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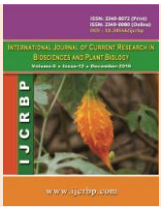

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Statistical optimization of culture conditions for the production of bioactive compounds by *Streptomyces spp*. isolated from vermicasts

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ABSTRACT

In the present work, the culture conditions appropriate for the maximum production of bioactive metabolites by *Streptomyces spp*. isolated from vermicasts were tested statistically. Two of the *Steptomyces sp*. isolated form vermicasts collected from Shervaroyan hills and Sirumalai hill ranges of southern Eastern Ghats were subjected to various environmental optimization conditions. These isolates were subjected to various physico-chemical parameters and the optimum conditions were identified for the maximum production of bioactive compounds with antimicrobial activity. AS1 showed a maximum biomass production in LB broth medium with a higher zone of inhibition against *Bacillus circulans*; whereas, AS3 showed a maximum biomass production and bioactive compound production in nutrient broth. Both isolates AS1 and AS3 showed maximum cell growth and zone of inhibition in the medium containing starch as a carbon source. For AS1, maximum growth and zone of inhibition was observed in the medium supplemented with casein as a nitrogen source.

Introduction

Earthworms are group of social animals and one of the important factors in the soil ecosystem. They play an important role in breaking down of organic material present the soil. Compared to termites the overall contribution of earth worm is more and their digestive system is more efficient. Microorganisms such as actinomycetes and protozoa are present in the gut of the earthworm (Jayakumar and Natarajan, 2012). *Actinomycetes* are group of Gram +ve bacteria with rich G+C content in their DNA. They produce large number of secondary metabolites such as antibiotics etc. *Actinomycetes* isolated from terrestrial environment have the potential use in the production of secondary metabolites (Saadoun and Gharaibeh, 2003). Majority of the antibiotics are produced by actionmycetes (Arifuzzaman et al., 2010; Holkar et al., 2013).

Streptomyces genus produces wide varieties of bioactive metabolites such as antibacterial, antifungal, antiviral, antitumor, anti-hypersensitivity and immunosuppressive (Omura et al., 2001; Paradkar et al., 2003; Patzer and

Volkmar, 2010). *Streptomyces* produces 75% of commercially and clinically valuable known bioactive compound which are broadly used in industries (Miyadoh, 1993). Antibiotic producing *Streptomyces* extensively present in soil and through antibiotic production they inhibit their competitors and pathogens. Discovery of bioactive compound from soil *Streptomyces* decrease in past two decades because search of new compounds directed towards unexplored environments (Ramazani et al., 2013).

Many of the microbes live in the tropical environment such as temperature, ph and high radiation etc. Usually the biotechnological production from the microorganism based on their special adaptations to their environment. Growth media composition and incubation condition have very strong influence in the production of secondary metabolites. Minor changes in medial composition exert a huge impact on quality and quantity of secondary metabolites. Secondary metabolism is regulated by carbon source, nitrogen sources, trace elements, precursors and others (Gao et al., 2009). In the present study, two of the *Streptomyces* species, isolated from vermicasts of forest floors of southern Eastern Ghats, Tamil Nadu were subjected to optimization of cultural conditions for maximizing the production of bioactive compounds with antimicrobial activity.

Materials and methods

The vermicast samples were collected from Shervaroyan and Sirumalai Hills of southern Eastern Ghats, Tamil Nadu, India. The samples were collected aseptically in dry and sterile plastic containers and were immediately transported to laboratory and stored at 4° C in the refrigerator until further use.

Isolation and screening of actinomycetes

One gram of sample was taken and serial dilutions were made up to 10^{-5}. From this, 0.1ml of sample was taken and spread over actinomycetes isolation agar plate, then incubated at 30°C for 5-10 days and were observed for the appearance of colonies (Athalye et al., 1981). Positive colonies were identified and further purified by streak- plate technique and the pure cultures were maintained on LB agar slants at 4°C for further use. The production of bioactive compounds was expressed by inhibition zone produced against test organisms. From this bioactive compound producing samples were subjected into various culture conditions for scaling up of bioactive compounds production.

