlier publication [17]. 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. The crude extract obtained from a 4-day-old culture exhibited high antimicrobial activity against the test microorganisms (Fig. 1). A previous report demonstrated high antimicrobial activity of crude extracts of a 4-day-old culture of *Arthrobacter kerguelensis* VL-RK_09 [18]. The metabolites collected from 4-day-old culture of *Streptomyces griseus* exhibited good antifungal activity [19]. Similarly, the extracts of 4-day-old cultures of *Streptomyces psammoticus* [20], *Streptomyces tendae* TKVL_ 333 [21], and *Nocardia levis* MK-VL_113 [22] were active against test bacteria and fungi.

Crude extracts of 5-day-old cultures of *Streptomyces purpeofuscus* [23] and *Streptomyces albidoflavus* [24] were active against Gram-positive as well as Gram-negative bacteria and fungi. Secondary metabolites extracted from 5-day-old cultures of *Streptomyces* sp. CDRIL-312 [25] and *Streptomyces* spp. [12] exhibited good antifungal activity. 5-day-old culture of *Streptomyces clavuligerus* was reported to produce a good yield of clavulanic acid [26] whereas 6-day-old culture of *Streptomyces* sp. 201 exhibited good antimicrobial activity [27].

Impact of pH and temperature on antimicrobial activity

The influence of initial pH on growth and bioactive metabolite production of the strain was determined by adjusting the pH of YMD broth from 4 to10. Maximum growth and antimicrobial metabolite production by the strain were found at pH 7 through the strain was able to grow over a wide range of pH (Fig. 2). The optimum pH for antibiotic production by several actinomycetes was reported to be 7 for *Streptomyces hygroscopicus* D1.5 [28], *Streptomyces torulosus* KH- 4 [29], *Streptomyces* sp. VITSVK 9[30], *Streptomyces cellulosae* VJDS-1 [31], *Rhodococcus erythropolis* VLK-12 [32], and *A. kerguelensis* VL-RK_09 [18].

The yield of bioactive metabolites of the strain was also recorded when grown at temperatures of 20-40°C, and the optimum was recorded at 30°C (Fig. 3). With the rise of incubation temperature from 20 to 30°C, there was an increase in bioactive metabolite production. However, further, increase in temperature (above 30°C) resulted in the declined production of bioactive metabolites. These results are in agreement with the earlier reports for *Streptomyces* [29].

Effect of carbon and nitrogen sources on antimicrobial activity

The effect of various carbon sources on antimicrobial metabolite production was tested by supplementing the YMD broth with several carbon sources at a concentration of 0.4% (replacing the dextrose) while making all other ingredients of the media same and incubated for 96 h at 30°C. The effects of carbon sources on the production of bioactive metabolites by *S. lavendulocolor* VHB-9 are presented in Fig. 4. Among the carbon sources tested, significant production of bioactive metabolites was obtained with lactose followed by sucrose. These results are supported by the reports of *S. hygroscopicus* strains AK-111-81 and CH-7, which utilized lactose as a carbon source for high antimicrobial metabolite production [33,34]. Since lactose supported a high yield of bioactive metabolites, different concentrations of lactose (0.5–4%) were tested to determine the optimal concentration. Lactose at a concentration of 0.5 % supported the highest yield of bioactive metabolites (Fig. 5).

The effect of various nitrogen sources on antimicrobial metabolite production was tested by supplementing the YMD broth with several nitrogen sources at a concentration of 0.4% to the YMD broth (replacing yeast extract). Peptone was found to be good as compared to other organic and inorganic nitrogen sources tested (Fig. 6). These results are comparable with *S. rochei* G164 [35] and *S. scabies* PK-A41 [36]. Since peptone enhanced the antimicrobial metabolite production by the strain, the effects of different concentrations of peptone were tested

