Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Effect of nutritional and cultural conditions on bioactive metabolite production by *Streptomyces variabilis* strain PO-178

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ARTICLE DETAILS

Article history:
Received on 15 February 2017
Modified on 29 March 2017
Accepted on 4 April 2017

Keywords:
Streptomyces variabilis,
Nutritional,
Cultural,
Agar well diffusion

ABSTRACT

Production of secondary metabolites by microorganisms is highly influenced by the nutritional and cultural conditions. The objective of the present study was to study the effect of nutritional and cultural conditions on the production of antibacterial metabolite by *Streptomyces variabilis* strain PO-178 isolated from Western Ghat soil of Agumbe, Karnataka, India. Starch casein nitrate broth was found to be the best medium for production of antibacterial agents by strain PO-178. The temperature 45°C and pH 9 was shown to be optimum for production of bioactive metabolites. Starch and casein was found to be best carbon and nitrogen source respectively. Increasing starch concentration resulted in better production of antibacterial agents. Sodium chloride at concentration 2% influenced maximum production of bioactive agents. The presence of all trace salts was required for better production of antibacterial agents. It is clear from the study that the production of bioactive agents by strain PO-178 is influenced by several nutritional and cultural conditions. Further, statistical approaches can be conducted to obtain the possible combinations of these conditions for better production of antibacterial agents.

INTRODUCTION

Production of bioactive agents by microorganisms is highly dependent on strains of microorganisms and nutritional and cultural conditions. Composition of media plays an important role in the production of bioactive agents by microorganisms. Minor variation made in the culture medium can exert huge impact on quantity of secondary metabolites produced by the microorganisms. The components of culture media and their optimum levels are crucial for the production of secondary metabolites. Hence, much effort is focused on designing and optimization of production medium, especially in the field of antibiotic production. Besides, better production of secondary metabolites is also influenced by conditions such as temperature, pH and aeration. The optimization experiments are generally carried out using non-statistical (one factor at a time) and statistical experimental design approaches. Design of an appropriate culturing system require a series of trials such as selection of basal medium, selection of carbon and nitrogen sources, trace elements and optimization of the physical parameters. In the classical one factor at a time approach, changing one independent variable while fixing all others at a fixed level is carried out[1-6].

Actinomycetes are Gram positive eubacteria characterized by high GC content and filamentous nature. They are involved in a range of important processes in a wide range of habitats. Actinomycetes are widespread in soil and degrade variety of complex polymers such as cellulose, pectin, lignocellulose and a variety of xenobiotics. They produce a range of bioactive metabolites owing to their greater metabolic capacities. The species belonging to the genus *Streptomyces* are dominant in *rhizosphere* region of soil and contribute to significant turnover of complex biopolymers. Besides, Streptomyces species are known to produce vast majority of bioactive metabolites being produced by actinomycetes. The metabolites produced by *Streptomyces* species are shown to display bioactivities such as antimicrobial, antiviral, antioxidant, insecticidal, plant growth promotory, herbicidal, analgesic, anti-inflammatory, antipyretic and other

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pharmacological activities[7-12]. During a screening programme, we have isolated *Streptomyces variabilis* strain PO-178 from Western Ghat soil of Agumbe, Shivamogga district, Karnataka, India. The strain was shown to exhibit antimicrobial, antioxidant, insecticidal, cytotoxic, anthelmintic, analgesic, anti-inflammatory, antipyretic and CNS depressant activities[13-15]. In the present study, we report optimization of nutritional and cultural conditions (by varying one factor at a time) for the production of antibacterial metabolite by *S. variabilis* strain PO-178.

MATERIALS AND METHODS

Isolation of *S. variabilis* strain PO-178

The strain PO-178 was isolated from a rhizosphere soil of Agumbe, Western Ghat region of Shivamogga district, Karnataka, India. The strain was identified as *S. variabilis* on the basis of cultural, microscopic, biochemical and 16S rDNA sequence analysis[15].

Optimization parameters

The influence of various nutritional and cultural conditions *viz*., culture media, incubation time, temperature, pH, carbon sources, nitrogen sources, sodium chloride, starch concentration and trace elements was studied by using non-statistical approach i.e., by varying one parameter at a time. During incubation, the inoculated flasks were constantly observed for growth of the strain and contamination, if any. After incubation, the contents were filtered through sterile Whatman filter paper No. 1, culture filtrates were extracted using butanol and the antibacterial activity of butanol extracts was assessed against *S. aureus* and *P. aeruginosa* by Agar well diffusion assay[16].

