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PHYLOGENETIC CHARACTERIZATION OF POTENTIAL BIOACTIVE METABOLITES PRODUCING ACTINOMYCETES FROM MANGROVE SEDIMENTS

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Keywords:

Mangrove ecosystem, Microbial diversity, Phylogenetic analysis and Antimicrobial activity

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ABSTRACT: An attempt has been made in the present study to isolate and identify actinomycetes, which can produce bioactive metabolites possessing antimicrobial activity. The soil samples were collected from mangrove sediments of Krishna district, Andhra Pradesh. The potent secondary metabolite producing strains were isolated and designated as VLK-15, VLK-24, and VLK-56. Identification of the strains was carried out basing on morphological, cultural, physiological and biochemical characters. Phylogenetic analysis of 16S rRNA gene sequence showed that the strains VLK-15, VLK-24, VLK-56 form distinct clade within the genus *Streptomyces*. The 16S rRNA sequences were deposited in the GenBank database of NCBI under the accession numbers MG309759 (*Streptomyces olivoverticillatus* VLK-15), MG309760 (*Strepto-myces rubrus* VLK-24), and MF952650 (*Streptomyces sampsonii*-VLK-56). The antimicrobial activity of the strains was evaluated by using agar well diffusion assay. The ethyl acetate extracts were highly effective against *Candida albicans*, *Staphylococcus aureus*, *Bacillus megaterium* followed by *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae*.

INTRODUCTION: Actinomycetes are sapro-phytic, free-living, Gram-positive bacteria widely distributed in different habitats, frequently filamentous and sporulating with DNA rich in G+C (55-75%). They are the potential source for many bioactive secondary metabolites [1]. The hunt for new antimicrobial agents is very useful to prevent infectious diseases [2]. They have been used in areas of medicine as well as in water sanitation to inhibit the growth of microorganisms in drinking water [3].

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Mangrove ecosystems are the unique woody plant communities in the tropical and subtropical coastal regions [4]. They are viewed as the ecosystems that comprise unexplored microbial diversity, including actinomycetes [5]. The mangrove ecosystems exist between terrestrial and marine environments that support a rich and diverse group of microorganisms [6]. There may be no other group of plants with such highly developed morphological, biological, ecological, and physiological adaptations to extreme conditions.

Mangrove derived actinomycetes remain as an unexploited and luxuriant source of pharmaceutical thrust. The expedition for the microbial groups with therapeutic possessions continues to receive great attention as researchers investigate mangrove

microbes for novel molecules with antimicrobial, antitumor, and biochemical activities [7].

Uncaged mangrove habitats provide more chances for isolating new species of streptomyces with unique chemical structures preventing many microbial diseases and cancers [8]. Hence, the present work was designed to study the diversity and polyphasic taxonomy of actinobacterial population of the mangrove ecosystems of the Krishna district of Andhra Pradesh, India.

MATERIALS AND METHODS:

Collection of Mangrove Sediment Sample: Sediment samples were collected at bimonthly intervals from mangrove ecosystems of the coastal region, Krishna district of Andhra Pradesh. The samples were collected from 6-10 cm depth and transported to the laboratory in sterile bags, and air-dried at room temperature.

Isolation of Actinomycetes: The air-dried and pretreated soil samples were suspended in sterile distilled water (1g in 100 ml), homogenized by vortexing, and 0.1ml of serially diluted sample (10^{-3} dilution) was spread over the surface of selective culture medium (starch-casein agar) containing 3% NaCl supplemented with tetracycline and secnidazole [9]. After incubation for one week at 30 °C, distinct strains were selected for subculturing to maintain pure culture on agar slants.

Extraction of Secondary Metabolites: Modified yeast extract, malt extract, and dextrose broth was used as a production medium for the extraction of crude secondary metabolites. All the actinobacterial isolates were inoculated separately and incubated at 30 °C in a rotatory shaker (120 rpm) for primary screening. The crude culture filtrate obtained from a five-day-old culture was extracted with equal volumes of ethyl acetate to extract the anti-microbial compounds. The solvent extracts were evaporated to dryness in a water bath at 40 °C, and the compound obtained was tested for its activity against the test microorganisms by agar well diffusion method [10].