Characterization of isolates

Smear was prepared by spreading the broth culture on a glass slide followed by heat drying. The smear was covered with crystal violet for 30-60 sec. and washed off with water. The smear was covered with Gram's iodine for 30-60 Sec, decolorized with alcohol, and washed with water. Finally the smear was stained with safranin counter stain for 2 min. After washing and drying, the slides were viewed at 100× under phase-contrast microscope (Arifuzzaman et al., 2010).

Selection of culture medium

The selected *Streptomyces* isolates were inoculated in different culture media such as nutrient broth, LB broth, malt extract broth (MEB) and yeast extract broth (YE). Crude bioactive compounds extracted from the culture broth and assayed for antibacterial activity to select an appropriate basal medium for subsequent statistical optimization.

Extraction of biologically active compounds by solvent extraction method

After the incubation period is over the fermented broth was aseptically transferred to sterile centrifuge tube and centrifuged at 5000 rpm for 30 mins. Then the supernatant was collected and transferred to separating funnel then added with ethyl acetate (1:3 ratio). After 30 mins, crude extract was collected and tested for its antimicrobial activity against test organisms (Ahmed, 2010). The bioactivity was determined by agar well diffusion method.

Determination of antimicrobial activity

Five test organisms including three Gram +ve (*Bacillus subtilis, Bacillus circulans* and *Staphylococcus aureus*) and two Gram -ve (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria were used for antimicrobial assay using crude extracts. Antagonistic activity of the extracts

was detected by the agar well diffusion (Muller Hinton Agar) method against the test organisms (Holder and Boyce, 1994). After incubation, the zone of inhibition was measured and recorded.

Optimization of potent *Streptomyces* spp. for maximum production of biomass and bioactive compound

Effect of carbon source on biomass and biologically active compound production

The isolated *Streptomyces* spp. were subjected to study the effect of different carbon sources glucose, fructose, starch, casein and maltose were used. One percent of each carbon source was added along with basal medium individually. Each flask containing different carbon sources were inoculated with *Streptomyces* spp. and incubated at 37°C for 7 to 10 days. Then biomass of each flask was measured (dry weight) and the production of bioactive compounds was recorded (Holder and Boyce, 1994).

Effect of nitrogen sources on biomass and biologically active compound production

The effect of nitrogen source was studied by using various nitrogen sources (yeast extract, casein, beef extract, peptone and ammonium chloride, and others). One percent of each nitrogen source were added along with basal medium in separate flasks and incubated at 37°C for 7 to 10 days. Flasks containing different nitrogen sources were aseptically inoculated with *Streptomyces* samples. After incubation measure the amount of biomass and bioactive compounds produced in each flask was recorded.

Effect of temperature sources on biomass and biologically active compound production

The samples were subjected into different temperature ranges (15°C to 45°C) to study the optimum temperature required for maximum growth and bioactive compound production. 25ml of basal medium was prepared and sterilized at 121°C for 45 min. Under aseptic condition the flasks were inoculated with *Streptomyces* samples and incubated at various temperatures (15°C to 45°C) for 10 to 14 days. After incubation the biomass and bioactive compounds produced by each flask were recorded.

Effect of pH on biomass and biologically active compound production

The effect of pH on growth and biologically active compound production by the *Streptomyces* spp. were analysed by different pH conditions (pH 4 to pH 8). Four different pH values containing LB broth was taken and inoculated with *Streptomyces* isolates and incubated at 37°C for 10-14 days. After incubation, the amount of biomass and bioactive compounds produced by each flask were recorded.

Results and discussion

The yield of bioactive compounds was increased by various parameters such as pH, temperature, carbon source and nitrogen sources. AS1 showed a maximum biomass production in LB broth medium with a higher zone of inhibition against *Bacillus circulans* (Fig. 1A). Whereas, AS3 showed a maximum biomass production and bioactive compound production in nutrient broth (Vilches et al., 1990). This was followed by yeast extract and malt extract. Low level of zone of inhibition was obtained from malt extract broth (Fig. 1B).

