

Original Research Article

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Optimization of Cultural Parameters for Pigment Production from *Streptomyces flavofuscus* ARITM02, Isolated from Rhizosphere Soil

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ABSTRACT

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Objective of the study is to enhance the productivity of pigmented compound of *Streptomyces flavofuscus* ARITM02 by optimizing its physiological conditions. *Streptomyces flavofuscus* ARITM02 was screened for its pigment producing ability. In order to improve its efficiency, the effects of various physiological conditions like, temperature, pH, incubation time, carbon and nitrogen sources were optimized and its productivity was examined by optical density measurement from UV-Vis spectrophotometer. Maximum pigment production was found at pH 7.5. The best nitrogen source for pigment production is peptone, similarly best carbon source is starch. The incubation time of 96 hours is found best to maximum yield of pigment and the optimum temperature was found 35 °C. The results from this study confirmed that the antibacterial substances produced by *Streptomyces* sp. were found to be more effective after its optimization.

Introduction

Actinomycetes are Gram-positive bacteria and they also known as a good source of microbial secondary metabolites producer in various pharma industries for therapeutic use. It is reported that many synthetic colors causes many serious problems related to environmental and it creates interest towards natural pigments from microorganisms. Natural pigments from actinomycetes are potentially good alternative of synthetic colors. Industrially many artificial synthetic colorants been used in foodstuff, dyestuff, cosmetic and pharmaceutical processes, which have many hazardous effects on health and ecosystems. Due to negative effect of

synthetic colorants, there is worldwide interest generated for the production of pigments from natural sources such as microorganisms (Berdy, 2005; Unagul *et al.*, 2005).

Pigments were primarily used as a coloring agent in various industries, researcher have focused the usage of pigments from coloring agents to antioxidants in various pharmaceutical and food industries from the past decade (Prakash *et al.*, 2001). Drug discovery from natural products has been traditionally focused on empirical exploitation of the most prolific microbial groups:

actinomycetes and filamentous fungi. The literature vividly displays that actinomycetes have been the origin of the largest number of new antibiotic drug candidates and also lead molecules with applications in many other therapeutic areas (Singh *et al.*, 2010). The great number of diverse antibiotics that produce these bacteria can be classified in different ways based on bacterial spectrum, the type of biological activity and the chemical structure. This latter way is the most useful and several classes of antibiotics can be distinguished, as: alkaloid derivatives which possess a diverse range of biological activities, e.g. anticancer and antimicrobial (Gebhardt *et al.*, 2002).

Continuous screening of secondary microbial products from bacteria is essential for discovery of novel chemicals which can be developed as new therapeutic agents. Researchers are exploring diverse untapped habitats in an attempt to discover microbes with potential for production of novel chemical moieties (Manivasagan *et al.*, 2013; Nakashima *et al.*, 2013).

In this work, the media for pigment production was optimized by various cultural parameters for best and enhanced production of pigment.

Materials and Methods

The soil samples were isolated from different sites of Chambal ravine area and other parts of Madhya Pradesh state. Out of 85 actinomycetes only seven actinomycetes showed pigment producing ability but one *Streptomyces flavofuscus* ARITM02 has been selected for further study because of its diffusible pigment producing ability and fast growth. The actinomycete was identified by morphological, biochemical and molecular basis.

Optimization of broth for pigment production

Streptomyces flavofuscus ARITM02 was inoculated in different concentration of starch casein broth with different optimization conditions and kept in incubator shaker at 150 rpm for 7 days.

Temperature

Optimum temperature was studied by varying the incubation temperatures at 15°C, 20°C, 25°C, 30°C, 40°C and 45°C. Isolate *Streptomyces flavofuscus* ARITM02 was inoculated in the starch casein broth medium and kept in incubator shaker at 150 rpm for 96 hours.

pH

Optimum pH was studied by varying the medium pH as 2, 4, 6, 7, 7.5, 8 and 10. The growth medium was adjusted to different pH using 1M HCl and 1M NaOH.

Loopful culture of *Streptomyces flavofuscus* ARITM02 was inoculated in the starch casein broth medium and kept in incubator shaker at 150 rpm for 7 days.

Carbon source

Streptomyces flavofuscus ARITM02 was inoculated in the starch casein broth medium and kept in incubator shaker at 150 rpm speed and 35°C temperature for 96 hours. Various carbon sources used in the medium were Glucose, Dextrose, Sucrose, fructose, Starch, lactose and Maltose.

Nitrogen source

Streptomyces flavofuscus ARITM02 was inoculated in the starch casein broth medium and kept in incubator shaker at 150 rpm and temperature for 7 days.

Nitrogen sources used in the medium were Peptone, Casein, Beef extract, Yeast extract and Malt extract.

Incubation period

Streptomyces flavofuscus ARITM02 was inoculated in the starch casein broth medium and incubated for different time interval to achieve high rate of antibiotic production. The incubation period varied from 24, 48, 72, 96, 120, 144 and 168 hours.

Results and Discussion

Different media was tested for fermentative production of pigment producing actinomycete but only starch casein broth was found suitable because the production of pigment found in this broth media only. The highest production of pigment was also found in starch casein broth. The optical density was measured at 480 nm (Figure 1).