Test Microorganisms: The antimicrobial metabolites produced by the strains were tested against test bacteria such as *Staphylococcus aureus* (MTCC 3160), *Escherichia coli* (ATCC 35218), *Bacillus subtilis* (ATCC 6633), *B. cereus* (MTCC-430), *B. megaterium* (NCIM2187) *Klebsiella pneumoniae (*ATCC10031*), Vibrio parahaemolyticus* (ATCC 43996) and fungi like *Candida albicans* (ATCC 10231) by agar well diffusion assay.

Identification: Micro-morphological, biochemical and physiological characteristics together with molecular (16S rRNA gene sequencing) analysis were carried out to identify the strains.

Molecular Identification: The Molecular identification of the potential strains was carried out by amplifying the bacterial genome. DNA samples were extracted using an Insta Genetm Matrix (BIO-RAD.). The primers 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3,' and 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3' were used for the PCR. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 µL reaction mixture using EF-Taq (SolGent, Korea) as follows: activation of Taq polymerase at 95 °C for 2 min, 35 cycles at 95 °C for one minute, 55 °C and 72 °C for one minute each were performed, finishing with a 10-min step at 72 °C.

The amplification products were purified with a multi-screen filter plate (Millipore Corp., Bedford, MA, USA). The sequencing reaction was performed using a PRISM Big Dye Terminator v3.1 Cycle Sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice, and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA).

Phylogenetic Analysis: The 16S rRNA sequence was compared with the known sequences in Gene Bank using the Basic Local Alignment Search Tool (BLAST) against the gene library available in the NCBI and the GenBank. The phylogenetic tree was constructed using the Maximum Parsimony method. The closely related homologous strains were identified, retrieved, and compared to the sequence of the isolated strains using CLUSTAL W available with the MEGA-X Version [11].

Gene Bank Accession Number: The 16S rRNA gene sequence of the strains are deposited in the National Center for Biotechnology Information (NCBI).

RESULTS AND DISCUSSION: The air-dried soil samples were pretreated with calcium carbonate [12]. The serial dilution plate technique was used for the isolation of actinobacteria. After incubation for a week at 30 °C, distinct colonies were selected based on smooth, granular, powdery appearance [9]. A total of 60 actinobacterial strains were isolated and designated as VLK-1 to VLK-60 from the mangrove ecosystem of coastal regions of the Krishna district. Some reports indicate the isolation of rare actinobacterial strains from Krishna mangrove ecosystems [14, 15]. The strains designated as VLK-15, VLK-24, and VLK-56 exhibited typical morphological characteristics of the genus Streptomyces [16]. The morphological, physiological, and biochemical characteristics of the strains are presented in **Table 1**.

TABLE 1: MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF STRAINS

Character	Response		
	VLK-15	VLK-24	VLK-56
Morphological characters			
Colour of aerial mycelium	White	Brown	Pale brown
Colour of substrate mycelium	Pale yellow	Dark brown	Dark brown
Sporophore morphology	Rectiflexible	Rectiflexible	Rectiflexible
Mycelial form	Branched	Branched	Branched
Physiological characters			
Gram's reaction	+	+	+
Production of melanin pigment	-	-	-
Range of pH for growth	4-9	4-10	4-9
Optimum pH for growth	7.0	7.5	7.0
NaCl tolerance	5%	7%	3%
Biochemical characters			
Catalase production	+	+	+
Urease production	+	+	+
Hydrogen sulphide production	-	-	-
Nitrate reduction	-	-	-
Starch hydrolysis	+	+	+
Gelatin liquefaction	-	-	-
Methyl red test	-	-	-
Voges proskauer test	-	-	-
Indole production	-	-	-
Citrate utilization	+	+	+

