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Original Article

OPTIMIZATION OF CULTURE CONDITIONS OF *STREPTOMYCES CARPATICUS* (MTCC-11062) FOR THE PRODUCTION OF ANTIMICROBIAL COMPOUND

***M. BHAVANA, VSSL PRASAD TALLURI, K. SIVA KUMAR, S. V. RAJAGOPAL**

Department of Biotechnology, GITAM Institute of Science, GITAM University, Visakhapatnam 530045, Andhra Pradesh, India.
Email: mandava.bhavana@gmail.com

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ABSTRACT

Objective: To improve the antimicrobial compound productivity of *Streptomyces carpaticus* (MTCC-11062) by optimizing its physical and chemical parameters

Methods: *Streptomyces carpaticus* (MTCC-11062) was isolated from Visakhapatnam sea coast of Bay of Bengal and was screened for its antimicrobial activity by using agar well diffusion method. To improve the production of antimicrobial compound the medium composition and physical parameters were optimized and its productivity was studied against *Bacillus cereus* (MTCC 430) *Escherichia coli* (MTCC 443), *Candida albicans* (MTCC 227) obtained from MTCC, Chandigarh, India.

Results: Optimum growth of mycelium and antimicrobial compound production occurred at pH 7.2, agitation 180 rpm and temperature 30^{0}C with glucose 10g/L, soyabean meal 2.5g/L, K_2HPO_4 2g/L, $MgSO_4$ 1g/L, NaCl 7.5g/L and trace salts.

Conclusion: The optimization of cultural conditions proposed in this paper has effetely improved the antimicrobial compound productivity of *Streptomyces carpaticus* (MTCC-11062).

Keywords: *Streptomyces carpaticus* (MTCC-11062), Optimization, Antimicrobial compound.

INTRODUCTION

Marine Actinomycetes are biotechnologically and economically valuable prokaryotes. They produce special bioactive secondary metabolites particularly antibiotics [1]. Marine actinomycetes are the potential source of novel antimicrobial compounds as the environmental conditions of the sea are completely different from that of terrestrial conditions [2] [3]. Marine streptomyces have an incomparable metabolic diversity and are excellent in producing new natural products. Approximately 2/3 of the well known antibiotics was produced by Streptomyces [4] [5]. About 75% of commercially and medically useful antibiotics are produced by the streptomyces spices [6] [7]. These actinomycetes are capable of breaking down complex biological polymers and are well adapted to marine environment [8]. The growth of actinomycetes can be enhanced by manipulating the cultural conditions like nutritional, physical and chemical parameters. In optimization, composition of the medium plays an important role in the productivity and economics [9] [10]. In the present study, *Streptomyces carpaticus* (MTCC-11062) was screened for its antimicrobial activity and are optimized for the higher yield of antimicrobial compound with reference to medium composition and other factors. Agar well diffusion method was used to check the efficiency of antibiotic production and the zone of inhibition was measured.

MATERIALS AND METHODS

Isolation of *Streptomyces carpaticus* (MTCC-11062)

The marine sediment samples were collected from Visakhapatnam sea coast of Bay of Bengal, India from a depth of 5-95mts. The sediment samples collected were transferred into sterilized zip locker bags. The actinomycetes strain, *Streptomyces carpaticus* (MTCC-11062) was isolated by serial dilution in starch casein agar medium with rifampicin (5mg/ml) and nystatin (25µg/ml) to inhibit bacterial and fungal contaminations respectively. The plates were incubated at 28^{0}C for 3 weeks.

Screening of *Streptomyces carpaticus* (MTCC-11062) for its antimicrobial activity

Streptomyces carpaticus (MTCC-11062) was grown in production broth (SS medium), containing (g/L): soluble starch-25, glucose-25, yeast extract-2, $CaCO_3$ and trace elements 1 ml and incubated at 28^{0}C in a shaker at 180rpm for 4 days. After incubation, the broth was centrifuged for 15 minutes at 5000 rpm and the supernatant was mixed with equal volume of ethyl acetate to extract the antimicrobial compound [11]. The antimicrobial study was carried out by agar well diffusion method [12]. 50µl of the ethyl acetate fraction was loaded in each well and the zone of inhibition was measured in mm.

Test organisms used

Bacillus cereus (MTCC 430) *Escherichia coli* (MTCC 443), *Candida albicans* (MTCC 227) were obtained from MTCC, Chandigarh.

