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OPTIMIZATION OF CULTURAL CONDITIONS TO ENHANCE BIOACTIVE METABOLITE PRODUCTION BY *STREPTOMYCES OLIVOVERTICILLATUS* VLK-15

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Keywords:

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ABSTRACT: The study was designed to optimize cultural conditions for enhanced production of bioactive compounds by *Streptomyces olivoverticillatus* VLK-15 isolated from mangrove ecosystem of South Coast of Andhra Pradesh, India. The soil dilution plate technique was employed for the isolation of actinobacteria using starch-casein agar (ISP-6) medium. The potent strain was identified as *Streptomyces olivoverticillatus* VLK-15 based on polyphasic taxonomy. The productivity of the strain was enhanced by amending the medium (ISP-2) with mannitol (0.5%), peptone (0.5%), and K_2HPO_4 (0.05%) adjusted to pH 7.0 and incubated with the culture at 30ºC for ninety-six hours. The secondary metabolites produced under optimized conditions exhibited high antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungi. Hence, it could be a possible source for novel and potential antimicrobial compounds.

INTRODUCTION: The mangrove ecosystem provides a unique environment to support the growth of different microorganisms which are involved in the production of potential secondary metabolites possessing pharmaceutical properties. Mangrove ecosystems are found in tropical and subtropical intertidal regions around the world [1]. Only 5% of microbes, including bacteria, fungi, and actinobacteria isolated from these ecosystems have been studied [2]. Among the microorganisms, marine actinobacteria produce unique and novel secondary metabolites, which play an extensive role in the pharmaceutical and medical industry due to their capacity to produce secondary metabolites.

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More than 10,000 bioactive compounds have been reported from actinobacteria [3], of which the major share (70%) is contributed by the genus *Streptomyces* alone [4]. There is an urgent need to access new actinobacterial species for the exploration of novel bioactive compounds to hit multi-drug-resistant pathogens. Hence, we focused on the isolation and identification of novel species as well as optimization of cultural conditions to enhance the bioactive compounds from rare actinobacterial strains from the mangrove ecosystem of the south coast of Andhra Pradesh.

MATERIALS AND METHODS:

Microorganism: The strain VLK-15 was isolated from the mangrove ecosystem of the south coastal region of Andhra Pradesh, India, and identified as *Streptomyces olivoverticillatus* VLK-15 by using polyphasic taxonomy including molecular analysis (16S r RNA). The gene sequence of the strain VLK-15 is deposited in the GenBank database of NCBI with the accession number MG309759 [5].

Antimicrobial Activity: The antimicrobial activity of strain VLK-15 was tested against bacteria such as *Bacillus megaterium* (NCIM2187), *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 35218) and *Candida albicans* (ATCC 10231) by agar well diffusion assay [6]. Nutrient agar and Czapek-Dox agar media were used for culturing the test bacteria and fungi, respectively. The culture filtrate was extracted with ethyl acetate, and the solvent extract (50 µL) was added to the well made on agar media seeded with test microbe. Wells with only ethyl acetate served as control. The diameter of the inhibition zone was measured after 24 h of incubation at 30°C for bacteria and 24-72 h for yeast.

Optimization of Cultural Conditions for Improved Production of Bioactive Compounds: An attempt was made to optimize the culture conditions such as pH, temperature, carbon sources, nitrogen sources and minerals to enhance the bioactive metabolite production by *Streptomyces olivoverticillatus* VLK-15. The secondary metabolite production by the strain was studied at regular intervals up to eight days. Fermentation was carried out in 250-mL Erlenmeyer flasks with constant shaking at 120 rpm.

The strain was cultured on ten different media such as ISP-1 to ISP-7 and Czapek-Dox, nutrient broth and yeast-starch broth to determine the ideal conditions for the maximum production of bioactive compounds. The bioactive metabolite production in each medium is evaluated [6].

The effect of initial pH on the bioactive compounds was determined by altering pH of the production medium from 4 to 10. Similarly, the optimal temperature for the production of bioactive compounds was evaluated by inoculating the strain at temperatures ranging from 25 to 45°C, while maintaining all other conditions at optimum levels [7].

The influence of carbon sources on bioactive compound production was determined by adding different carbon sources to the fermentation medium, such as dextrose, galactose, maltose, sucrose, mannitol, lactose, starch, cellulose, fructose, and xylose, each at a concentration of 0.4% (w/v) [8]. Impact of different concentrations of the best carbon source (0.1-1% w/v) on bioactive compound production was also studied.