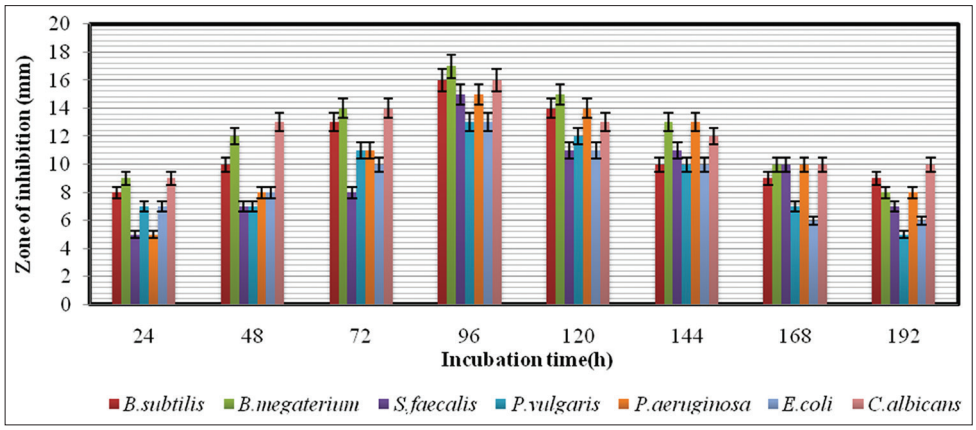

Fig. 1: Time course of bioactive metabolite production by *Streptomyces lavendulocolor* VHB-9. The data were statistically analyzed and found to be significant at a 5% level

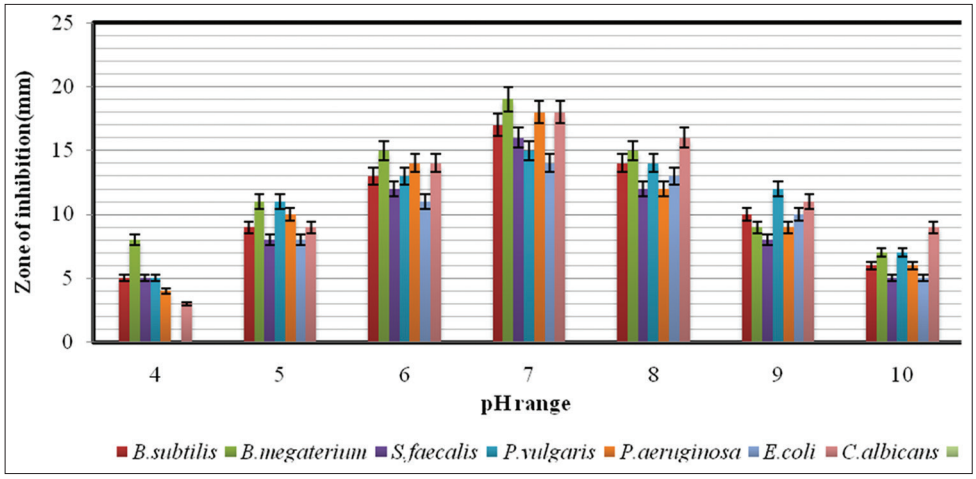

Fig. 2: The effect of pH on the bioactive metabolite production by *Streptomyces lavendulocolor* VHB-9. The data were statistically analyzed and found to be significant at a 5% level

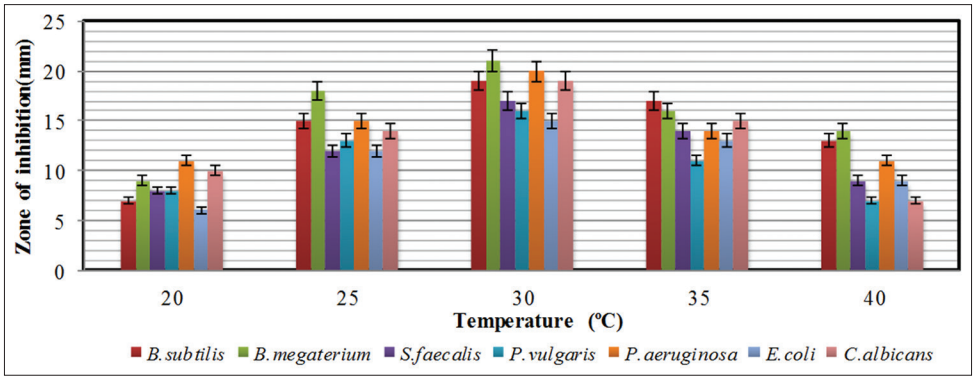

Fig. 3: The effect of temperature on the bioactive metabolite production by *Streptomyces lavendulocolor* VHB-9. The data were statistically analyzed and found to be significant at a 5% level