Effect of Culture media

The strain PO-178 was inoculated aseptically into Erlenmeyer flasks containing sterile Starch casein nitrate broth (SCNB), Inorganic salts-starch broth (ISSB), Yeast extract-malt extract broth (YEMEB) and Oat meal broth (OMB) media. The inoculated flasks were incubated at 30°C for 10 days[17,18]. SCNB was found to be best medium for production of antibacterial metabolites. Hence, SCNB was chosen for further optimization studies.

Effect of incubation period

The strain PO-178 was inoculated aseptically into Erlenmeyer flasks containing sterile Starch casein nitrate broth media and incubated at 30°C for up to 13 days. Antibacterial activity of culture filtrate was assessed after 3, 5, 7, 9, 11 and 13 days. During incubation, the flasks were observed for growth and contamination, if any[19]. Maximum antibacterial activity was observed in culture filtrate obtained on 7th day of incubation. Hence, the harvesting of culture was carried out on 7th day of incubation.

Effect of pH

The strain PO-178 was aseptically inoculated into Erlenmeyer flasks containing sterile SCN broth adjusted with different pH values *viz*., 3, 5, 7, 9 and 11 using 0.1N Hydrochloric Acid and 0.1N Sodium hydroxide. The flasks were then incubated at 30°C for 7 days[19].

Effect of temperature

The strain PO-178 was inoculated aseptically into Erlenmeyer flasks containing sterile SCN broth. The flasks were incubated at different temperature regimes *viz*., 4°C, 20°C, 30°C and 45°C for 7 days[19].

Effect of carbon sources

The strain PO-178 was inoculated into Erlenmeyer flasks containing sterile SCN broth with glucose, galactose, lactose, maltose and starch as sole carbon sources (1%). The flasks were incubated at 30°C for 7 days[19,20].

Effect of nitrogen source

The individual effect of different nitrogen sources *viz*., beef extract, yeast extract, urea, casein and peptone was studied on antibacterial metabolite production by strain PO-178. The flasks containing SCNB with different nitrogen sources were inoculated with isolate PO-178 and incubated at 30°C for 7 days[19].

Effect of starch concentration

The influence of starch concentration on antibacterial metabolite production was checked by inoculating strain PO-178 into SCN broth adjusted with different concentrations of starch *viz*., 0, 0.5, 1.0, 2.0 and 5.0%. The flasks were incubated at 30°C for 7 days[20,21].

Effect of sodium chloride

The strain PO-178 was inoculated aseptically into Erlenmeyer flasks containing SCN broth with different concentrations of sodium chloride *viz*., 0.0, 0.2, 0.5, 1.0, 2.0 and 3.0% and incubated at 30°C for 7 days[19].

Effect of trace salts

The effect of trace salts *viz*., Magnesium sulphate, Calcium carbonate and Ferrous sulphate on production of antibacterial metabolite by strain PO-178 was studied by modifying SCN broth. The strain PO-178 was inoculated into Erlenmeyer flasks containing SCN broth with all trace salts, without trace salts and broth lacking each trace salt. The flaks were incubated at 30°C for 7 days. Media containing all the three trace salts served as control[20,22].

RESULTS AND DISCUSSION

Effect of culture media

The production of bioactive metabolites by actinomycetes is influenced by the culture media to a large extent. The result of effect of culture media on production of antibacterial metabolites by strain PO-178 is shown in Figure 1. The production and accumulation of bioactive agents was observed in all broth media. SCNB was found to be the best medium for production of antibacterial metabolites by isolate PO-178 as indicated by wider zones of inhibition produced by butanol extract from SCNB. Least inhibitory activity was observed in case of butanol extract obtained from OMB. Similar result was observed in earlier studies. *Streptomyces* Ds-104 was found produce inhibitory compounds optimally in SCNB when compared to other media[17]. Starch casein broth was the best medium for production of antimicrobial metabolites by *Streptomyces* sp. MP 525[18]. SCN broth appeared to be good for the production of antimicrobial metabolites by *S. fradiae*[23].