The effect of various nitrogen sources such as ammonium nitrate, peptone, tryptophan, L-proline, tyrosine, urea, yeast extract, histidine, cysteine, and alanine were studied by supplementing nitrogen source (0.4%) to the medium. Further, the best nitrogen source (0.1–1% w/v) for improved production of bioactive metabolites was also recorded [9]. The optimized medium was supplemented with mineral salts such as KH_2PO_4, K_2HPO_4, $FeSO_4$, $ZnSO_4$ and $MgSO_4$ to measure the impact of minerals on the production of bioactive metabolites [10]. The metabolites produced by the strain grown under optimal conditions were tested for antimicrobial activity.

Statistical Analysis: Data obtained on antimicrobial activity under different culture conditions are statistically analyzed with one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION:

Influence of Incubation Time on Biomass and Antimicrobial Activity: The growth pattern and bioactive compound production by the strain VLK-15 were studied at regular intervals up to eight days. The stationary phase of *Streptomyces olivoverticillatus* VLK-15 continued from 72 h to 120 h of incubation **Fig. 1**. The bioactive compounds obtained from the four-day-old culture exhibited good antimicrobial activity against the test microorganisms. This is in conformity with the earlier reports that the extracts of 4-day old cultures of *Streptomyces lavendulocolor* VHB-9 [11], *S. cheonanensis* VUK-A [12], and *S. tendae* TK-VL_333 [13] exhibited high antimicrobial activity against the test microorganisms.

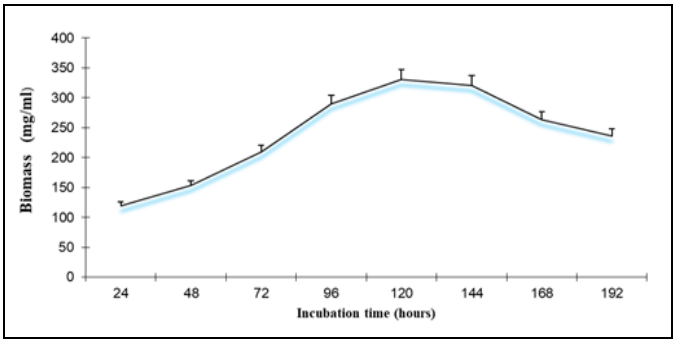

FIG. 1: GROWTH CURVE OF *STREPTOMYCES OLIVOVERTICILLATUS* VLK-15. DATA ARE STATISTICALLY ANALYZED AND FOUND TO BE SIGNIFICANT AT 5%

Effect of Culture Media on Antimicrobial Compounds: Bioactive compound production by the strain VLK-15 was studied in different culture media **Fig. 2**. Among the ten media tested, YMD (ISP-2) supported high levels of bioactive metabolites followed by starch-casein (ISP-6) and nutrient broth.YMD broth for *Streptomyces violaceoruber* VLK-4 [6] and Czapek- Dox broth for *Streptomyces* sp. MNK-7 [14] supported high rates of antibiotic production.

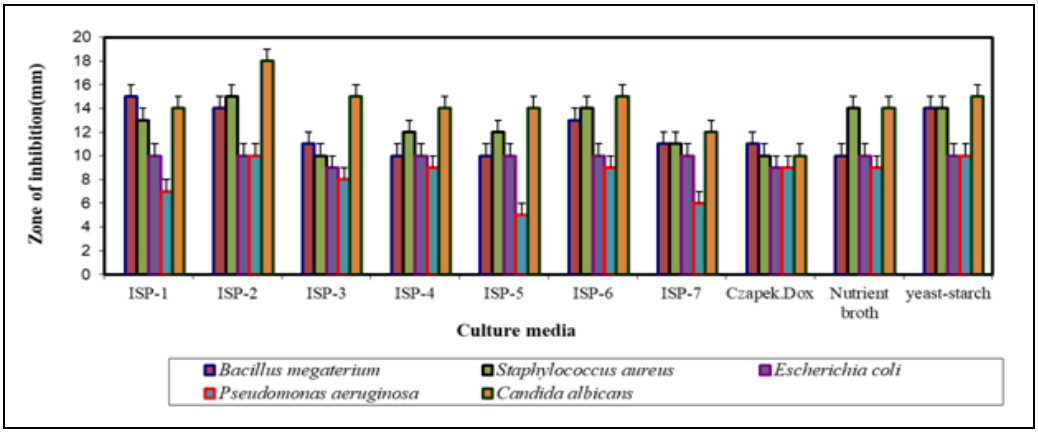

FIG. 2: EFFECT OF DIFFERENT CULTURE MEDIA ON BIOACTIVE COMPOUNDS PRODUCTION BY *STREPTOMYCES OLIVOVERTICILLATUS* VLK-15. Data are statistically analyzed and found to be significant at 5%.