Fig. 2A and Fig. 2B show the impact of carbon sources on production and increases in zone of inhibition respectively. Among various carbon sources tested, Starch was the best carbon source for both biomass and production of bioactive compounds. Both the isolates AS1 and AS3 showed maximum cell growth and zone of inhibition in the medium containing starch as a carbon source (Fig. 2A and Fig. 2B). Starch is a complex and slowly utilized nutrient source. On the other hand glucose, casein and maltose also showed maximum cell growth but with very poor bioactive compound production which coincides the previous studies (Huck et al., 1991; Iwai and Omura, 1982; Reddy et al., 2011).

During nutrient excess condition the metabolism of actinomycetes directed towards generation of cell mass rather than the production of secondary metabolites. Glucose act as catabolite repressor, in which production of enzymes of secondary metabolite synthesis is inhibited (Oskay, 2011). From this results starch has found to be best

carbon sources among other carbon sources.

This study also reveals the effect of different nitrogen sources on biomass and bioactive metabolite production by *Streptomycetes* isolates. For AS1, maximum growth and zone of inhibition was observed in the medium supplemented with casein as a nitrogen source (Fig. 3A), whereas AS3 showed a maximum biomass production and bioactive metabolite production in the medium supplemented with peptone as a nitrogen source (Fig. 3B).

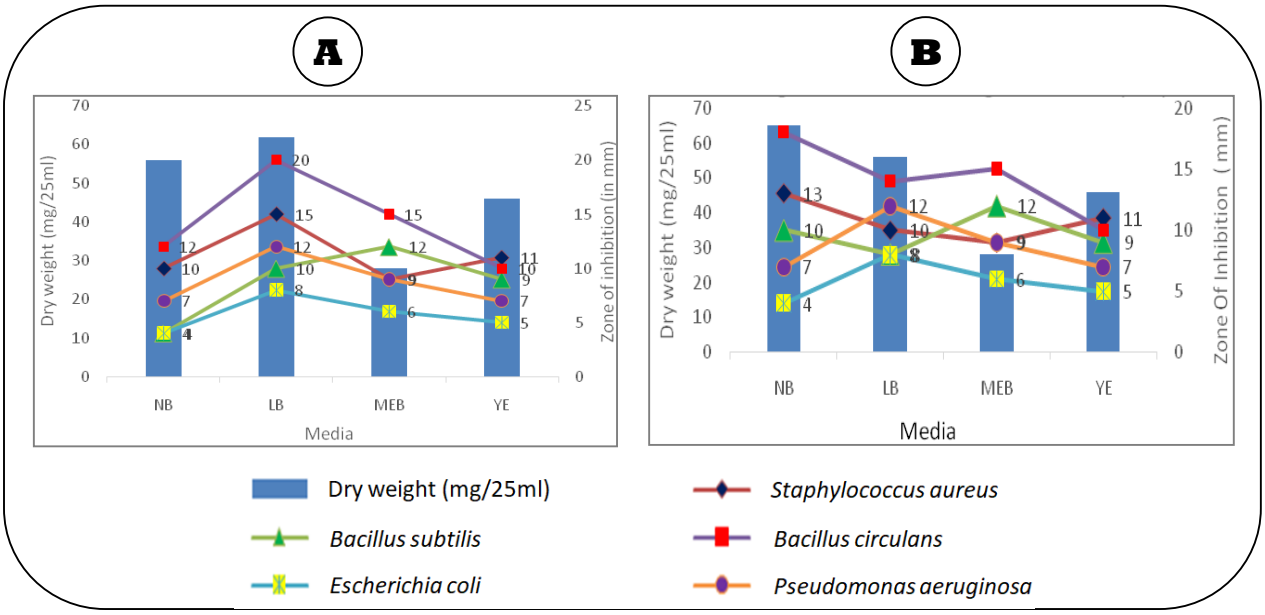

Fig. 1: Effect of different growth media on: (A) AS1 and (B) AS3.