Effect of incubation time

Among various cultural conditions, the time of incubation is crucial for production and harvesting the bioactive agents. It is highly varied among actinomycetes. In the present study, it is observed that antibacterial metabolites production by the isolate was started after 3rd day of incubation. The high antibacterial activity was observed on 7th day of incubation and then production was gradually declined (Figure 2). There was no antibacterial metabolite production on 3rd day as indicated by no inhibition of test bacteria. It has been found that *Streptomyces* KEH23[24] and *Streptomyces plicatus*[21] produced high antimicrobial metabolites on 4th day of incubation. Ripa *et al*.[19] observed maximum production of antimicrobial metabolite by *Streptomyces* sp. RUPA-08PR after 10 days of incubation. Saha *et al*.[25] observed production of antimicrobial metabolite production by *Streptomyces* sp. MNK7 on 3rd day which reached maximum level after 10 days of incubation and later declined. In the study by Mangamuri *et al*.[26], secondary metabolites obtained from six day old culture of *S. tritolerans* produced high antimicrobial activity.

Effect of pH

The initial pH of the production medium has a direct influence on the production of bioactive agents by actinomycetes. Figure 3 shows the effect of initial pH of medium on production of bioactive metabolite by the strain PO-178. At pH 3, there was no inhibitory activity. Maximum inhibitory activity was observed in case of extract from medium adjusted with pH 9. The result is in accordance with an earlier study in which *S. sannanensis* strain RJT-1 was found to produce antimicrobial metabolites optimally at pH 9.0[27]. Above pH 9, a decline in the antibacterial activity was observed. The optimum pH for growth and metabolite production by actinomycetes is highly varied and usually ranges from 6.0 to 8.0[28]. The production of antimicrobial metabolite by *Streptomyces* sp. MNK7 was highest at pH 5[25]. Optimum production of bioactive agents by *Streptomyces violatus* occurred at pH 7.0[29]. A pH of 7.5 was found to be optimum for bioactive metabolite production by *S. tritolerans*[26]. Ripa *et al*.[19] found optimum production of bioactive agents by *Streptomyces* sp. RUPA-08PR at pH 8.0.

Effect of temperature

The temperature of incubation influences significantly the growth and bioactive metabolite production by actinomycetes. Figure 4 shows the influence of incubation temperature on production of bioactive metabolite. There was no production of inhibitory agents in broth incubated at 4°C. Increasing the temperature of incubation resulted in higher antibacterial activity of the isolate. High inhibitory activity was observed in case of extract obtained from culture incubated at 45°C. However, the optimum temperature for most actinomycetes ranges from 23 to 37°C respectively[28]. It has been show that incubation temperature 30°C was found optimum for maximum metabolite production by *S. violatus*[29], *S. albidoflavus* C247[30] and *Streptomyces* KEH23[24]. 35°C was found to be optimum for bioactive metabolite production by *Streptomyces* sp. MNK7[25]. Ripa *et al*.[19] observed high antimicrobial metabolite production by *Streptomyces* sp. RUPA-08PR at 39°C. The production of bioactive agents by *S. tritolerans* was highest at 35°C[26].

