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 *Streptomyces 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 spectrometry, $^1$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 (2R, 3R)-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.

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 *Streptomyces* 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 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 63880), _Pseudomonas aeruginosa_ (ATCC 9027), _Escherichia coli_ (ATCC 9027), _Aspergillus niger_, _Fusarium solani_, _Fusarium oxysporum_, and _Candida albicans_ (MTCC 1893) 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$_2$PO$_4$, K$_2$HPO$_4$, NaCl, KC, MgSO$_4$·7H$_2$O, FeSO$_4$·7H$_2$O, 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 various test fungi for testing antifungal activity.

**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$_2$HPO$_4$ - 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 ([2R, 3R]-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, 70E spectrophotometer and nuclear magnetic resonance (NMR) (1H NMR and 13C NMR) model: Varian Gemini 200 and samples were made in CDCl$_3$ with trimethyl salin 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 (10⁵ 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), E. oxyaspurum (MTCC 3075), and Penicillium citrinum (MTCC 6489) were used for testing antifungal activity.

Statistical analysis
The results of bioactive metabolite production by S. lavendulicolor 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. lavendulicolor 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 psammaticus [20], Streptomyces tendae TKVL, 333 [21], and Nocarcia levis MK-VL-L13 [22] were active against test bacteria and fungi.

Crude extracts of 5-day-old cultures of Streptomyces purpureofuscus [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 sp. [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 to 10. 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 cellulose VJS-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. lavendulicolor 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. rochet G164 [35] and S. scabies PKA41 [36]. Since peptone enhanced the antimicrobial metabolite production by the strain, the effects of different concentrations of peptone were tested
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$_2$HPO$_4$ supported the highest antimicrobial activity. Similar results were reported for *S. albidaflavorus* [24] Ripa et al. (2009) and Usha et al. (2011) reported that K$_2$HPO$_4$ supported antibiotic production by *Streptomyces* sp. RUPA-08PR and *Pseudonocardia* spp. [38,39].
The strain VHB-9 is grown in the optimized fermentation medium containing lactose (0.5%), peptone (0.5%), K$_2$HPO$_4$ (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.
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 CHCl₃, MeOH, DCM, and DMSO. The IR absorption maxima Vₘₐₓ at 3437/cm suggested the presence of functional OH group. In ESIMS, the compound showed molecular ions at m/z = 108 inferring the molecular weight of C₄H₂₈O₂[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).¹³C NMR depicted peaks at δ 70.81 (2C) and δ 16.90 (2C). (α) D₂⁵ = −12.5 (c=1, CHCl₃). 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 CHCl₃, MeOH, DCM, and DMSO. The IR absorption maxima Vₘₐₓ 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 C₁₉H₃₈O₂[M+1]+. The proton NMR of the compound displayed proton 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 liquid soluble in CHCl₃, MeOH, DCM, and DMSO. The compound showed molecular ions at m/z = 108 inferring the molecular weight of C₄H₂₈O₂[M+NH3]+. The proton NMR of the compound displayed proton signals 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).¹³C NMR depicted peaks at δ 70.81 (2C) and δ 16.90 (2C). (α) D₂⁵ = −12.5 (c=1, CHCl₃). 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 CHCl₃, MeOH, DCM, and DMSO. The IR absorption maxima Vₘₐₓ 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 C₁₉H₃₈O₂[M+1]+. The proton NMR of the compound displayed proton 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 liquid soluble in CHCl₃, 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

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**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                      |
| 12     | *Fungi*                     | 25                      |
| 13     | *Candida albicans*           | 25                      |
| 14     | *Aspergillus niger*          | 21                      |
| 15     | *Aspergillus flavus*         | 19                      |
| 16     | *Fusarium solani*            | 18                      |
| 17     | *Fusarium oxysporum*         | 17                      |
| 18     | *Penicillium citrinum*       | 18                      |
| 19     | *Alternaria* sp.             | 17                      |

