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Research Article

OPTIMIZATION OF PROCESS PARAMETERS FOR IMPROVED PRODUCTION OF ANTIMICROBIAL METABOLITES FROM *NOCARDIOPSIS FLAVESCENS* VJMS-18 ISOLATED FROM SOUTH-COASTAL REGIONS OF ANDHRA PRADESH, INDIA

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ABSTRACT

Objective: The objective of the present study is to optimize the cultural parameters for *Nocardiopsis flavescens* VJMS-18 strain isolated from marine sediment samples of the south-coast of Andhra Pradesh, India.

Methods: The strain *N. flavescens*VJMS-18 was identified based on morphological, physiological, biochemical and molecular approaches. The effect of environmental parameters such as incubation period, pH, temperature and salt concentration and the effect of various nutrients such as carbon and nitrogen sources and minerals on the bioactive metabolite production by *N. flavescens* VJMS-18 was evaluated by employing agar well diffusion assay.

Results: The nutritional requirements and cultural conditions to enhance the yield of secondary metabolites are optimized under shake flask conditions. ISP-2 medium supplemented with sodium chloride at 3% maintained at pH 7.0 supported the maximum yield of secondary metabolites by the strain when incubated at 35°C for 8 days. The strain exhibited a broad spectrum of antagonistic activity against Gram-positive (*Staphylococcus aureus* and *Bacillus megaterium*) and Gram-negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*) as well as fungus (*Candida albicans*).

Conclusion: It was found that the antimicrobial metabolite production by the strain was positively influenced by carbohydrates, nitrogen sources and minerals.

Keywords: Marine actinomycetes, Nutritional parameters, Antagonistic activity, *Nocardiopsis flavescens*VJMS-18.

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INTRODUCTION

The ocean sediment is an untouched source of rare microorganisms whose strange evolutionary environment could be appeared in the development of novel secondary metabolites used for pharmaceuticals. In India, about 1000 natural products were derived from marine microbes [1], in which marine actinomycetes are proven as a potential source of bioactive compounds and the richest source of secondary metabolites.

Marine actinomycetes are amenable to the demanding marine environment, to attain a particular advantage and to contend with competitors, they have developed unique metabolic and physiological potentiality not found in terrestrial ones. It is now clear that specific populations of marine-adapted actinomycetes not only exist but add significant new diversity to actinomycete taxa [2,3]. Actinomycetes are steady and enduring microbes of the marine ecosystem that are well-marked for their potency to produce bioactive metabolites with complex diversity and various potent biological activities.

Actinomycetes are present in various ecological habitats and marine environments [4] and to cope with environmental stress, they have advanced complex stress management for their survival, which is being hidden for multiple purposes [5]. They are being exploited for various commercial applications in the environmental, biomedical and industrial sectors [6]. A huge number of structurally attractive and biologically active secondary metabolites produced by microorganisms have been reported, many of them being tapped by the pharmaceutical industry as potent antibiotics [7,8]. Focus on rare resources such as marine actinomycetes has attracted the special attention in recent years due to their scope to produce biologically active secondary metabolites, which have not been discovered in terrestrial microorganisms [9-14].

One of the pharmaceutically and biotechnologically important genera that attract natural products research is *Nocardiopsis*, mainly for its ability to produce a wide variety of secondary metabolites accounting for its wide range of biological activities. *Nocardiopsis* species are Gram-positive, aerobic, halotolerant and catalase-positive actinomycetes. They possess nocardioform mycelia with long chains of spores on the aerial parts. They are valuable treasures to the industry as they could produce bioactive agents such as griseusin D, apoptolidin and methyl pendolmycin [15-17]. Hence, the discovery of additional species to this genus will tend to both understanding of their ecological roles and the stipulation of bioresources for industrial applications.

Our continuous screening program for potent *Actinobacteria* resulted in the isolation of a promising strain, VJMS-18, with high antimicrobial activity, which was further identified as *Nocardiopsis flavescens*. The strain was isolated from marine sediment samples of south coastal regions of Andhra Pradesh, India. 16S rDNA sequence of the strain was deposited in GenBank with accession number MH423862 [18]. The objectives of the present study include the optimization of process parameters for enhancing the production of bioactive metabolites by strain and the evaluation of antimicrobial activity.