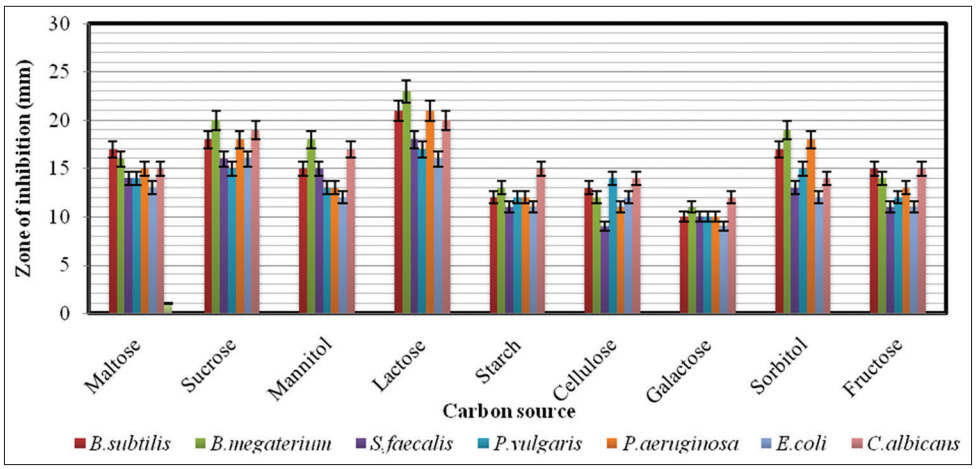

Fig. 4: The effect of carbon sources supplemented in the modified yeast extract-malt extract-dextrose broth on bioactive. Metabolite production by *Streptomyces lavendulocolor* VHB-9. The data were statistically analyzed and found to be significant at a 5% level

(Fig. 7). An enhanced level of bioactive metabolite production was found with peptone at a concentration of 0.5%. L-asparagine (0.09%) was reported as the suitable nitrogen source for optimum production of bioactive metabolites by *Streptomyces* sp. [27] *S. rubrolavendulae* ICN3 was reported to exhibit best anti-MRSA and cytotoxic activity when glucose and sodium nitrate were amended to the medium as carbon and nitrogen sources, respectively [37].

The effect of mineral salts on antimicrobial activity

The effect of mineral salts on secondary metabolite production by the strain VHB-9 is shown in Fig. 8. Among the mineral salts tested, K_2HPO_4 supported the highest antimicrobial activity. Similar results were reported for *S. albidoflavus* [24] Ripa *et al.* (2009) and Usha *et al.* (2011) reported that K_2HPO_4 supported antibiotic production by *Streptomyces* sp. RUPA-08PR and *Pseudonocardia* spp. [38,39].

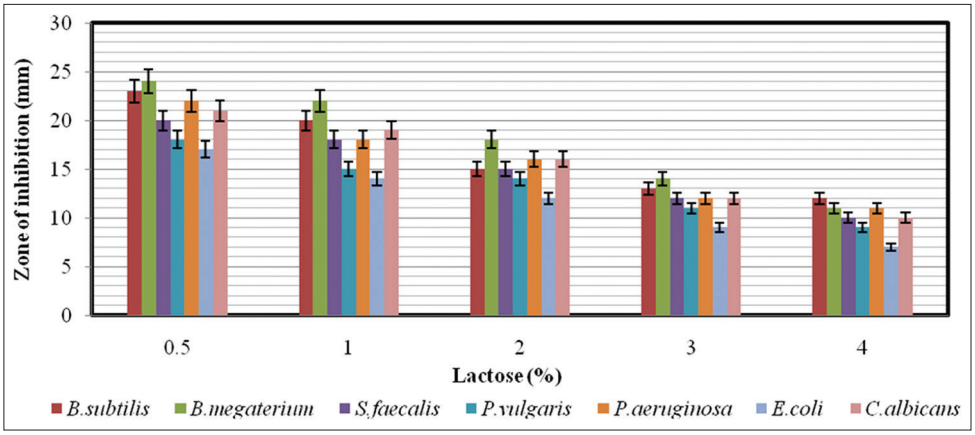

Fig. 5: The effect of different concentrations of lactose as carbon source on antimicrobial activity of *Streptomyces lavendulocolor* VHB-9. The data were statistically analyzed and found to besignificant at a 5% level