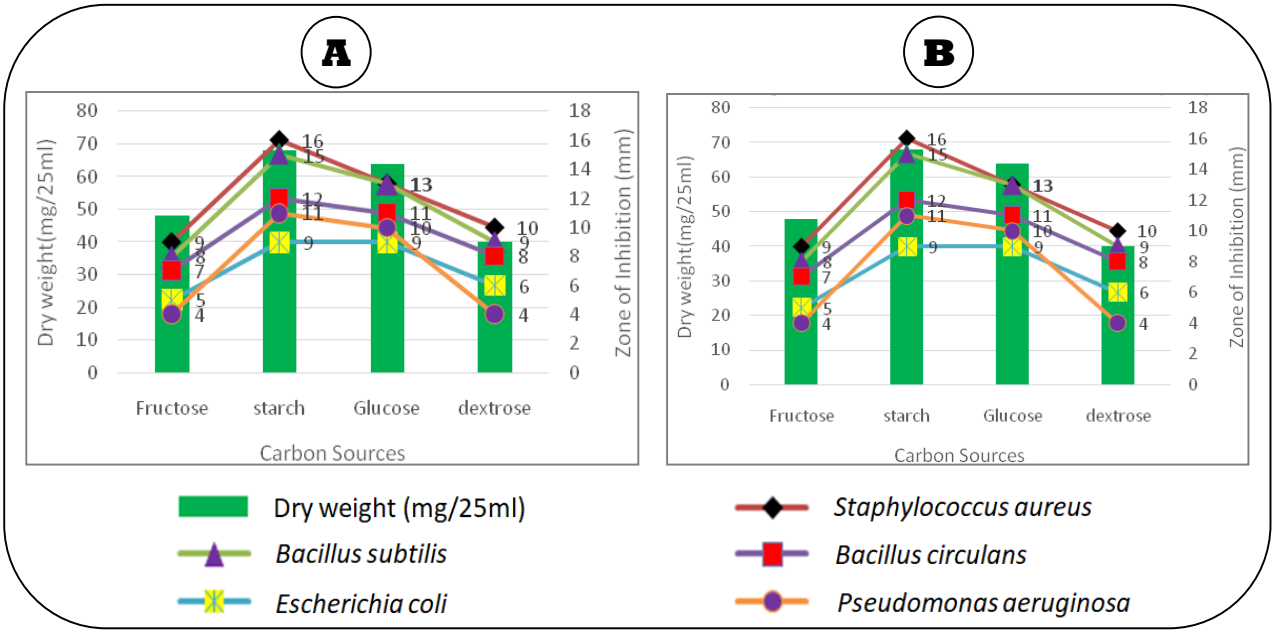

Fig. 2: Effect of different carbon source on: (A) AS1 and (B) AS3.