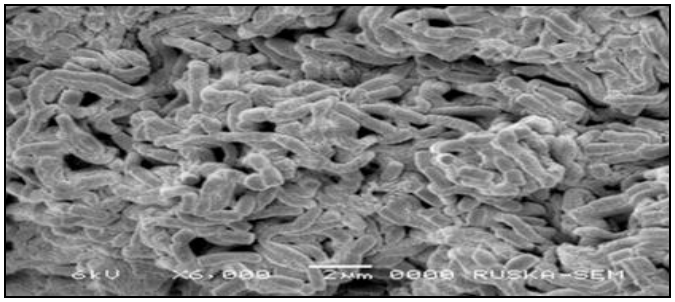

FIG. 1: SCANNING ELECTRON MICROSCOPY PHOTOGRAPH OF VLK-15

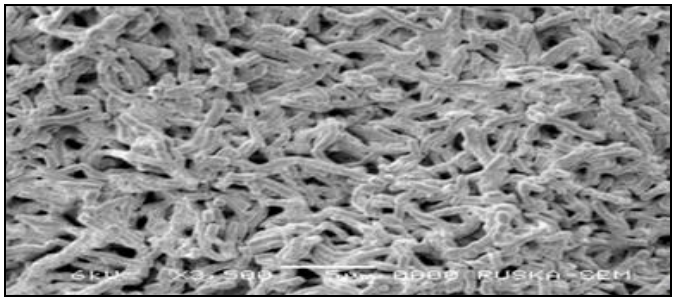

FIG. 2: SCANNING ELECTRON MICROSCOPY PHOTOGRAPH OF VLK-24

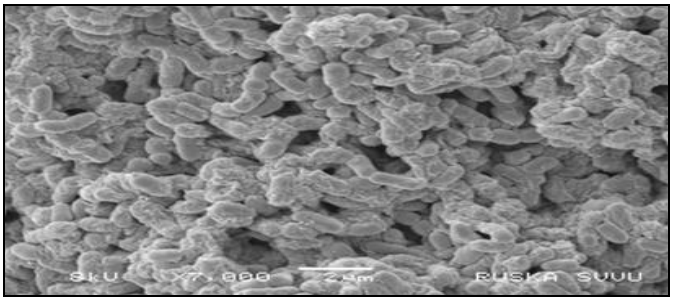

FIG. 3: SCANNING ELECTRON MICROSCOPY PHOTOGRAPH OF VLK-56

The strains exhibited good growth on ISP-1, ISP-2, ISP-7, starch casein agar, Czapek-Dox agar, and maltose tryptone agar. The growth was moderate on ISP-4 and ISP-5 and nutrient agar media, while it was poor on ISP-3 agar. The color of aerial mycelium varied from white to brown, while the substrate mycelium varied from brown to dark brown. Production of melanin pigment was not found by the strains on ISP-7 medium.

Micromorphology of the strains was observed by the coverslip culture method. The cultures showed extensively branched aerial mycelium with a long chain of spores. The strains may be placed in the rectus-flexibilis group of Streptomyces [17, 18]. Spore characters of the strains were recorded with a Scanning Electron Microscope (SEM) **Fig. 1-3**.

The utilization of different carbon sources by the strains indicated its wide pattern of carbon source consumption **Table 2**. The strains efficiently utilized the carbon sources such as glucose, maltose, sucrose, lactose, and starch.

TABLE 2: UTILIZATION OF CARBON SOURCES BY THE STRAINS

Carbon source	Response		
	VLK-15		VLK-15
Glucose	+++	Glucose	+++
Maltose	+++	Maltose	+++
Sucrose	++	Sucrose	++
Lactose	+++	Lactose	+++
Fructose	++	Fructose	++
Galactose	+	Galactose	+
Starch	++	Starch	++
Mannitol	-	Mannitol	-

(+++) = Efficient, (++) = Good, (+) = Moderate (-) = poor

The partial gene sequence of 16S rRNA of the strains was blasted against the nucleotide database of the NCBI. The library search reported matching strains, and the sequences were aligned with the set of published sequences on the basis of by nucleotide BLAST similarity search analysis.