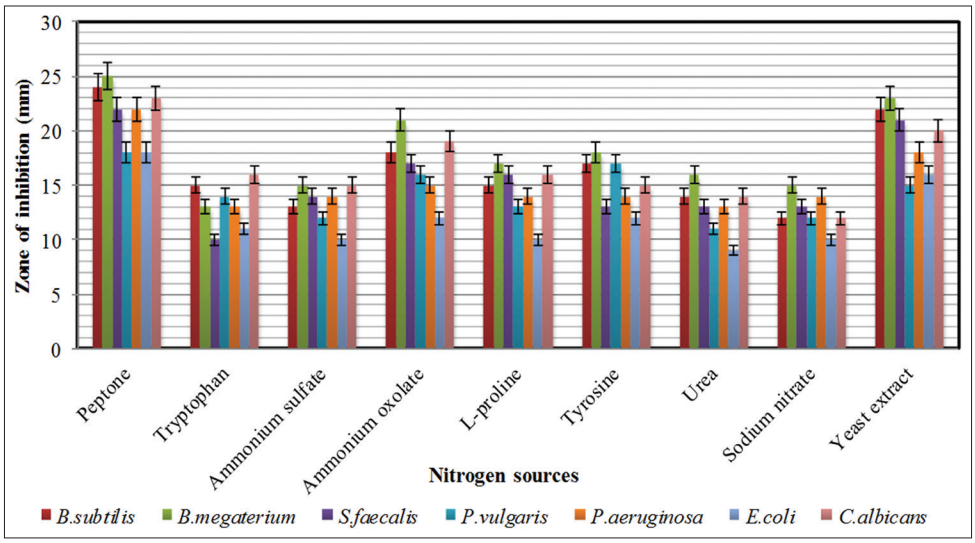

Fig. 6: The effect of various nitrogen sources supplemented in the modified yeast extract-malt extract-dextrose broth on the antimicrobial activity of *Streptomyces lavendulocolor* VHB-9. The data were statistically analyzed and found to be significant at a 5% level

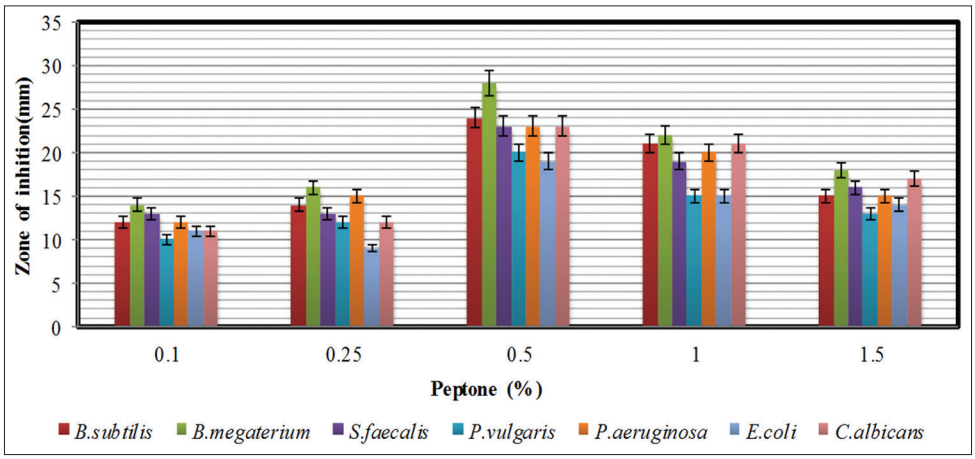

Fig. 7: The effect of different concentrations of peptone as nitrogen source on the antimicrobial activity of *Streptomyces lavendulocolor* VHB-9. The data were statistically analyzed and found to be significant at a 5% level

The strain VHB-9 is grown in the optimized fermentation medium containing lactose (0.5%), peptone (0.5%), K_2HPO_4 (0.05%), and $CaCO_3$ (0.2%) with pH adjusted to 7.0 and incubated at 30°C. After 96 h of incubation, the fermentation broth extracted with ethyl acetate exhibited good antimicrobial activity against Gram-positive as well as Gram-negative bacteria and fungi (Table 1). Among the bacteria tested, *B. megaterium* was highly sensitive to the metabolites followed by *B. subtilis*, *S. aureus*, *P. aeruginosa*, *S. flexneri*, *L. Casei*, and *L. acidophilus*.