METHODS

The strain *N. flavescens*VJMS-18 was isolated from marine sediment samples of south coastal regions of Andhra Pradesh, India, by using serial dilution plate technique on yeast extract malt extract dextrose agar (ISP-2) with pH 7.0 [19]. The pure culture was maintained on ISP-2 agar slants at 4°C [20]. The strain has been deposited in the NCBI GenBank with accession No. MH423862.

Nutritional parameters affecting the bioactive metabolite production by the strain

Bioactive metabolite production by the strain was optimized using different parameters such as incubation period, pH, temperature, sodium chloride, carbon, nitrogen sources and minerals.

Growth pattern and effect of incubation time on bioactive metabolite production by the strain

The growth pattern of *N. flavescens*VJMS-18 and its antimicrobial activity against Gram-positive bacteria (*Bacillus megaterium*, *Streptococcus mutans* and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Xanthomonas campestris* and *Pseudomonas aeruginosa*) and fungi (*Aspergillus flavus*, *Candida albicans,* and *Penicillium citrinum*) were recorded by culturing the strain in ISP-2 broth for 10 days. The strain was inoculated into 250 mL flasks containing 100 mL ISP-2 broth and incubated at 35°C on a rotary shaker at 120 rpm. At every 24 h interval, the biomass of strain and production of antimicrobial metabolites were determined. Biomass was measured as the dry weight of the cell mass (mg/100 mL culture medium). The supernatant was extracted with ethyl acetate, vacuum dried in a rotavapor and used for testing the antimicrobial activity against bacteria and fungi through agar well diffusion method [21].

Influence of initial pH and incubation temperature on bioactive metabolite production by the strain

The influence of initial pH on bioactive metabolite production by the strain was determined by adjusting the pH of the production medium from 5.0 to 9.0. The optimal pH achieved at this step was used for further study [22]. Similarly, the optimum temperature for bioactive metabolite yield was measured by incubating the production medium at temperatures ranging from 20 to 40°C [23].

Influence of sodium chloride on bioactive metabolite production by the strain

The influence of salinity on bioactive metabolite production by the strain was recorded by culturing the strain in the fermentation medium amended with different concentrations of sodium chloride (0–12%) at optimum pH and temperature for 8 days. The salt concentration in which the strain exhibits the optimum level of bioactive metabolites was fixed for further studies.

Influence of carbon and nitrogen sources on bioactive metabolite production by the strain

Various carbon sources such as arabinose, galactose, dulcitol, maltose, mannitol, starch, sucrose, lactose, fructose, cellulose and sorbitol at 1% were added to the optimized medium by replacing the carbon source. The influence of varying concentrations of the best carbon source (0.5–2.0%) on bioactive metabolite production was also examined.Similarly, the impact of different nitrogen sources on the yield of antimicrobials of the strain was studied by supplementing the nitrogen source in the medium with different nitrogen sources like peptone, glycine, urea, glutamine, asparagine, cystiene, L-arginine, ammonium sulfate, tryptone, beef extract and sodium pyruvate in the optimized medium by replacing the nitrogen source. Further, the impact of different levels of optimized nitrogen source (0.5–2.0%) was studied to enhance antimicrobial metabolite production [24].

Test organisms

The antimicrobial metabolites produced by the strain under optimized conditions were tested against bacteria *(S. aureus (MTCC 3160), B. megaterium (NCIM 2187), S. mutans (MTCC 497), X. campestris (MTCC 2286), K. pneumoniae (MTCC 109), P. aeruginosa (ATCC 9027) and E. coli (ATCC 9027)) and fungi A. flavus (ATCC 189), C. albicans (MTCC 183) and P. citrinum (MTCC 6849)* using agar plate diffusion assay.

Statistical analysis

Statistical analysis was carried out for antimicrobial metabolite production by the strain using One-way analysis of variance.

RESULTS AND DISCUSSION

Growth pattern and antimicrobial profile of the strain

The growth pattern and antimicrobial profile of *N. flavescens*VJMS-18 were studied at regular intervals up to 10 days in batch culture. The stationary phase of the strain extended from 192 h to 216 h of incubation (Fig. 1). The secondary metabolites obtained from the 8-day-old culture showed high antimicrobial activity against the test microbes. The antimicrobial metabolites produced from 11-day-old culture of *Nocardiopsis litoralis* VSM-8 [25], 5-day-old culture of *Nocardiopsis halotolerans* VJPR-2 [26] and 4-day-old culture of *Nocardia metallicus* VJSY-14 [27] exhibited high antagonistic activity. The secondary metabolites obtained from 6-day-old culture of *Streptomyces vinaceusdrappus* VJMS-4, *Streptomyces rectiverticillatus* VJMS-8 [28] and 5-day-old culture of *Streptomyces albogriseolous* VJMS-7 [29] showed high antimicrobial activity against the test microbes.