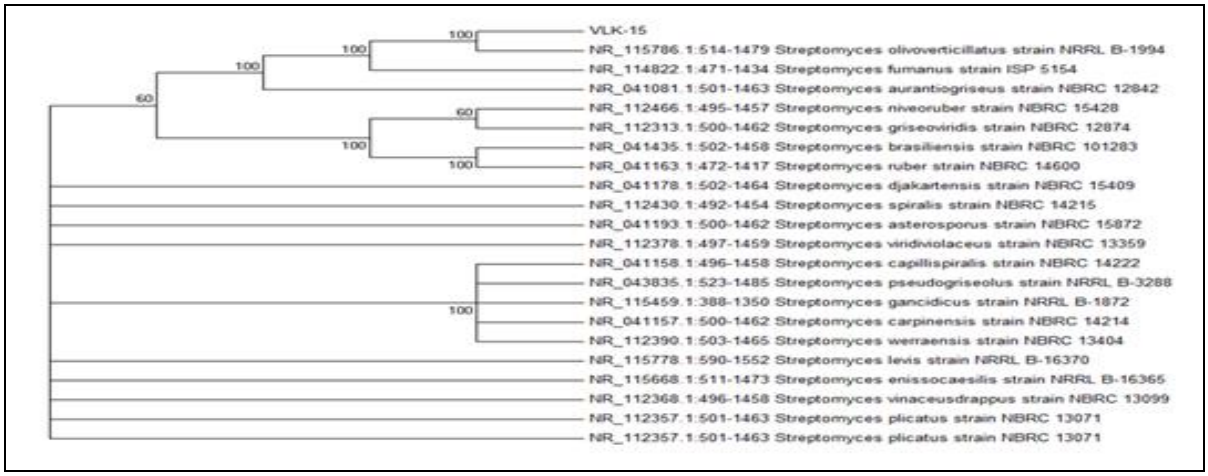

FIG. 4: MAXIMUM PARSIMONY TREE BASED ON PARTIAL 16S rRNA GENE SEQUENCE SHOWING RELATIONSHIP BETWEEN THE ISOLATE VLK-15 AND RELATED MEMBERS OF THE GENUS STREPTOMYCES

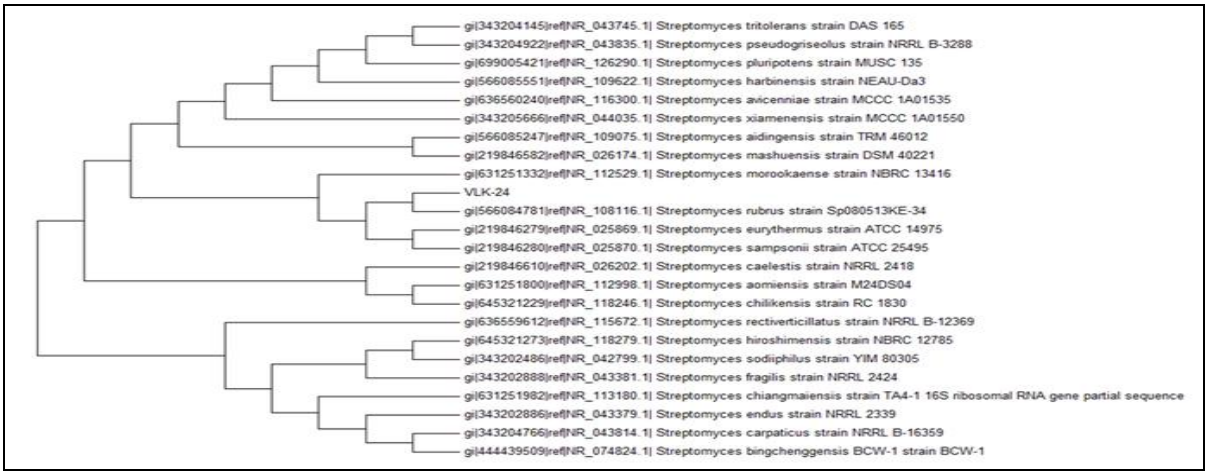

FIG. 5: MAXIMUM PARSIMONY TREE BASED ON PARTIAL 16S rRNA GENE SEQUENCE SHOWING RELATIONSHIP BETWEEN THE ISOLATE VLK-24 AND RELATED MEMBERS OF THE GENUS STREPTOMYCES