Optimization of growth and antimicrobial compound production

Effect of carbon source

Streptomyces carpaticus (MTCC-11062) was inoculated in the Pridham and gottlieb inorganic salt medium [13] by varying medium carbon sources. The different carbon sources 1% (w/v) used in the medium were Glucose, Fructose, Galactose, Xylose, Arabinose, Glycerol, Starch, Maltose, Lactose, sucrose, meso-inositol and Mannitol. The flasks were incubated at 180 rpm for 4 days.

Optimization of best carbon source concentration

Streptomyces carpaticus (MTCC-11062) was inoculated in the optimized medium with varying concentrations of carbon source. The concentrations were (g/L) 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20. These flasks were incubated at 180 rpm for 4 days.

Effect of nitrogen source

The effect of various nitrogen sources on antimicrobial compound production and growth of mycelium was studied by adding organic and inorganic nitrogen sources at 1% (w/v) level into optimization medium.

The organic nitrogen sources employed were soyabean meal, yeast extract, beef extract and peptone. The inorganic nitrogen sources used were ammonium nitrate, ammonium sulfate, potassium nitrate and sodium nitrate.

Optimization of best nitrogen source concentration

Streptomyces carpaticus (MTCC-11062) was inoculated in the optimized medium with varying concentrations of nitrogen source. The concentrations were 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0(g/L) and the flasks were incubated at 180 rpm for 4 days.

Effect of K_2HPO_4 concentration

Streptomyces carpaticus (MTCC-11062) was inoculated in optimized medium with varying concentrations of K_2HPO_4. The different concentrations employed in the medium were 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 g/L

Effect of $MgSO_4.7H_2O$ concentration

Streptomyces carpaticus (MTCC-11062) was inoculated in optimized medium with varying concentrations of $MgSO_4$. The different concentrations used in the medium were 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75 and 2.0g/L

Effect of Sodium chloride concentration

Streptomyces carpaticus (MTCC-11062) was inoculated in optimized medium with varying concentrations of sodium chloride. The different concentrations used in the medium were 2.5, 5.0, 7.5, 10 and 12.5g/L

Effect of temperature

Streptomyces carpaticus (MTCC-11062) was inoculated in optimized medium and incubation at different temperatures ranging from 15 - 50^0C at 180rpm for 4 days.

Effect of pH

Optimum pH was studied by adjusting the pH of growth medium at 6, 6.4, 6.8, 7.2, 7.6, 8.0, 8.4 and 8.8. *Streptomyces carpaticus* (MTCC-11062) was inoculated in the optimized medium and incubated at 180 rpm for 4 days.

Effect of incubation time

To obtain the high rate of antibiotic production, *Streptomyces carpaticus* (MTCC-11062) was inoculated in optimized medium for different incubation period of 24 hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs, 144 hrs, 168 hrs and 192 hrs.

Effect of agitation

Streptomyces carpaticus (MTCC-11062) was inoculated in optimized medium at different agitation rates of 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220 and 240 rpm. All the optimized flasks were incubated for 7 days.

Antimicrobial activity of *Streptomyces carpaticus* (MTCC-11062) after optimization

After incubation *Streptomyces carpaticus* (MTCC-11062) was extracted using ethyl acetate and the antimicrobial activity was carried out by agar well diffusion method. 50µl of the ethyl acetate fraction was loaded in each well and the zone of inhibition was measured.

Measurement of growth of mycelium

The growth of *Streptomyces carpaticus* (MTCC-11062) was measured as dry weight of the mycelium (MDW). A whatmann No.1 filter paper was taken and washed twice with distilled water and dried. The weight of the filter paper was noted.

The mycelial content in the culture flask was filtered through the whatmann No.1 filter paper which is previously weighed. The mycelia content along with the filter paper was dried in the hot air oven for 18-24 hrs. The filter paper was cooled and the dry weight of the mycelium was measured.

Statistical Analysis

The experiments were carried out in triplicates and the results obtained were expressed in mean ± standard deviation

RESULTS AND DISCUSSION

Isolation of *Streptomyces carpaticus* (MTCC-11062)

The actinomycetes strain, *Streptomyces carpaticus* (MTCC-11062) was isolated from the marine soil of Visakhapatnam sea coast and it was stored in glucose yeast extract malt extract medium for further analysis.

Screening of *Streptomyces carpaticus* (MTCC-11062) for its antimicrobial activity

Among the bacterial and fungal strains tested, *B. cereus* showed highest zone of inhibition of 24 mm followed by *E. coli* (22 mm) and *C. albicans* showed zone of inhibition of 20 mm.