Influence of initial pH and incubation temperature on bioactive metabolite production by the strain

The various environmental requirements influence growth and bioactive metabolite production by actinomycetes. Maximum growth and antimicrobial metabolite production were obtained at pH 7.0 (Fig. 2). The actinomycetal strains such as *N. metallicus* DSM 44598 [30], *Nocardiopsis dassonvillei* [31] and *Nocardiopsis luteus* [32] showed optimum level of antibiotic production at 7.0 pH. The influence of temperature on bioactive metabolite production by the strain is presented in Fig. 3. Good growth as well as antimicrobial metabolite production was obtained at 35°C. The organism appeared to

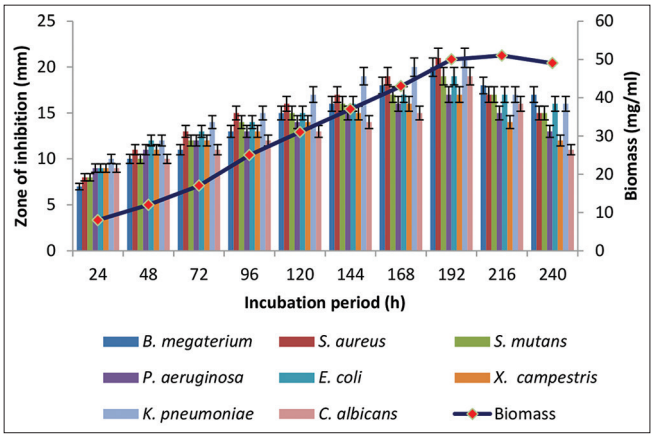

Fig. 1: Influence of incubation period on bioactive metabolite production by *Nocardiopsis flavescens*VJMS-18. (Data are statistically analyzed and found significant at 5%)

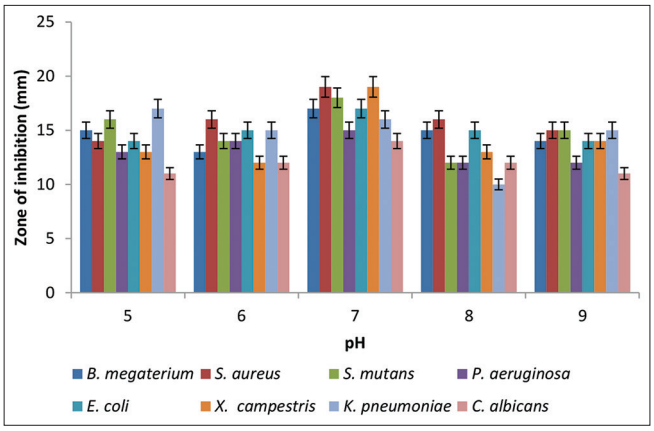

Fig. 2: Influence of pH on bioactive metabolite production by *Nocardiopsis flavescens*VJMS-18. (Data are statistically analyzed and found significant at 5%)

be mesophilic in terms of its optimum temperature for growth. Several strains of *Actinobacteria* belonging to the genus *Nocardiopsis* including *Nocardiopsis algeriensis* [33] and *N. halotolerans* [34], showed optimum levels of antibiotic production at 35°C.

Influence of sodium chloride on bioactive metabolite production by the strain

Optimum salt requirement for bioactive metabolite production was examined by supplementing the production medium with different salt concentrations ranging from 0 to 12%. Sodium chloride at the concentration of 3% was found to be optimum for antimicrobial compound production by *N. flavescens*VJMS-18 (Fig. 4). Further increase in salt concentration reduced the antimicrobial activity. The requirement of sodium chloride for the production of bioactive metabolites seems to be different among actinomycete strains. The optimum sodium chloride concentration for antimicrobial metabolite production was reported to be 3% for *Nocardiopsis nanhaiensis* [35] and *Nocardiopsis fildesensis* [36].