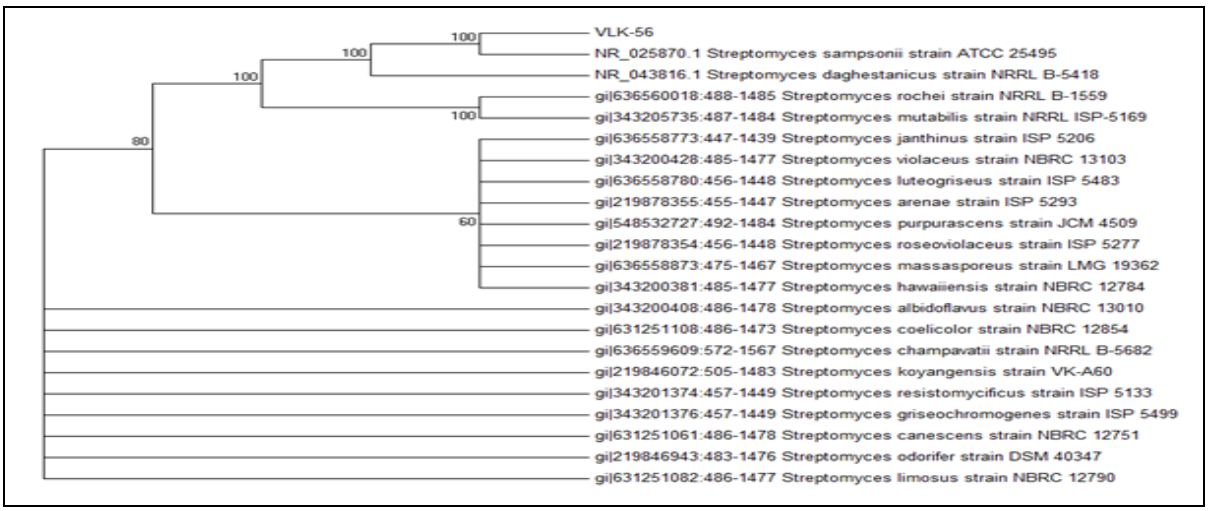

FIG. 6: MAXIMUM PARSIMONY TREE BASED ON PARTIAL 16S rRNA GENE SEQUENCE SHOWING RELATIONSHIP BETWEEN THE STRAIN VLK-56 AND RELATED MEMBERS OF THE GENUS STREPTOMYCES

Based on 16S rRNA gene sequences of the strains showed a close relation with *Streptomyces* sp. **Fig. 4-6**. The 16S rRNA sequences are deposited in the GenBank database of NCBI with the accession numbers MG309759 (*Streptomyces olivoverticillatus* VLK-15), MG309760 (*Streptomyces rubrus* VLK-24), and MF952650 (*Streptomyces sampsonii*-VLK-56).

The antimicrobial activity of the strains was evaluated by using ethyl acetate extract. The crude ethyl acetate extracts were highly effective against *Candida albicans*, *Staphylococcus aureus*, *Bacillus megaterium* followed by *B. subtilis*, *Escherichia coli*, and *Klebsiella pneumonia* **Fig. 7**.

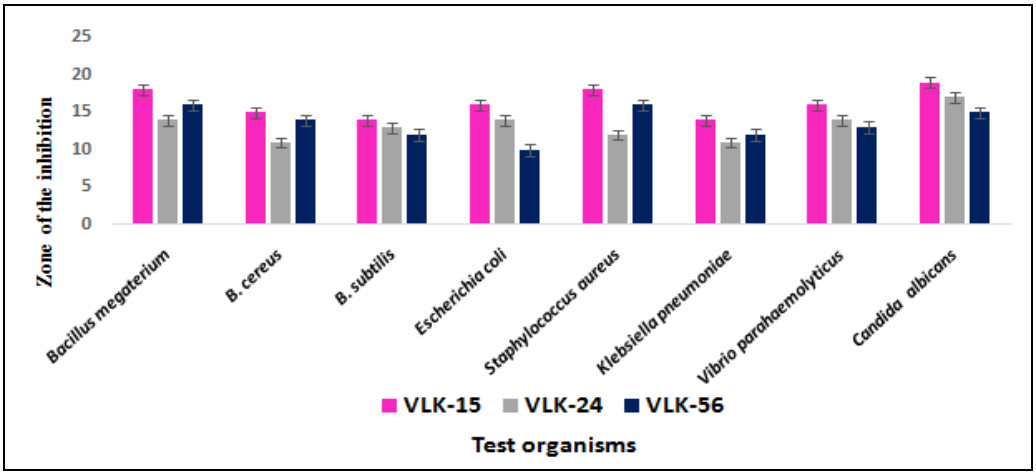

FIG. 7: ANTIMICROBIAL ACTIVITY OF ETHYL ACETATE EXTRACTS OF STRAINS AGAINST THE TEST ORGANISMS

CONCLUSION: The present findings revealed that the bioactive compounds produced by *Streptomyces* spp. possessed high antimicrobial activity. The potent actinobacteria *Streptomyces olivoverticillatus* VLK-15, *Streptomyces rubrus* VLK-24 and *Streptomyces sampsonii*-VLK-56 are reported from the mangrove ecosystem of the coastal region, Krishna district of Andhra Pradesh. It is evident from the study that mangrove habitats serve as a good source of potent actinobacteria with broad-spectrum antimicrobial activity.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding this paper's publication.