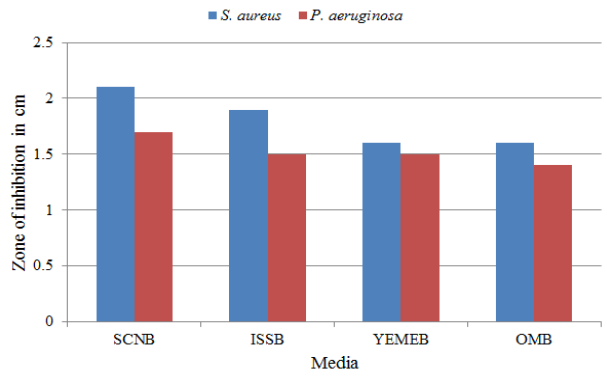

Figure 1: Effect of culture media on production of antibacterial metabolite

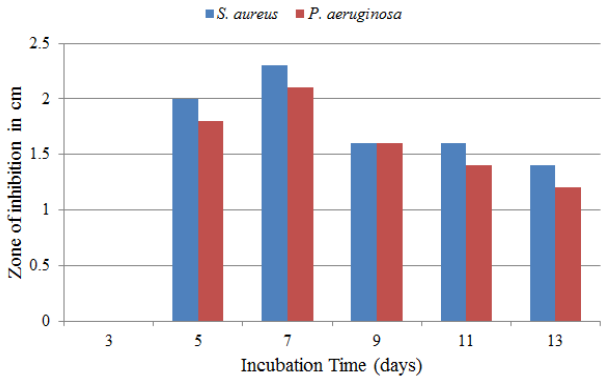

Figure 2: Effect of incubation time on production of antibacterial metabolite

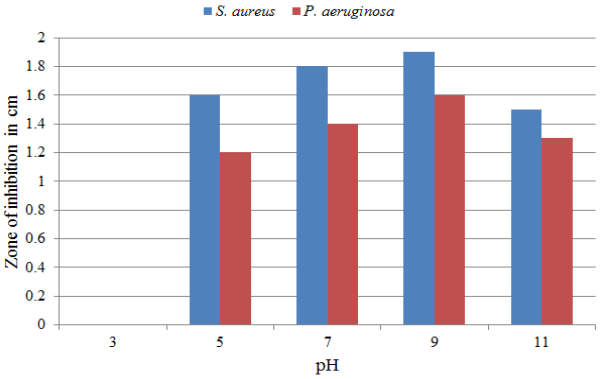

Figure 3: Effect of pH on production of antibacterial metabolite

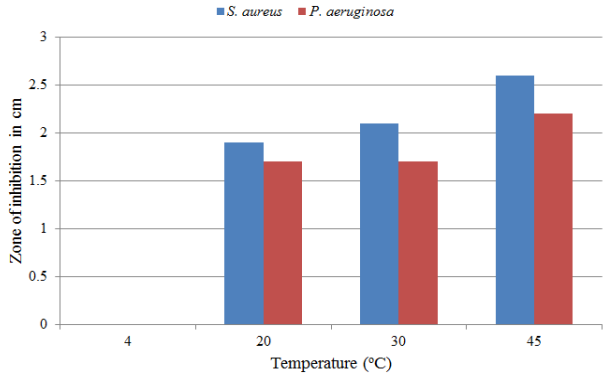

Figure 4: Effect of temperature on production of antibacterial metabolite

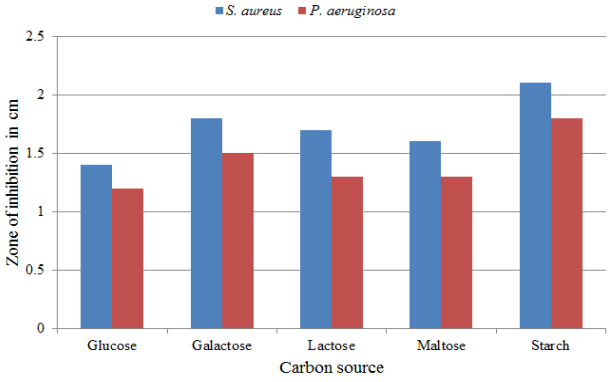

Figure 5: Effect of carbon sources on production of antibacterial metabolite

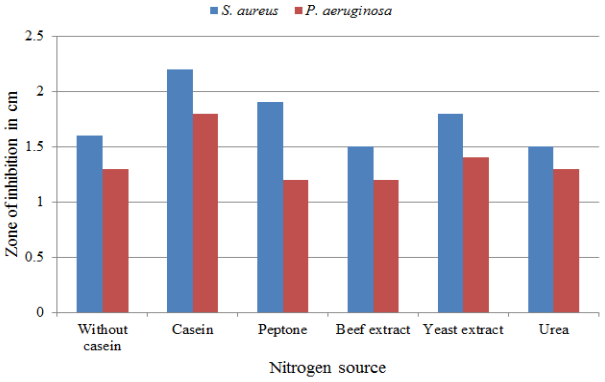

Figure 6: Effect of nitrogen sources on production of antibacterial metabolite

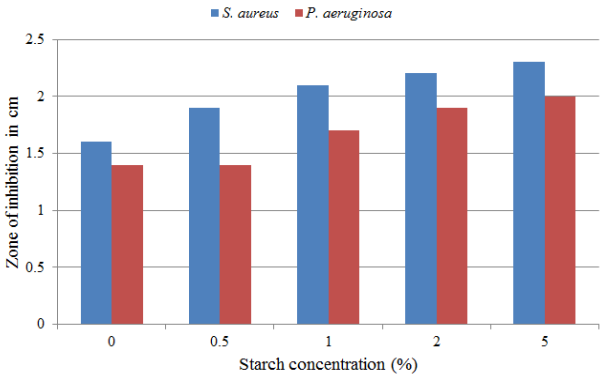

Figure 7: Effect of starch concentration on production of antibacterial metabolite