Influence of carbon sources on bioactive metabolite production by the strain

The effect of different carbon sources was evaluated for their impact on antimicrobial metabolite production (Fig. 5). Among the various carbon sources tested, mannitol was the best one for bioactive metabolite production. Kavitha *et al*., reported that *Nocardia levis* MK-VL_113 isolated from laterite soils utilized sucrose as the sole carbon source for antibiotic production. As mannitol was the most preferred carbon source for biomass and bioactive metabolite production by the strain, different levels of mannitol (0.5–2.0%) were tested to determine the optimal concentration for bioactive metabolite production (Fig. 6). Mannitol at 1.0% supplemented in the medium promoted the bioactive metabolite production.

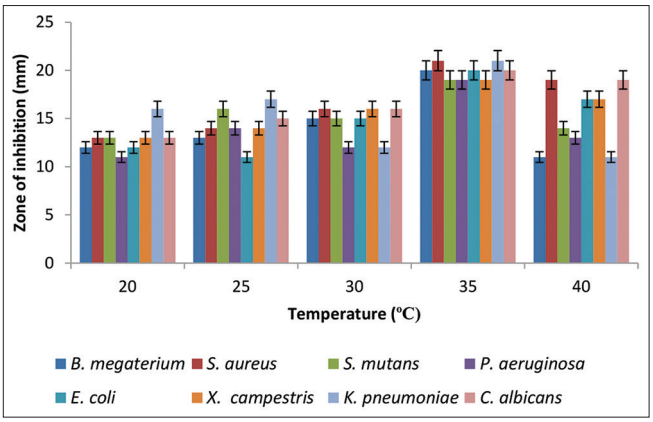

Fig. 3: Influence of Temperature on bioactive metabolite production by *Nocardiopsis flavescens* VJMS-18. (Data are statistically analyzed and found significant at 5%)

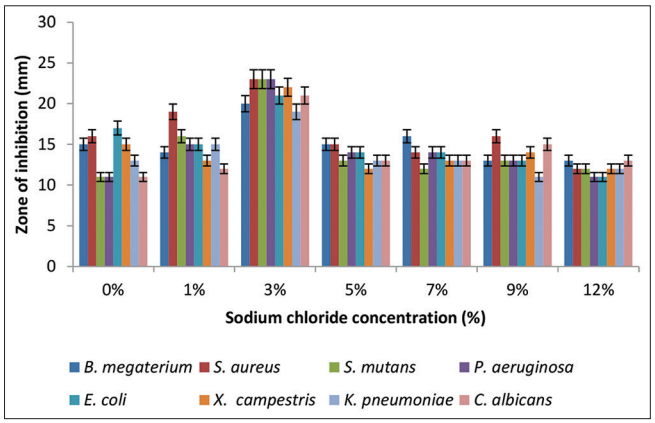

Fig. 4: Influence of sodium chloride concentration on bioactive metabolite production by *Nocardiopsis flavescens*VJMS-18. (Data are statistically analyzed and found significant at 5%)

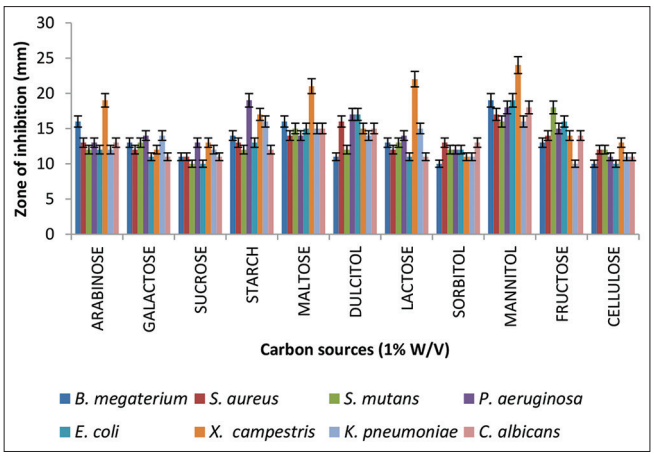

Fig. 5: Influence of carbon sources on bioactive metabolite production by *Nocardiopsis flavescens*VJMS-18. (Data are statistically analyzed and found significant at 5%)