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

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

**Fig. 10: Molecular structure of nonadecanoic acid**
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.

**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                       | 3.60   | 3.97           | 52            |
| 4       | Propanoic acid                       | 1.47   | 4.56           | 25            |
| 5       | Phenol, 2,4-bis-(1,1-dimethylethyl)   | 3.97   | 5.22           | 96            |
| 6       | 2,2-Diisopropyl-1,3-diol                 | 1.02   | 5.60           | 35            |
| 7       | 2-B-Hexadecene                       | 3.96   | 6.14           | 86            |
| 8       | Hexadecene                           | 4.50   | 6.24           | 90            |
| 9       | 4-Ethynyl-1,3-dioxolane              | 9.10   | 7.03           | 25            |
| 10      | 6-Methylquinoline 1-oxide            | 3.11   | 7.45           | 38            |
| 11      | 2-Phenyldiacetate 4-ethyl             | 12.44  | 7.83           | 12            |
| 12      | 5-Octadecene                         | 1.25   | 8.20           | 99            |
| 13      | Octadecane                           | 0.83   | 8.30           | 93            |
| 14      | n-Hexadecanoic acid                  | 4.43   | 9.84           | 98            |
| 15      | 1-Tricosanol                          | 0.52   | 10.20          | 91            |
| 16      | 5-Octadecene                         | 1.90   | 11.52          | 91            |
| 17      | 1,3-Dicesnamide                      | 2.47   | 16.56          | 93            |
| 18      | Stigmaster-3,5-diene                  | 1.26   | 19.23          | 91            |
| 19      | No match found                       | 2.36   | 25.59          | -             |

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

**Table 3: (Continued)**

| Test organism B1A | MIC (µg/ml) | Positive control* |
|-------------------|-------------|-------------------|
|                   | B1B | B4 (PPF†) |          |
| **Bacteria**      |     |           |          |
| Bacillus megaterium | 65       | 40       | 75       | 30       |
| Bacillus subtilis  | 75   | 55       | 80       | 30       |
| Serratia marcescens | 90   | 60       | 100      | 25       |
| Xanthomonas campestris | 75   | 65       | 85       | 40       |
| Proteus vulgaris   | 100  | 80       | 100      | 50       |
| Pseudomonas aeruginosa | 70   | 55       | 75       | 20       |
| Escherichia coli   | 90   | 60       | 95       | 25       |
| Enterococcus faecalis | 95   | 75       | 100      | 25       |
| Streptococcus mutans | 75   | 55       | 90       | 30       |
| Lactobacillus casei | 85   | 55       | 100      | 25       |
| Staphylococcus aurous | 80   | 55       | 95       | 25       |
| Yeast              |     |           |          |
| Candida albicans   | 85   | 55       | 100      | 50       |
| Fungi              |     |           |          |
| Aspergillus niger  | 90   | 55       | 125      | 5        |
| Aspergillus flavus | 80   | 70       | 100      | 10       |

Test organism B1A

| MIC (µg/ml) | B1B | B4 (PPF†) | Positive control* |
|-------------|-----|-----------|-------------------|
| Fusarium oxysporum | 90  | 75       | 100      | 10       |
| Fusarium solani | 90  | 65       | 125      | 10       |
| Penicillium citrinum | 100 | 75       | 125      | 10       |

*MIC: Minimum inhibitory concentration, †Positive control: Tetracycline against bacteria, Griseofulvin against yeast and Carbendazim against fungi.

**Bacterial assay**

MICs of compounds B1A, B1B, and B4 obtained from the strain against different microorganisms including bacteria and fungi in terms of available library data NIST MS search (ver. 2.0) (Included with NIST’02 mass spectral library, Agilent p/n G 1033 A).

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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 V. vulgare 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.