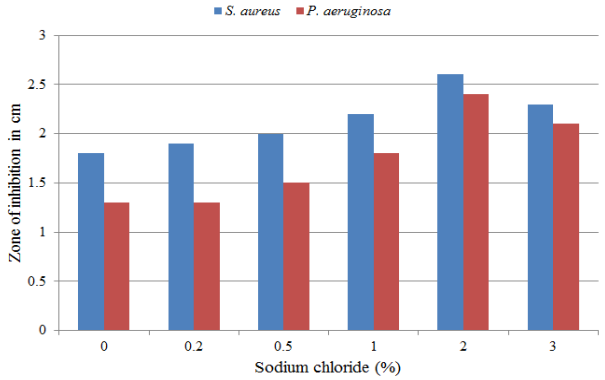

Figure 8: Effect of NaCl on production of antibacterial metabolite

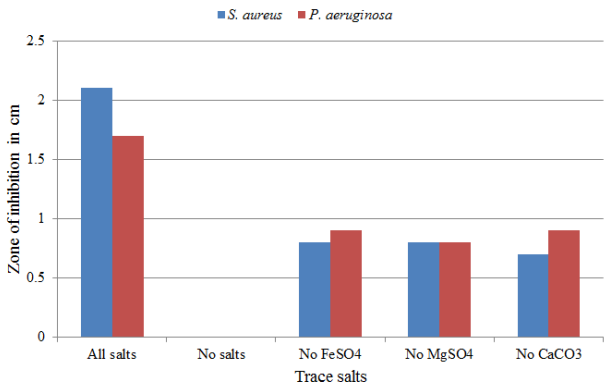

Figure 9: Effect of trace salts on production of antibacterial metabolite

Effect of carbon sources

A variety of carbon sources are known to influence growth and metabolite production by actinomycetes. Monosaccharides, disaccharides, polysaccharides and sugar alcohols are known to be utilized by actinomycetes for bioactive metabolite production. The influence of different carbon sources on production of antibacterial metabolites is shown in Figure 5. Marked inhibitory activity was observed in case of extract obtained from culture grown in broth containing starch as sole carbon source. Similarly, *S. plicatus*[21], *Streptomyces* species[31], *S. fradiae*[23] and *Streptomyces* sp. MS-266 Dm4[20] preferred starch as the best carbon source. However, starch was found to be poor carbon source for *S. kanamyceticus* M27[32]. Next to starch, galactose was the better carbon source for production of bioactive metabolites by strain PO-178. Least inhibitory activity was observed in case of extract of strain PO-178 obtained from broth containing glucose as carbon source. However, glucose was found to positively influence production of antibiotic by *Streptomyces psammoticus* BT-408[22], *S. sannanensis* strain RJT-1[27] and *Streptomyces* sp. RUPA-08PR[19]. Disaccharides such as lactose, maltose and sucrose were preferred for metabolite production by *S. sannanensis* strain RJT-1[27], *S. albidoflavus* C247[30] and *S. tritolerans*[26] respectively. Glycerol was found to be suitable for bioactive metabolite production by *Streptomyces* KEH23[24] and *Streptomyces* species A2[33]. Inositol was best carbon source for *Streptomyces* sp. MNK7[25].

Effect of nitrogen sources

Actinomycetes are known to use both organic and inorganic nitrogen sources for better growth and metabolite production. The requirement of nitrogen is highly varied among actinomycetes. Some prefer organic nitrogen sources while others yield higher quantity of bioactive metabolites by using inorganic nitrogen sources. It is shown that complex nitrogenous compounds increase the rate of production of antibiotics by *Streptomyces* species. These sources strengthen high antibiotic titer possibly linking the slow release of nitrogenous components during the course of fermentation[26]. The influence of different organic nitrogen sources on antibacterial metabolite production by the isolate is shown in Figure 6. Casein was found to be the best nitrogen source followed by other nitrogen sources. Inhibitory activity was also observed in SCN broth medium without casein. Organic nitrogen sources are known to influence growth and metabolite production by *Streptomyces* species. Peptone and soybean meal appeared to be best nitrogen sources for metabolite production by *Streptomyces* species A2[33]. Peptone was preferred by *Streptomyces* sp. MS-266 Dm4[20]. *S. tritolerans* preferred soya peptone[26]. Yeast extract, L-Asparagine and tryptone were preferred by *Streptomyces* sp. RUPA-08PR[19] and *Streptomyces* sp. MNK7[25].