Effect of carbon source

Microorganisms have a capacity to utilize a variety of carbon sources and can adapt to the changes in the osmotic strength nutrients and oxygen limitation and stress conditions [14][15][16]. The presence of carbon source which is easily metabolized, especially glucose, results in a coordinated change of metabolic function in many bacteria [17]. Glucose as a component in the production media provides carbon atoms for the mycelium and produce generations. Maximum antimicrobial compound and mycelium growth was observed with Glucose followed by Glycerol and Starch. In contrast Fructose, Galactose, Xylose, Arabinose, Maltose, Lactose, Sucrose, Meso-inositol and Mannitol showed low antimicrobial compound production and mycelium growth (figure-1).

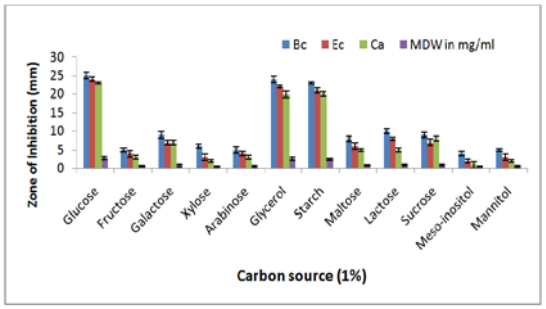

Fig. 1: Effect of different carbon sources on growth and antimicrobial compound production of *Streptomyces carpaticus* (MTCC-11062)

Optimization of best carbon source concentration

The growth and antimicrobial compound production of *Streptomyces carpaticus* (MTCC-11062) was increasing continuously from 2.5g/L to 10g/L of glucose concentration. Further increase in the glucose concentration showed a gradual decrease in the production of antimicrobial compound and the growth of the mycelium. Therefore optimum glucose concentration for the maximum production of antibiotic activity was at 10g/L (figure-2).

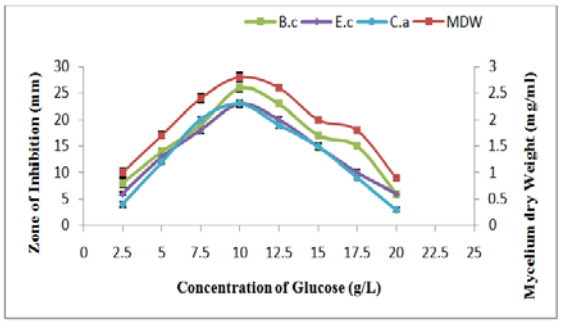

Fig. 2: Effect of glucose concentration on growth and antimicrobial compound production of *Streptomyces carpaticus* (MTCC-11062)

Effect of nitrogen source

Maximum antibiotic production and mycelium growth was observed with organic nitrogen source when compared to inorganic nitrogen source. Among organic nitrogen sources, maximum growth and production was shown by soyabean meal followed by peptone and ammonium nitrate (figure-3).

Some investigators reported that, soyabean meal and peptone are the most excellent organic nitrogen sources for the oxytetracycline production by *Streptomyces rimosus* 93060 [18]. Soyabean meal was regarded as appropriate medium component for antibiotic production for the strain S*treptomyces capoamus* [19]. It was also reported that the medium which is supplemented with soyabean meal as nitrogen source was found to produce maximum antimicrobial compound [20].

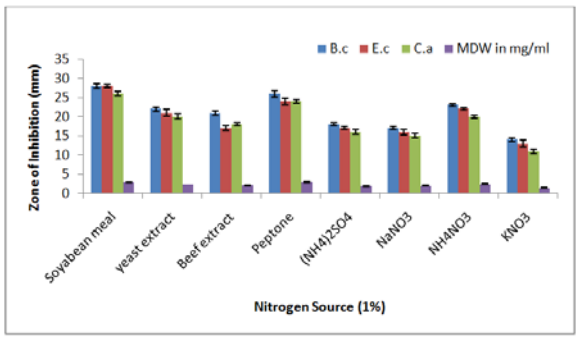

Fig. 3: Effect of different nitrogen sources on growth and antimicrobial compound production of *Streptomyces carpaticus* (MTCC-11062)

Optimization of best nitrogen source concentration

The antimicrobial compound production and growth of *Streptomyces carpaticus* (MTCC-11062) was increasing continuously from 0.5g/L to 2.5g/L of soyabean meal concentration. Further increase in the soyabean meal concentration showed a gradual decrease in the production of antimicrobial metabolite and the growth of mycelium. Therefore optimum soyabean meal concentration for the maximum production of antimicrobial compound was at 2.5g/L (figure-4).