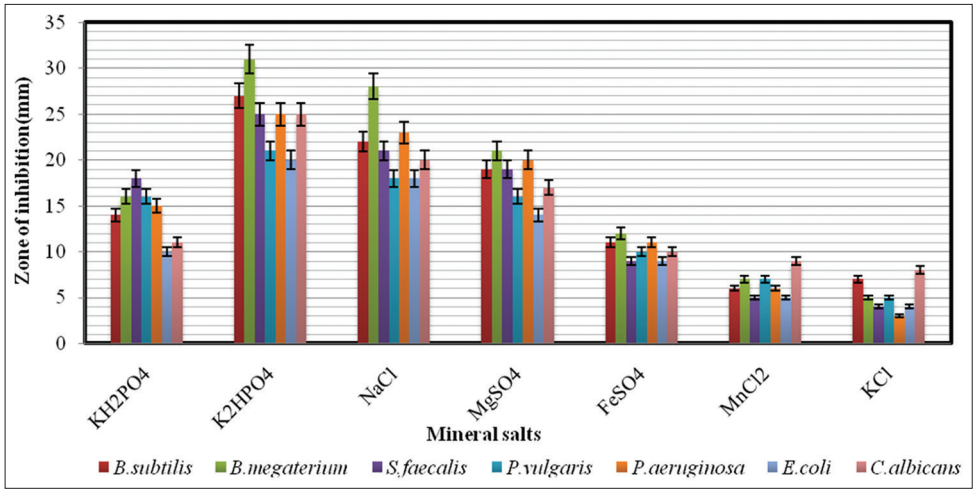

Fig. 8: The effect of mineral salts on the antimicrobial activity of *Streptomyces lavendulocolor*

Table: 1 Antimicrobial activity of *S. lavendulocolor* VHB-9 under optimized culturing conditions

S. No.	Test organisms	Zone of inhibition (mm)
1	*Staphylococcus aureus*	27
2	*Bacillus megaterium*	31
3	*Shigella flexneri*	25
4	*Bacillus subtilis*	28
5	*Proteus vulgaris*	21
6	*Pseudomonas aeruginosa*	26
7	*Escherichia coli*	21
8	*Streptococcus mutans*	22
9	*Vibrio cholera*	21
10	*Lactobacillus casei*	25
11	*Lactobacillus acidophilus*	23
	Fungi	
12	*Candida albicans*	25
13	*Aspergillus niger*	21
14	*Aspergillus flavus*	19
15	*Fusarium solani*	18
16	*Fusarium oxysporum*	17
17	*Penicillium citrinum*	18
18	*Alternaria* sp.	17

Among the fungi tested, *C. albicans* exhibited high sensitivity followed by *A. niger*, *A. flavus*, *F. solani, Penicillium citrinum, F. oxysporum,* and *Alternaria* sp.

Isolation, purification, and structural elucidation of active metabolites

The culture filtrates (30 L) 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 subjected to silica gel column chromatography. The crude extract was applied to a silica gel G column (25 cm ×5 cm, Silica gel, Merck, Mumbai, India) for the isolation and purification of bioactive compounds.

Among the fractions collected, two fractions (B1 and B4) collected at gradient solvent system of Hexane: ethyl acetate (70–30 v/v and 90–10 v/v) were analyzed. The B1 fraction was re-chromatographed (22 cm × 2.5 cm, Silica gel 100; Merck) to get two pure compounds, B1A (25 mg) and B1B (20 mg). The fraction B4, obtained as a mixture was analyzed by GC-MS system.