REFERENCES

1. Demain AL, Sanchez S. Microbial drug discovery: 80 years of progress. J Antimicrob Chemother 2009;62:5-16.
2. de Lima Procópio RE, Da Silva IR, Martins MK, De Azevedo JL, De Araújo JM. Antibiotics produced by Streptomyces. Braz J Infect Dis 2012;16:466-71.
3. Levy SB, Marshall B. Antibacterial resistance worldwide: Causes, challenges and responses. Nat Med 2004;10:122-9.
4. Nathwani D, Tigecycline: Clinical evidence and formulary position. Int J Antimicrob Agents 2005;25:185-92.
5. Alanis AJ. Resistance to antibiotics: Are we in the post-antibiotic era? Arch Med Res 2005;36:697-705.
6. Livermore DM. Bacterial resistance: origins, epidemiology and impact. Clin Infect Dis 2003;36:11-23.
7. Miyadoh S. Research on antibiotic screening in Japan over the last decade: A producing microorganisms approach. Actinomycetologica 1993;9:100-6.
8. Munaganti RK, Naragani K, Muvva VL. Antimicrobial profile of Rhodococcus erythropolis VL-RK_05 isolated from Mango Orchards. Int J Pharm Sci Res 2015;6:1805-12.
9. Srinivasan MC, Laxman RS, Deshpande MV. Physiology and nutritional aspects of actinomycetes: An overview. World J Microbiol Biotechnol 1991;7:171-84.
10. Saurav K, Kannabiran K. Diversity and optimization of process parameters for the growth of Streptomyces VITSVK 9 sp. Isolation from Bay of Bengal. India J Nat Environ Sci 2010;1:56-65.
11. Elliah P, Srinivasulu B, Adinarayana K. Optimization studies on neomycin production by a mutant strain of Streptomyces marinusensis in solid state fermentation process. Biochemistry 2000;39:529-34.
12. Kathiresan K, Balagurunathan R, Selvam MM. Fungicidal activity of marine actinomycetes against phytopathogenic fungi. Ind J Biotechnol 2005;4:271-7.
13. Farid MA, El-Enshasy HE, El-Diwany AI, El-sayed EA. Optimization of cultural conditions for the growth of Streptomyces VITSVK 9 sp. Isolation from Bay of Bengal. India J Nat Environ Sci 2010;1:56-65.
14. Elliah P, Srinivasulu B, Adinarayana K. Optimization studies on neomycin production by a mutant strain of Streptomyces marinusensis in solid state fermentation process. Biochemistry 2000;39:529-34.
15. Farid MA, El-Enshasy HE, El-Diwany AI, El-sayed EA. Optimization of the cultivation medium for Natamycin production by Streptomyces natalensis. J Basic Microbiol 2000;40:157-66.
16. Konda S, Raparthi S, Bhaskar K, Munaganti RK, Giguloth V, Nagarapu L, et al. Synthesis and antimicrobial activity of novel benzoxazine sulfonamide derivatives. Bioorg Med Chem Lett 2015;25:1643-6.
17. Naragani K, Mangamuri K, Muvva V, Poda S, Munaganti RK. Antimicrobial potential of Streptomyces cheonanensis VUK-A from mangrove origin. Int J Pharm Pharm Sci 2016;8:53-7.
18. Bossuza O, Ammar A, Saidana D, Chraa J, Chraif I, Dami M, et al. Chemical composition and antimicrobial activity of volatile components from capitula and aerial parts of Rhamnus punicana DC growing wild in Tunisia. Microbiol Res 2008;163:87-95.
19. Bindhu BS, Muvva VL, Munaganti RK, Naragani K, Konda S, Dorigondla KR. A study on production of antimicrobial metabolites by Streptomyces lavendulocolor TK-VL_333. Int J Cur Res 2010;10:110-4.
20. Sujatha P, Bapiraju KV, Ramana T. Studies on a new marine streptomycete BT-408 producing polypeptide antibiotic SBR-22 effective against methicillin resistant Staphylococcus aureus. Microbiol Res 2005;160:119-26.
21. Kavitha A, Vijayalakshmi M. Production of amylases by Streptomyces tendae TK-VL_333. Int J Cur Res 2010;10:110-4.