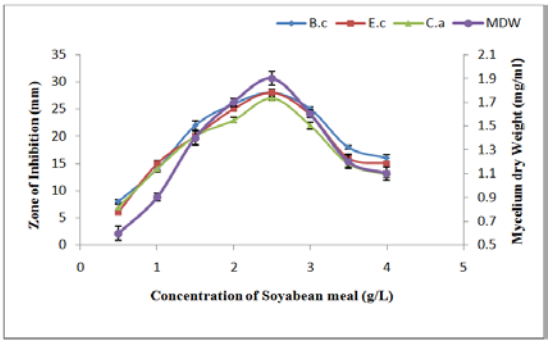

Fig. 4: Effect of Soyabean meal concentration on growth and antimicrobial compound production of *Streptomyces carpaticus* (MTCC-11062)

Effect of K_2HPO_4 concentration

Addition of inorganic phosphates in large amount induces the consumption of nitrogen and carbon source and respiration in accelerated. This results in the good growth of the microorganisms but the antibiotic production is usually reduced.

Optimum K_2HPO_4 concentration required for the production of antimicrobial compound was 2.0g/L. Further increase in the K_2HPO_4 concentration showed a gradual decrease in the production of antimicrobial compound and growth of mycelium (figure-5).

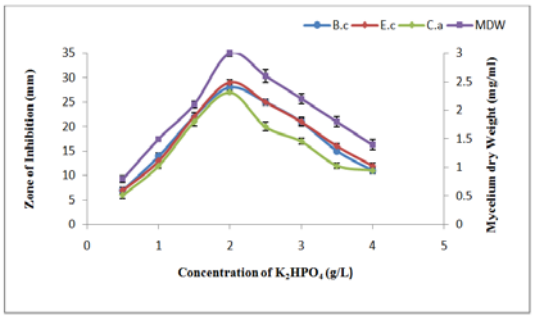

Fig. 5: Effect of K2HPO4 concentration on growth and antimicrobial compound production of *Streptomyces carpaticus* (MTCC-11062)

Effect of $MgSO_4$ concentration

Optimum concentration of $MgSO_4$ required for the production of antimicrobial compound was 1g/L. Further increase in $MgSO_4$ concentration showed a gradual decrease in the production of antimicrobial compound and growth of mycelium (figure-6).

The effect of $MgSO_4$ and other metal salts on the antibiotic production was investigated and reported by several authors [21][22][23].

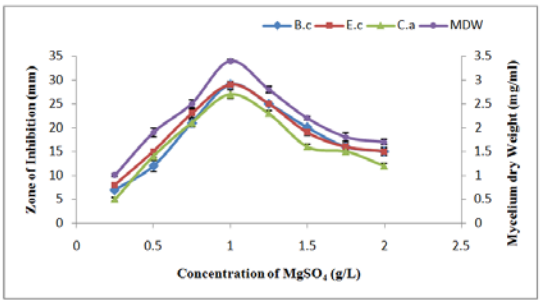

Fig. 6: Effect of MgSO4 concentration on growth and antimicrobial compound production of *Streptomyces carpaticus* (MTCC-11062)

Effect of sodium chloride concentration

Optimum NaCl concentration required for the production of antimicrobial compound was 7.5g/L. Further increase in NaCl concentration showed a drastic decrease in the production of antimicrobial compound and growth of mycelium (figure-7).

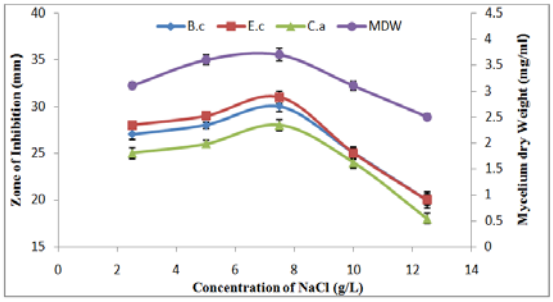

Fig. 7: Effect of NaCl concentration on growth and antimicrobial compound production of *Streptomyces carpaticus* (MTCC-11062)

Effect of temperature

The amount of secondary metabolite production *Streptomyces* highly depend on the temperature and growth rate [24]. The optimum growth and antimicrobial compound production was observed at 30^{0}C and beyond optimum temperature the growth and antimicrobial metabolite production was decreased [25]. However higher temperature showed adverse effect on both growth and antimicrobial compound production (figure-8).