Impact of pH and Temperature on Bioactive Compounds: Maximum antimicrobial production by the strain VLK-15 was found at pH 7 **Fig. 3**. Bioactive metabolites obtained from *Streptomyces lavendulocolor* VHB-9 [11], *S. cheonanensis* VUK-A [12], *S. violaceoruber* VLK-4 [6,] and *Streptomyces* sp. VITSVK9 [7] exhibited good antimicrobial activity when grown at pH 7.

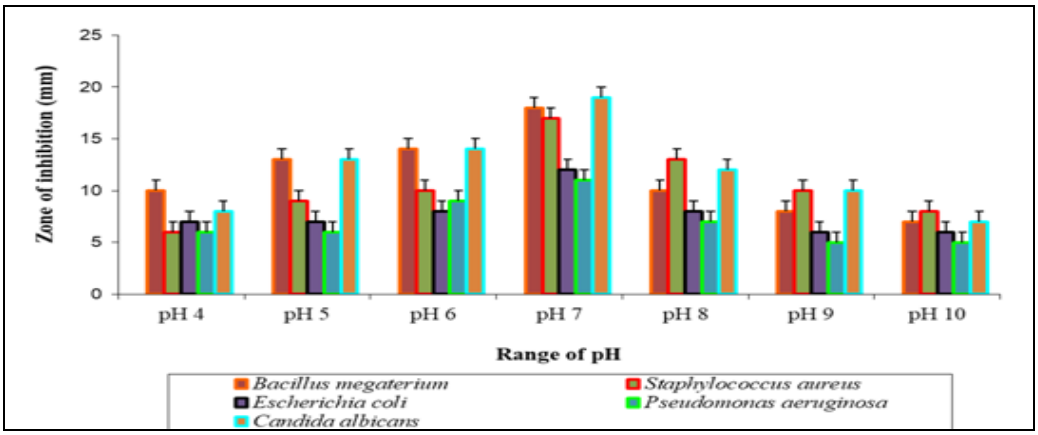

FIG. 3: INFLUENCE OF pH ON SECONDARY METABOLITE PRODUCTION BY *STREPTOMYCES OLIVOVERTICILLATUS* VLK-15. Data are statistically analyzed and found to be significant at 5%.

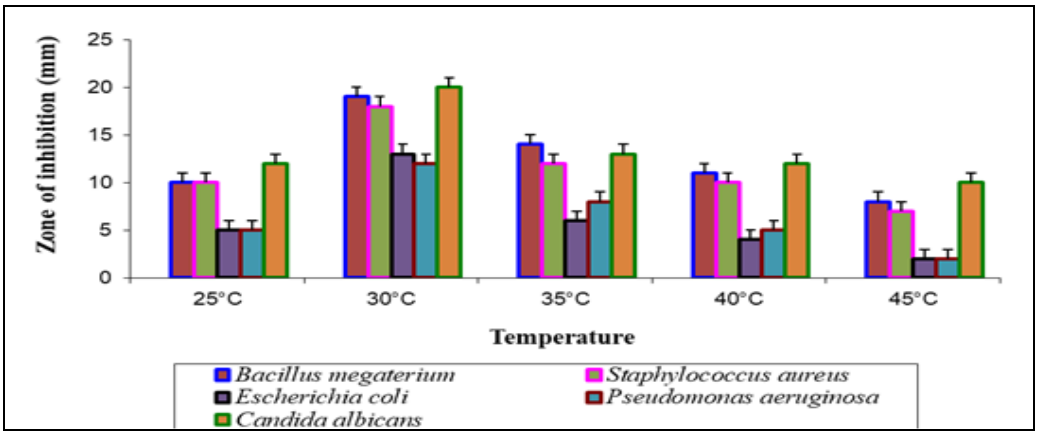

FIG. 4: EFFECT OF TEMPERATURE ON BIOACTIVE METABOLITE PRODUCTION BY *STREPTOMYCES OLIVOVERTICILLATUS* VLK-15. Data are statistically analyzed and found to be significant at 5%.