22. Kavitha A, Vijayalakshmi M. Cultural parameters affecting the production of bioactive metabolites by Nocardia levis MK-VL-113. J Appl Sci Res 2009;5:2138-47.
23. Anupama M, Narayana KJ, Vijayalakshmi M. Screening of Streptomyces purpurascens for antimicrobial metabolites. Res J Microbiol 2007;4:1-3.
24. Narayana KJP, Vijayalakshmi M. Production of extracellular α-amylase by Streptomyces albidoflavus. Asian J Biochem 2008;3:194-7.
25. Harindran J, Gupta TE, Naik SR. HA-1-92, a new antifungal antibiotic produced by Streptomyces CDRIL-312: Fermentation, isolation, purification and biological activity. World J Microbiol Biotechnol 1999;15:425-30.
26. Parag SS, Rekha SS. Optimization of nutrional requirements and feeding strategies for Clavulanic acid production by Streptomyces clavuligerus. Biores Technol 2007;98:2010-7.
27. Thakur D, Bora TC, Bordoloi GN, Mazumdar S. Influence of nutrition and culture conditions for optimum growth and antimicrobial metabolite production by Streptomyces sp. 201. J Med Mycol 2009;19:161-7.
28. Battacharyya BK, Pal SC, Sen SK. Antibiotic production by Streptomyces hygroscopicus. D1.5: Cultural effect. Rev Microbiol 1998;29:49-52.
29. Atta HM, Bayoumi R, El-Sehwaw A, Aboshady A, Al-Humayun A. Biotechnological application for producing some antimicrobial agents byactinomycetes isolates from Al-Khurmah governorate. Eur J Appl Sci 2010;2:98-107.
30. Kumar S, Krishnan K. Bioactivity guided extraction of 5,2,4-dimethylbenzylpyruvolidine-2-one from marine Streptomyces VITSVK5 spp. and its anti- Aspergillus activity against drug resistant clinical isolates. Pharm Lett 2013;5:178-84.
31. Indupalli MD, Vijayalakshmi M, Kumar MR. Streptomyces cellulaseus VJDS-1, a promising source for potential bioactive compounds. Int J Pharm Pharm Sci 2015;7:57-61.
32. Naragani K, Kumar MR, Kiranmayi MU, Vijayalakshmi M. Optimization of culture conditions for enhanced antimicrobial activity of Rhodococcus erythropolisVLK-12 isolated from South Coast of Andhra Pradesh, India. Brit Microbiol Res J 2014;4:63-79.
33. Gesheva V, Ivanova V, Gesheva R. Effect of nutrients on the production of AK-111-81 microbile antibiotic by Streptomyces hygroscopicus. Microbiol Res 2005;160:243-8.
34. Konstantinovic SS, Veljkovic VB, Savic DS, et al. The impact of different carbon and nitrogen sources on antibiotic production by Streptomyces hygroscopicus CH-7. Cur Res Tech Edu Top Appl Microbiol Microbiol Biotechnol 2010;2:1337-42.
35. Chattopadhyay D, Sen SK. Optimization of cultural conditions for antifungal antibiotic accumulation by Streptomyces rochei G164. Hindustan. Antibiot Bull 1997;39:64-71.
36. Han WC, Lee JY, Park DH, Lim CK, Hwang BK. Isolation and antifungal and antioomycete activity of Streptomyces scabiae strain PK-a41, the causal agent of common scab disease. Plant Pathol J 2004;20:115-26.
37. Kannan RR, Iniyam AM, Vincent SG. Production of a compound against methicillin resistant Staphylococcus aureus (MRSA) from Streptomyces rubroaventae ICN3 and its evaluation in zebrafish embryos. Indian J Med Res 2014;139:913-20.
38. Ripa FA, Nikkon F, Zaman S, Khondkar P. Optimal conditions for antimicrobial metabolites production from a new Streptomyces sp. RUPA-08PR isolated from Bangladeshi soil. Microbiology 2009;157:214-21.
39. Kiranmayi MU, Sudhirak P, Vijayalakshmi M. Production and optimization of L-asparaginase by an actinobacterium isolated from Nizamtpatnam mangrove ecosystem. J Environ Biol 2014;35:799-805.