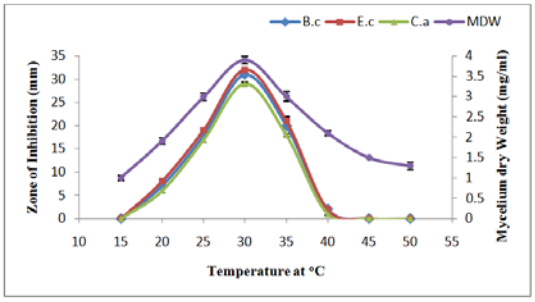

Fig. 8: Effect of temperature on growth and antimicrobial compound production of *Streptomyces carpaticus* (MTCC-11062)

Effect of pH

The changes in pH levels affect the cellular regulation processes and biosynthesis of secondary metabolites in streptomyces species [26][27]. Most of the commercially used streptomyces species showed optimum pH range from 7-8 [28]. The growth and antimicrobial metabolite production of *Streptomyces carpaticus* (MTCC-11062) was maximum at pH 7.2 (figure-9).

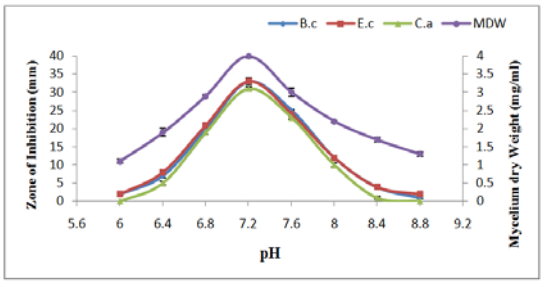

Fig. 9: Effect of pH on growth and antimicrobial compound production of *Streptomyces carpaticus* (MTCC-11062)

Effect of incubation time

The antimicrobial compound production and growth of *Streptomyces carpaticus* (MTCC-11062) was increasing continuously from 24 hrs to 120 hrs. Further increase in incubation time showed a gradual decrease in the production of antimicrobial compound and the growth of mycelium. Therefore optimum incubation time for the maximum production of antimicrobial metabolite was at 120 hrs (figure-10).

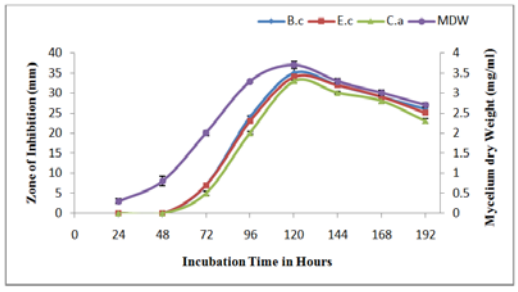

Fig. 10: Effect of incubation time on growth and antimicrobial compound production of *Streptomyces carpaticus* (MTCC-11062)

Effect of agitation

Agitation facilitates greater aeration to the cells and provides favorable conditions for the greater availability of the nutrients to the culture. The optimum growth and antimicrobial compound production was observed at 180rpm. Beyond 180 rpm growth and antimicrobial compound production decreased gradually (Fig. 11).

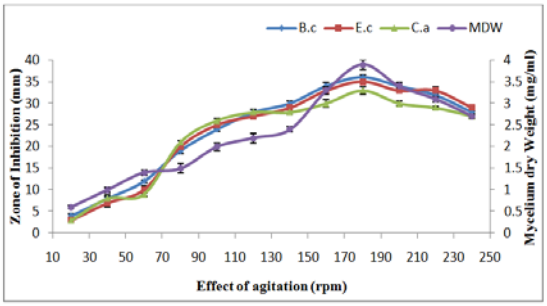

Fig. 11: Effect of agitation on the growth and antimicrobial compound production of *Streptomyces carpaticus* (MTCC-11062)

CONCLUSION

The synthetic medium formulated in the present investigation, for the maximum production of antimicrobial compound by *Streptomyces carpaticus* (MTCC-11062) contain the following medium composition (g/L): 10 glucose, 2.5 soyabean meal, 2.0 K_2HPO_4, 1 $MgSO_4$, 7.5 NaCl, and trace salt solution. The cultural conditions required are temperature 30^{0}C, pH 7.2, incubation period 120h and agitation 180rpm. Hence, overall data indicates a significant increase in the yield by using newly formulated production medium and optimized cultural conditions.

CONFLICT OF INTERESTS

Declared None

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