The production of antimicrobial compounds by the strain VLK-15 increased with rise in the incubation temperature from 25 -30°C **Fig. 4**. Further increase in temperature (above 30°C) resulted in the decline in the production of bioactive metabolites. Earlier reports on *Streptomyces* species also indicated that culturing the strains at 30°C could lead to high levels of bioactive metabolites [11, 12, 15, 16].

Impact of Carbon and Nitrogen Sources on Antimicrobial Activity: The effect of carbon sources on production of secondary metabolites by the strain VLK-15 is shown in **Fig. 5**. Significant production of bioactive compounds was found in mannitol amended medium (ISP-2) followed by dextrose, lactose and starch. As mannitol appeared as the most favored carbon source for secondary metabolite production, different concentrations of mannitol (0.1-1%) were added to find the optimal concentration. Mannitol @ 0.5% exhibited high yields of bioactive compounds **Fig. 6**. Mannitol was reported as a best carbon source to enhance the bioactive metabolite production by *Streptomyces violaceoruber* VLK-4 [6], whereas *S. lavendulocolor* VHB-9 [11] and *Streptomyces cheonanensis* VUK-A [12] utilized lactose as carbon source for secondary metabolite production.

Different nitrogen sources were tested for their impact on bioactive metabolite production by the strain VLK-15. Among the nitrogen sources used, peptone followed by yeast extract and tryptophan was found to be the best nitrogen source for the production of bioactive compounds. As Peptone improved the bioactive compound production by the strain VLK-15, the influence of various concentrations of peptone was assessed, and 0.5% was found to be good for the production of bioactive metabolites **Fig. 8**. These results are comparable with *Streptomyces lavendulocolor* VHB-9 [11] and *Streptomyces cheonanensis* VUK-A [12].

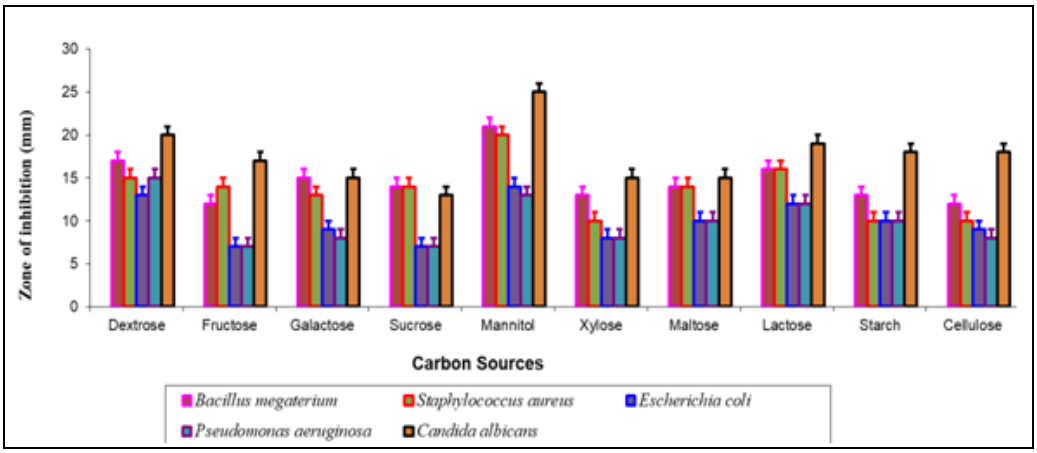

FIG. 5: EFFECT OF DIFFERENT CARBON SOURCES ON BIOACTIVE METABOLITES PRODUCTION BY *STREPTOMYCES OLIVOVERTICILLATUS* VLK-15. Data are statistically analyzed and found to be significant at 5%.