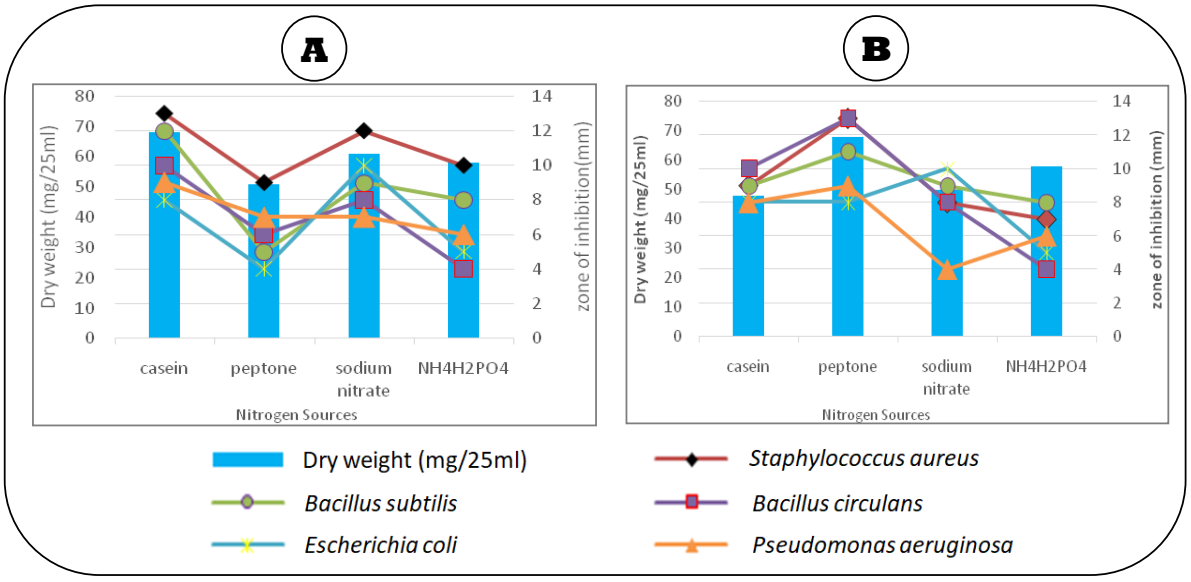

Fig. 3: Effect of different nitrogen source on: (A) AS1 and (B) AS3.

Maximum biomass production and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was observed in the culture media supplemented with casein and peptone as a nitrogen source which is in accordance with the study by Ghosh and Prasad (2010). The present study also showed the effect of sodium nitrate and ammonium di-hydrogen phosphate on biomass production and bioactive compound production. Casein extract followed by ammonium di-hydrogen phosphate also act as very good nitrogen sources. Peptone also acts as very good nitrogen source by other authors. The impact of temperature on biomass and bioactive compound production of the isolate was studied. 30°C was found to be optimum temperature for the growth of isolate AS1 (Fig. 4A); whereas, in AS3, the maximum growth was attained at 32°C (Fig. 4B). Biomass production and zone of inhibition was gradually reduced by increasing the temperature from 28°C-37°C. Hassan et al. (2004) has also observed maximum antibiotic production by *Streptomyces violatus* at 30°C.