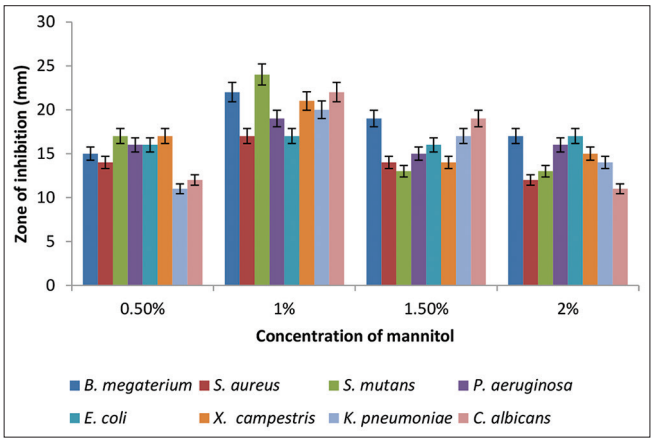

Fig. 6: Influence of mannitol concentration on bioactive metabolite production by *Nocardiopsis flavescens*VJMS-18. (Data are statistically analyzed and found significant at 5%)

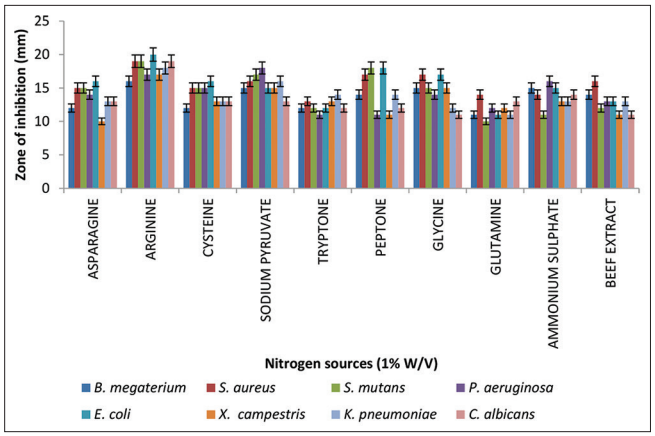

Fig. 7: Influence of different nitrogen sources on bioactive metabolite production by *Nocardiopsis flavescens*VJMS-18. (Data are statistically analyzed and found significant at 5%)

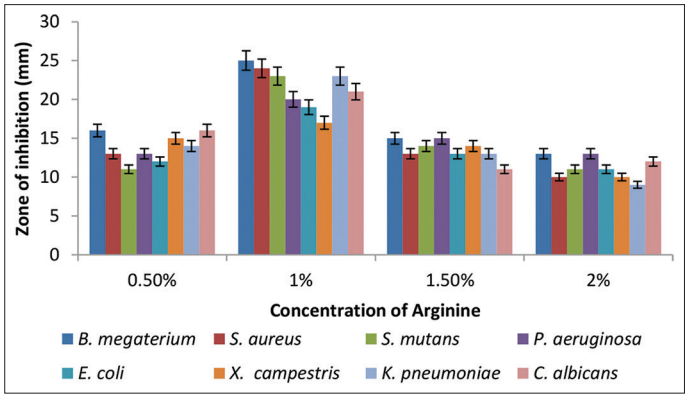

Fig. 8: Influence of arginine concentration on bioactive metabolite production by *Nocardiopsis flavescens*VJMS-18. (Data are statistically analyzed and found significant at 5%)

Influence of nitrogen sources on bioactive metabolite production by the strain

Different nitrogen sources were found to have a significant effect on secondary metabolite production by *N. flavescens*VJMS-18. Maximum antimicrobial activity was obtained in culture filtrates supplemented with arginine followed by peptone and glutamine (Fig. 7). Arginine at 1% supported high metabolite production (Fig. 8). Antibiotic production was found to be governed by nitrogen sources [37] and the utilization of nitrogen sources for the production of bioactive metabolites seems to be different among actinomycete strains.

CONCLUSION

In the present study, *N. flavescens*VJMS-18 isolated from south-coastal regions of Andhra Pradesh, India, exhibited high antimicrobial activity when cultured in modified ISP-2 broth with malt extract (1%), mannitol (1%), arginine (1%) and sodium chloride (3%) with pH 7.0 and incubated at 35°C for 216 h. Among the bacteria tested, *E. coli*, *S. aureus* and *K. pneumoniae* were highly sensitive to the metabolites followed by *X. campestris*, *S. mutans* and *B. megaterium* while *C. albicans* exhibited high sensitivity followed by *A. flavus* and *P. citrinum* with respect to fungi.

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AUTHORS CONTRIBUTION

The concept and design of the study, data collection, data analysis and manuscript writing were done by the first and corresponding author.

CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

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