REFERENCES:

1. Yang N and Song F: Bioprospecting of novel and bioactive compounds from marine actinomycetes isolated from south China sea sediments. Curr Microbiol 2018; 75: 142-49.
2. Gupte S, Kaur M and Kaur M: Novel approaches to developing new antibiotics. J BacteriolMycol 2017; 4: 00089.
3. Westh H, Zinn CS and Rosdahl VT: Sarisa Study Group: An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. Microbial Drug Resistance 2004; 10: 169-76.
4. Xu DB, Ye WW, Han Y, Deng ZX and Hong K: Natural Products from Mangrove Actinomycetes. Mar Drugs 2014: 12: 2590-2613.
5. Hong K, Gao AH, Xie QY, Gao H, Zhuang L, Lin HP, Yu HP, Li J, Yao XS, Goodfellow M and Ruan JS: Actinomycetes for marine drug discovery isolated from mangrove soils and plants in China. Marine Drugs 2009; 7: 24-44.
6. Undabarrena A, Beltrametti F, Claverías FP, González M, Moore ER and Seeger M: Exploring the diversity and antimicrobial potential of marine actinobacteria from the comau fjord in Northern Patagonia, Chile. Front Microbiol 2016; 7: 1135.
7. Mangamuri U, Muvva V, Poda S, Naragani K, Munaganti RK, Chitturi B and Yenamandra V: Bioactive metabolites produced by *Streptomyces cheonanensis* VUK-A from Coringa Mangrove sediments: Isolation, structure elucidation and bioactivity.3 Biotech 2016; 6: 63.
8. Jose PA and Jebakumar SRD: Unexplored hyper saline habitats are sources of novel actinomycetes. Front Microbiol 2014; 5: 1-3.
9. Kiranmayi MU, Sudhakar P, Krishna N, Yellamanda B and Vijayalakshmi M: Taxonomic characterization of potential bioactive metabolite producing Actinomycetes from mangrove sediments of Coringa. J Phar Res 2011; 4: 4650-53.
10. Munaganti RK, Muvva V, Konda S, Naragani K, Mangamuri UK, Reddy DK and Akkewar DM: Antimicrobial profile of *Arthrobacter kerguelensis* VL-RK_09 isolated from Mango Orchards. Brazilian Journal of Microbiology 2016; 47: 1030-38.
11. Kumar S, Stecher G, Li M, Knyaz C and Tamura K: MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 2018; 35: 1547-49.
12. Bindu BSSNH, Muvva V, Munaganti RK, Naragani K, Konda S and Reddy DK: A study on production of antimicrobial metabolites by *Streptomyces lavendulocolor* VHB-9 isolated from Granite Mines. Brazilian Archives of Biology and Technology 2017; 60: 1-13.
13. Indupalli M, Muvva V, Mangamuri U, Kumar MR and Krishna N: Bioactive compounds from mangrove derived rare *Actinobacterium saccharomonosoraoceani* VJDS-3. 3 Biotech 2017; 8: 103.
14. Naragani K and Muvva V: Actinobacteria from Mangrove Habitats: Diversity and Antimicrobial Activity. European Journal of Biomedical and Pharmaceutical Sciences 2016; 3: 460-67.
15. Hensy WR: Bergey's manual of systematic bacteriology 9th edition. John G. Holt and Stanley T. Williams (EDS).Williams and Wilkins, Baltimore, Philadelphia, Hong Kong, London Munich, Sydney, Tokyo 1994.
16. Pridham TG and Gottlieb D: The utilization of carbon compounds by some Actinomycetales as an aid for species determination. J Bacteriol 1948; 56: 107-14.
17. Naragani K, Mangamuri U, Muvva V, Poda S and Munagant RK: Antimicrobial potential of Streptomyces cheonanensis VUK-A from Mangrove origin. International Journal of Pharmacy and Pharmaceutical Sciences 2016; 8: 53-57.

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