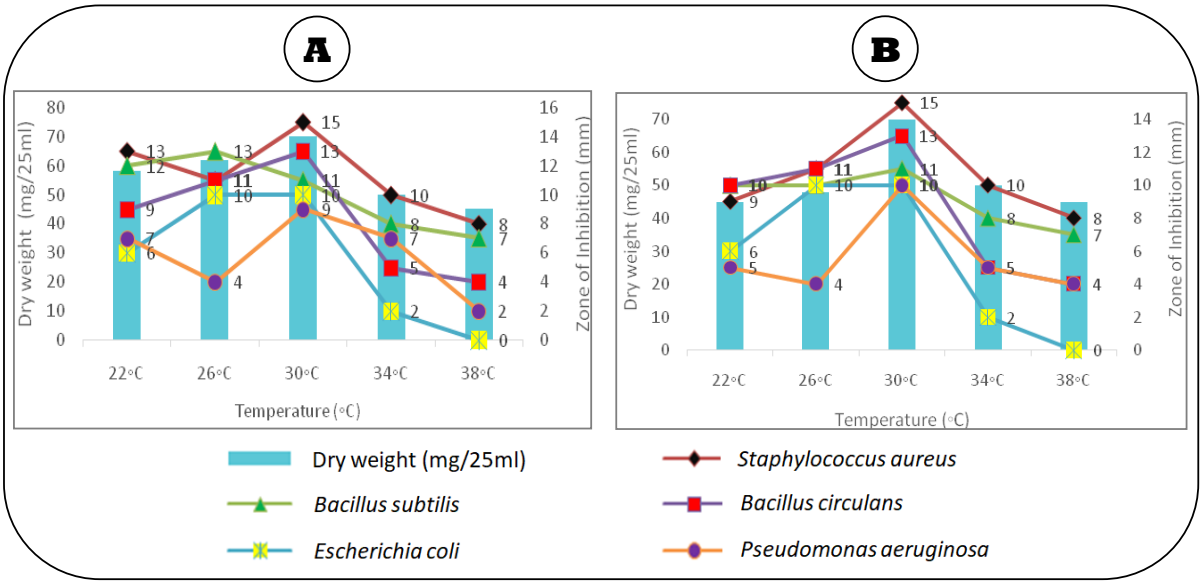

Fig. 4: Effect of different temperature on: (A) AS1 and (B) AS3.

The pH of the culture medium is one of the important factors to improve the biomass production and bioactive compound production. The pH is one of the most important environmental factors. Changes in the initial external pH affect many cellular processes such as regulation and biosynthesis of secondary metabolites (Chen et al., 2011). Generally, in most published literature, optimum pH for antibiotic production in *Streptomyces* cultures has been reported to be near neutral. For the isolate AS1, where pH 7 was proved to be optimum for growth and bioactive metabolite production (Fig. 5A) which is in line with Usha Kiranmayi et al. (2012) and Narayana and Vijayalakshmi (2008). But for the isolate AS3, pH 6 was found to be satisfactory for maximum biomass production and zone inhibition (Fig. 5B).

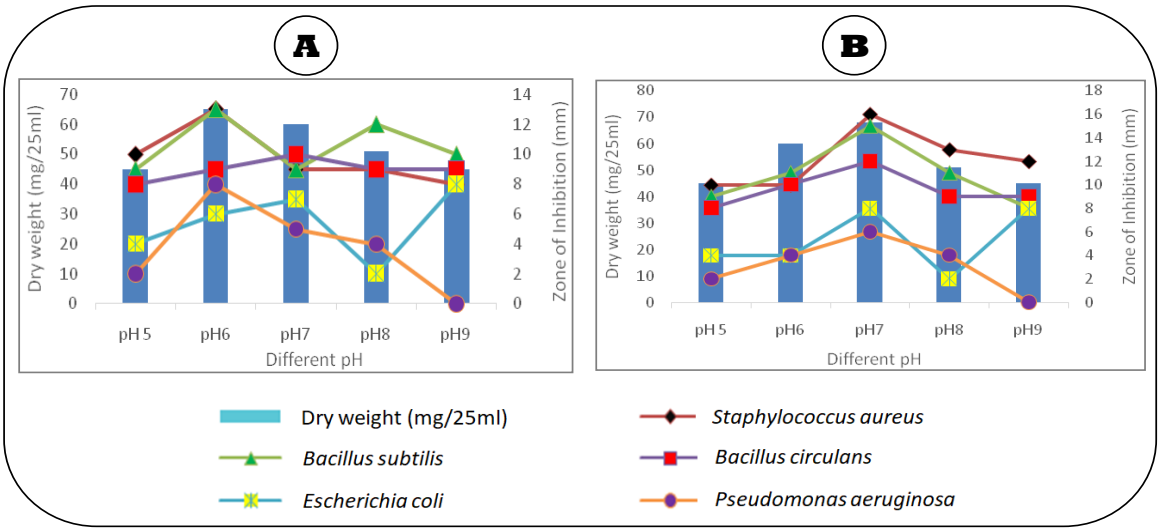

Fig. 5: Effect of different pH on: (A) AS1 and (B) AS3.

Conclusion

The present study concluded that the optimum conditions required for the production of bioactive metabolite by *Streptomyces* species, AS1 and AS3 isolated from vermicasts require different cultural conditions. The maximum production of bioactive compounds was achieved by optimizing various parameters like production media, carbon source, nitrogen source, temperature and pH. Both the isolates, AS1 and AS3 showed better antimicrobial activity against human pathogens in optimum production levels of bioactive compounds. Further studies on purification, characterization and identification of bioactive compounds from these *Streptomyces* isolates are required for scaling-up of production levels.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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