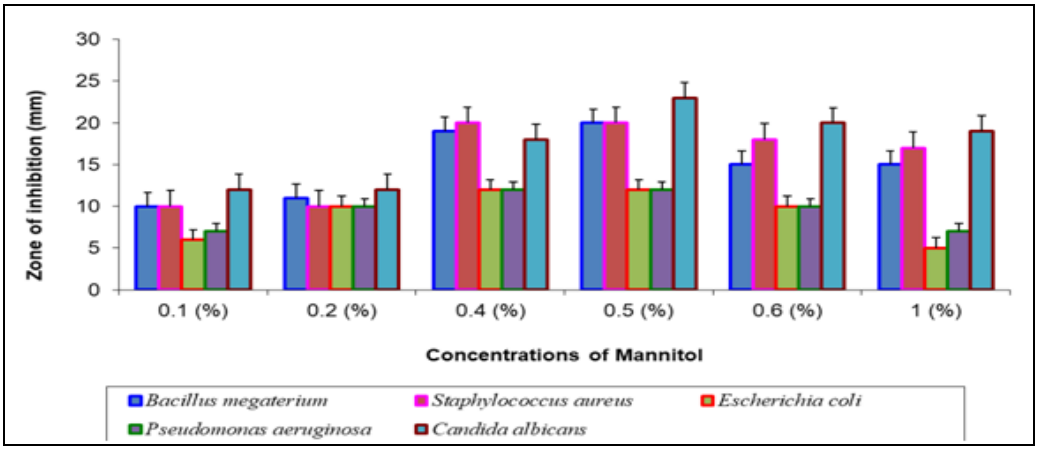

FIG. 6: IMPACT OF DIFFERENT CONCENTRATIONS OF MANNITOL ON BIOACTIVE METABOLITES PRODUCTION BY *STREPTOMYCES OLIVOVERTICILLATUS* VLK-15. Data are statistically analyzed and found to be significant at 5%.

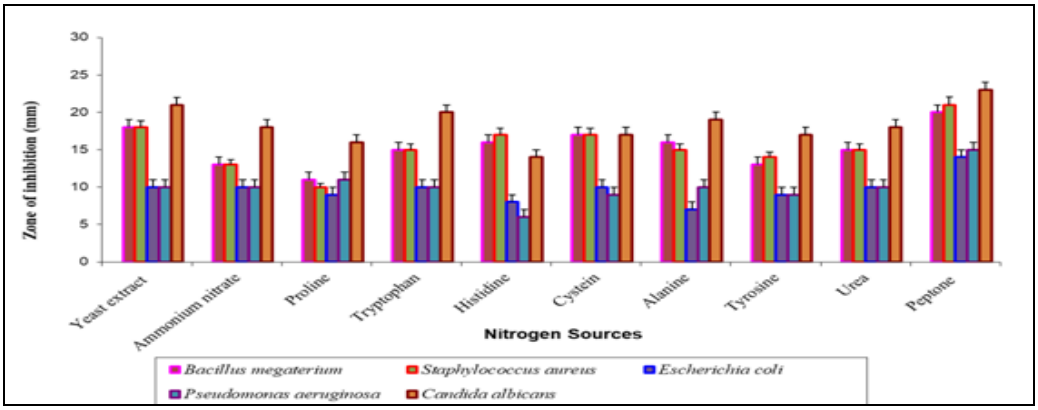

FIG. 7: EFFECT OF NITROGEN SOURCES ON SECONDARY METABOLITE PRODUCTION BY *STREPTOMYCES OLIVOVERTICILLATUS* VLK-15. Data are statistically analyzed and found to be significant at 5%.

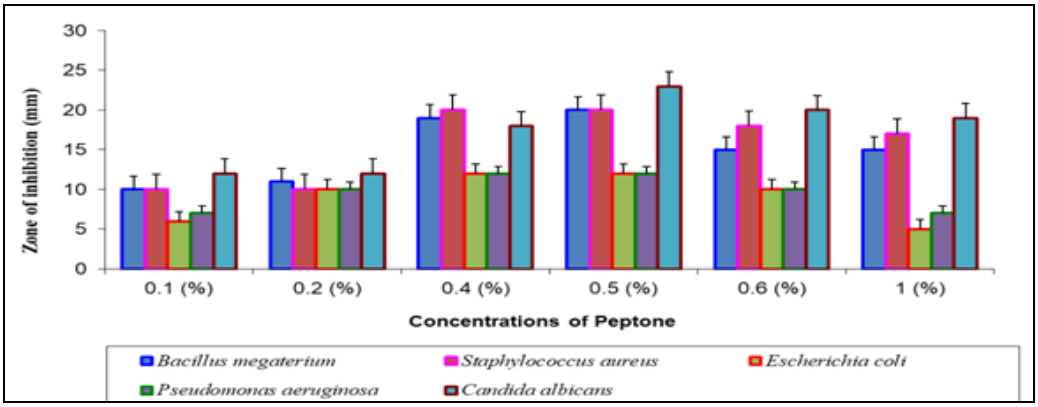

FIG. 8: INFLUENCE OF DIFFERENT CONCENTRATIONS OF PEPTONE ON BIOACTIVE METABOLITE PRODUCTION BY *STREPTOMYCES OLIVOVERTICILLATUS* VLK-15. Data are statistically analyzed and found to be significant at 5%.