B1A eluted with 30% ethyl acetate appeared as light brown liquid soluble in $CHCl_3$, MeOH, DCM, and DMSO. The IR absorption maxima V*max* at 3437/cm suggested the presence of functional OH group. In ESIMS, the compound showed molecular ions at *m/z* = 108 inferring

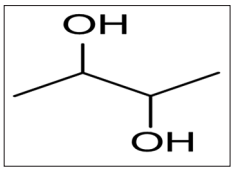

Fig. 9: Molecular structure of (2*R*, 3*R*)-2, 3-butanediol

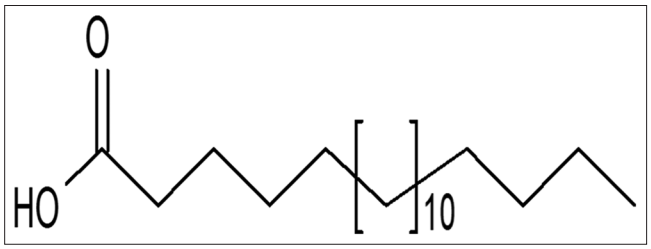

Fig. 10: Molecular structure of nonadecanoic acid

the molecular weight of $C_4H_{28}O_2$ [M+NH3]+. The proton NMR of the compound displayed proton signals at δ 3.81 (2H, Qd, *J*=6.04Hz) due to methylene protons bearing hydroxyl group, two exchangeable protons at δ 1.93 (br s, OH), at δ 1.67 (br s, OH) and two methyl groups at δ 1.15 (6H, d, *J*=6.04 Hz).[13]C NMR depicted peaks at δ 70.81(2C) and δ 16.90 (2C). (α) D25 = −12.5 (c=1, $CHCl_3$). Based on the spectral data and optical rotation, B1A was identified as B1A Fig. 9). This is the first report of this compound from *S. lavendulocolor* VHB-9. The second fraction B1B in pure form appeared as brown liquid soluble in $CHCl_3$, MeOH, DCM, and DMSO. The IR absorption maxima V*max* at 1708/cm suggested the presence of the carboxylic group. In ESIMS, the compound showed molecular ions at *m/z*=298 inferring the molecular weight of $C_{19}H_{38}O_2$ [M+1]+. The proton NMR of the compound displayed signals at δ 1.65–1.55 (30H, m), 1.25–1.99 (m, 2H) for aliphatic methylene protons, at δ 2.35 (t, 2H, *J*=7.2 Hz) for alpha methylene protons, at δ 1.25–1.99 (m, 2H) for methylene protons, and at δ 0.82 (t, 3H, *J*=6.1 Hz) for methyl protons. 13C NMR depicted peak at δ 180.8 for the carboxylic group. Based on spectral data, the B1B was identified as nonadecanoic acid (B1B) (Fig. 10). This is the first report of this compound from the strain VHB-9.

The active nonpolar fraction B4 appeared as light brown was soluble in $CHCl_3$, MeOH, DCM, and DMSO. The proton NMR of the compound revealed the presence of a mixture of compounds. The components of partially purified fourth fraction (B4) were analyzed on Agilent GC-MS system. The peaks of components in gas chromatography were subjected to mass spectral analysis. The spectra were analyzed from the

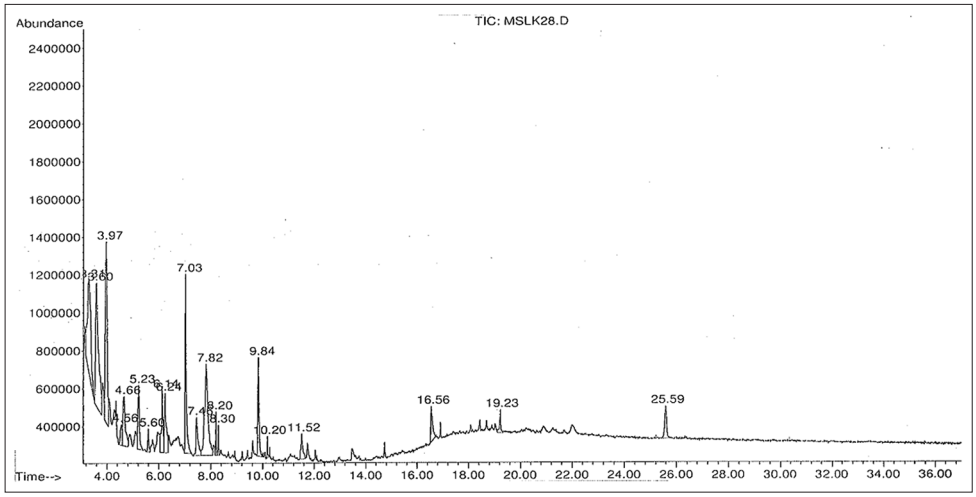

Fig. 11: Gas chromatographic spectrum of fraction B4 produced by *Streptomyces lavendulocolor* VHB-9