Effect of starch concentration

Starch concentration in the production medium is shown to influence the growth as well as production of antimicrobial substances by various *Streptomyces* species. The concentration of initial starch concentration was found to exhibit marked effect on production of bioactive agents by the isolate. It was observed that increasing starch concentration resulted in an increase of antibacterial metabolite production as indicated by high inhibitory activity of extract against test bacteria. Maximum bioactivity was observed at high starch concentration i.e., 5%. Antibacterial activity was also observed in case of broth completely lacking starch (Figure 7). However, Osman *et al.*[21] observed maximum metabolite production by *S. plicatus* at 2% starch concentration. Similarly, Ababutain *et al.*[20] maximum growth and antibiotic production by *Streptomyces* sp. MS-266 Dm4 at 2% starch.

Effect of sodium chloride

Sodium chloride is known to exhibit a marked effect on growth and metabolite production by actinomycetes. Figure 8 shows the effect of different concentrations of sodium chloride on production of bioactive agents by the isolate. The isolate was able to produce bioactive agents in the absence of sodium chloride. However, the production of inhibitory agents increased on

increasing the sodium chloride concentration. Maximum activity was observed at 2% concentration beyond which activity declined. The result is in justification with the study of Arasu *et al*.[5] in which *Streptomyces* species from soils of Western Ghat region of Kanyakumari district, India displayed marked activity at sodium chloride concentration of 2% beyond which the activity was not decreased. However, Vasavada *et al*.[27] found 3% sodium chloride as optimum for production of antibiotic by *S. sannanensis* strain RJT-1. Mangamuri *et al*.[26] observed high antimicrobial metabolite production by *S. tritolerans* at 5% sodium chloride concentration. The study of Ripa *et al*.[19] and Saha *et al*.[25] found 1% sodium chloride as ambient for maximum production of antimicrobial metabolites by *Streptomyces* sp. RUPA-08PR and *Streptomyces* sp. MNK7 respectively. In another study, sodium chloride concentration of >0.2% repressed metabolite production by *S. fradiae*[23].

Effect of trace salts

Trace salts have profound effect of production of antimicrobial metabolites by actinomycetes. In this study, the effect of trace salts *viz*., ferrous sulfate, magnesium sulfate and calcium carbonate on metabolite production by strain PO-178 was investigated. It was found that all trace salts had a positive influence on metabolite production as indicated by higher inhibitory activity. Omission of each salt resulted in drastic lowering of inhibitory activity of extract. Inhibitory activity was not observed in case of extract obtained from medium lacking all trace salts (Figure 9). Hassan *et al*.[29] found improved antibiotic production on addition of ferrous sulphate and manganese chloride. Ababutain *et al*.[20] and Sujatha *et al*.[22] found positive influence of magnesium sulfate on antibiotic production by *Streptomyces* sp. MS-266 Dm4 and *S. psammoticus* BT-408 respectively. Gunda and Charya[34] found a positive influence of manganese and iron on the antibiotic production by actinobacteria. Manganese chloride and calcium carbonate positively influenced metabolite production by *Streptomyces* species A2[33]. In a study, Gautham[23] found antimicrobial metabolite production by *S. fradiae* only in the presence of all trace salts indicating the significance of trace salts for production of antimicrobial metabolites.

CONCLUSION

In the present study, we determined the effect of nutritional and cultural conditions on antibacterial metabolite production by *S. variabilis* by varying one factor at a time protocol. It is evident from the present study that the production of bioactive agents by strain PO-178 is influenced by various nutritional and cultural conditions. Starch casein nitrate broth was the best medium for production of antibacterial metabolites. The optimum temperature and pH was 45^{o}C and 9 respectively. Starch and casein was found to be ideal carbon and nitrogen source respectively. Increasing starch concentration in the medium enhanced the production of antibacterial agents. 2% sodium chloride resulted in maximum production of bioactive agents. All trace salts were required for maximum production of antibacterial metabolites. Further studies are required to determine the effect of various combinations of these parameters for better production of antibacterial agents.

ACKNOWLEDGEMENTS

Authors thank Principal, Sahyadri Science College (Autonomous) for providing facilities to conduct work and moral support.

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