Influence of Minerals on Production of Antimicrobial Compounds: The effect of minerals on secondary metabolite production by the strain VLK-15 is shown in **Fig. 9**. Among the minerals tested, K_2HPO_4 followed by $FeSO_4$, KH_2PO_4 and $ZnSO_4$ supported a high yield of bioactive compound production. Similar results were found for *Streptomyces lavendulocolor* VHB-9 [11], *S. violaceoruber* VLK-4 [6], and *Streptomyces cheonanensis* VUK-A [12].

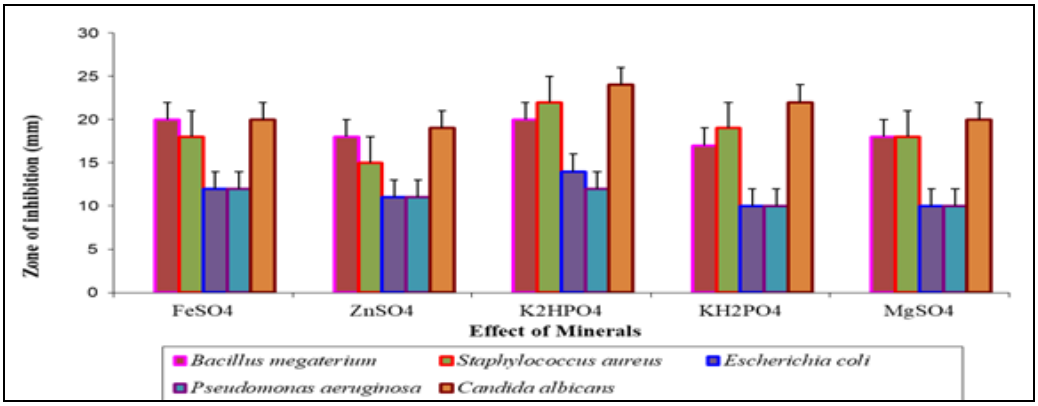

FIG. 9: IMPACT OF DIFFERENT MINERALS ON BIOACTIVE METABOLITE PRODUCTION BY *STREPTOMYCES OLIVOVERTICILLATUS* VLK-15. Data are statistically analyzed and found to be significant at 5%.

The bioactive compounds obtained from optimized fermentation broth exhibited high antimicrobial activity against test bacteria and fungi **Table 1**. Among the bacteria tested, *Bacillus megaterium* was highly sensitive to the compounds produced by the strain VLK-15 followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli*. *Candida albicans* showed high sensitivity to the metabolites.

TABLE 1: ANTIMICROBIAL ACTIVITY OF ETHYL ACETATE EXTRACT OF *STREPTOMYCES OLIVOVERTICILLATUS* VLK-15 UNDER OPTIMIZED CONDITIONS

Test microorganisms	Zone of inhibition (mm)
Bacillus megaterium	32
Staphylococcus aureus	30
Pseudomonas aeruginosa	27
Escherichia coli	26
Candida albicans	30

CONCLUSION: In the present study a potent strain *Streptomyces olivoverticillatus* VLK-15 isolated from the mangrove ecosystem of the south coast of Andhra Pradesh, India, possessing antagonistic activity, revealed its potential to inhibit several pathogenic bacteria and fungi. Bioactive metabolite production was often influenced by various environmental factors, including nutrients (nitrogen and carbon source), temperature and pH. Maximum bioactive compound production by the strain was found when cultured on ISP-2 medium amended with mannitol @ 0.5% and peptone @ 0.5% adjusted to pH 7 for 96 hours at 30ºC. Further studies on purification, characterization, and identification of bioactive metabolites of *Streptomyces olivoverticillatus* VLK-15 are in progress. This is the first report on the optimization of culture conditions to enhance the bioactive metabolites by the strain *Streptomyces olivoverticillatus* VLK-15.

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CONFLICTS OF INTEREST: All authors declare that there are no conflicts of interest.

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