Table 2. List of compounds obtained from GC-MS analysis of fraction B4 produced by *S. lavendulocolor* VHB-9

Peak No.	Compound name	Area	Retention time	Percent match
1	2-Ethoxy pentane	13.05	3.30	38
2	Methoxy acetic acid	14.19	3.60	43
3	Pentanoic acid	13.60	3.97	52
4	Propanoic acid	1.47	4.56	25
5	Benzene acetic acid	4.57	4.66	25
6	Phenol, 2,4-bis-(1,1-dimethylethyl)	3.97	5.22	96
7	2,2-Diisopropyl1-1,3Dioxolane	1.02	5.60	35
8	Z-8-Hexadecene	3.96	6.14	86
9	Hexadecene	4.50	6.24	90
10	4-Ethynyl-4-vinyl-1,6-heptadecene	9.10	7.03	25
11	6-Methylquinoline 1-oxide	3.11	7.45	38
12	2-Phenylpent-4-enol	12.44	7.83	12
13	5-Octadecene	1.25	8.20	99
14	Octadecane	0.83	8.30	93
15	n-Hexadecanoic acid	4.43	9.84	98
16	1-Tricosanol	0.52	10.20	91
17	Cis-9-Hexadecenal	1.90	11.52	91
18	13-Docsenamide	2.47	16.56	93
19	Stigmastan-3,5-diene	1.26	19.23	91
20	No match found	2.36	25.59	-

GC-MS: Gas chromatography-mass spectroscopy, *S. lavendulocolor: Streptomyces lavendulocolor*

Table 3: MIC values of the bioactive compounds produced by *S. lavendulocolor* VHB-9

Test organism B1A	MIC (µg/ml)			
	B1B	B4 (PPF†)		Positive control#
Bacteria				
Bacillus megaterium	65	40	75	30
Bacillus subtilis	75	55	80	40
Serratia marcescens	90	60	100	25
Xanthomonas campestris	75	65	85	40
Proteus vulgaris	100	80	100	50
Pseudomonas aeruginosa	70	55	75	40
Escherichia coli	90	60	95	25
Enterococcus faecalis	95	75	100	25
Streptococcus mutans	75	55	90	30
Lactobacillus casei	85	55	100	25
Lactobacillus acidophilus	80	55	95	25
Staphylococcus aureus	65	50	80	25
Yeast				
Candida albicans	85	55	100	50
Fungi				
Aspergillus niger	90	55	125	5
Aspergillus flavus	80	70	100	10
Fusarium oxysporum	90	75	100	10
Fusarium solani	90	65	125	10
Penicillium citrinum	100	75	125	10

(Contd...)

Table 3: *(Continued)*

*MIC: Minimum inhibitory concentration, #Positive control: Tetracycline against bacteria, Griseofulvin against yeast and Carbendazim against fungi. B1A: (2R, 3R)-2, 3-Butanediol, B1B: Nonadecanoic acid, B4: PPF, PPF: Public provident fund, *S. lavendulocolor: Streptomyces lavendulocolor*

available library data NIST MS search (ver. 2.0) (Included with NIST'02 mass spectral library, Agilent p/n G 1033 A).

Compounds present in partially purified fraction were tentatively identified. The GC analysis revealed the presence of 20 compounds (Fig. 11). The list of compounds with their retention times is listed in Table 2.

Biological assay

MICs of compounds B1A, B1B, and B4 obtained from the strain against different microorganisms including bacteria and fungi in terms of

MIC are shown in Table 3. B1B is more effective than B1A and B4. *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. MIC values of B1A B1B, public provident fund and tetracycline against the test bacteria varied from 65 to 100 µg/ml, 40–80 µg/ml, 75–100 µg/ml, and 25–50 µg/ml, respectively. For fungi, these values ranged from 90 to 100 µg/ml for B1A, 55–75 µg/ml for B1B, 100–125 µg/ml for partially purified fraction, 5–10 µg/ml for carbendazim, and 50 µg/ml for griseofulvin. All the compounds showed good activity against *Candida.*

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