Cultural parameters for optimization of medium for isolate ARITM02

Incubation time

The results clearly showing (Figure 2) that the ARITM02 isolate produced maximum level of pigment after 96 hours of incubation. The increase in pigment production observed from 24 hours to 96 hours and then showed decline phase of isolate growth. So it is concluded that growth and pigment production increased at incubation time of 96 hours and 96 hours is best incubation time for maximum pigment yield.

Temperature

Temperature is an important parameter for growth of actinomycete. The growth varies with different temperature. Temperature played an important role in the pigment

production and activity. Starch casein broth with selected isolate was incubated at different temperature (15, 20, 25, 30, 35 and 40°C) on rotary shaker at 150 rpm. It was found that 35 °C was the optimum temperature for growth and pigment production (Figure 3). Deviation from optimum temperature affects the efficiency of cultural growth.

pH

The result showed that optimization of actinomycetes could produce pigment over a range of pH from pH 4-10 and maximum pH was found 7.5. It is noticed that pigment production declined along with high pH 2 and very less production on pH 4. Similarly very high alkaline pH 10 does not support the pigment production. pH 8 showed some production of pigment according to increase in incubation time up to 96 hours incubation. pH 7 was found also good after pH 7.5. So it is concluded that range of pH 7-7.5 was optimum pH conditions of medium for pigment production by ARITM02 isolate (Figure 4).

Carbon sources

Effect of carbon sources on production of pigment was detected by fermentation media with selected isolate ARITM02 at different carbon sources (with carbon source as starch, fructose, maltose, glucose, lactose, xylose, sucrose). Starch casein broth with standard composition used as a control without inoculation of isolate *Streptomyces flavofuscus* ARITM02. The results of the present study showed that the optimal carbon source for pigment production was starch (Figure 5).

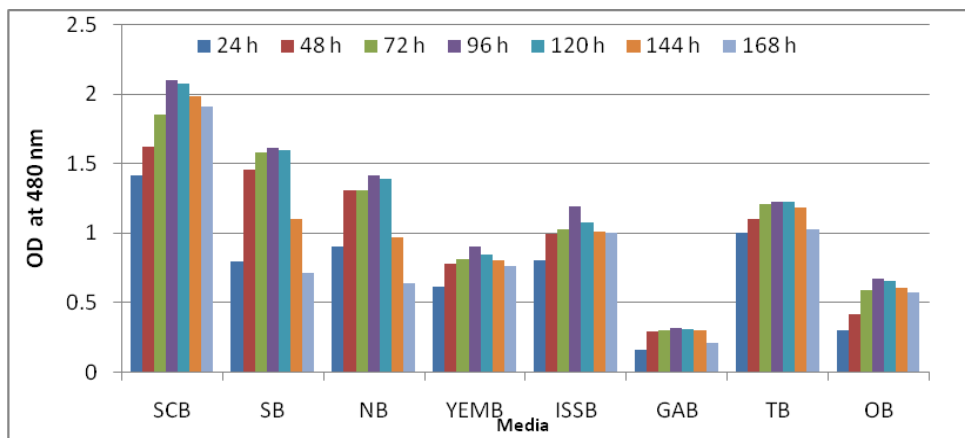
Nitrogen sources

Effect of nitrogen sources on growth of actinomycete was detected by fermentation

media with selected isolate ARITM02 at different carbon sources (potassium nitrate, casein, ammonium nitrate, peptone, beef extract, yeast extract and malt extract). The

results obtained (Figure 6) demonstrated that the optimal nitrogen source for pigment production was peptone.

Fig.1 The growth of *Streptomyces flavofuscus* ARITM02 on different broth media



Starch Casein broth (SCB), Starch broth (SB), Nutrient broth (NB), Yeast extract-malt extract broth (YEMB), actinomycetes isolation broth (AIB), Inorganic starch salt broth (ISSB), Tyrosine broth, Oatmeal agar (OB), Glycerol asparagines broth (GAB)

Fig.2 Effect of different incubation time on growth of *Streptomyces flavofuscus* ARITM02

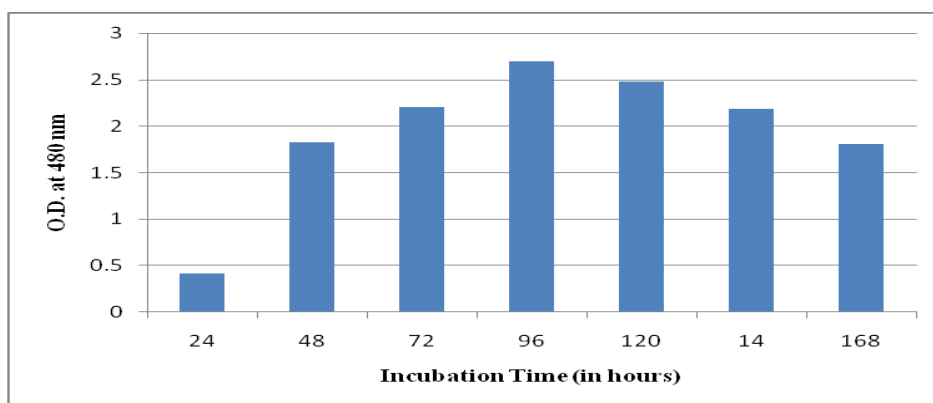


Fig.3 Effect of different temperatures on growth of *Streptomyces flavofuscus* ARITM02

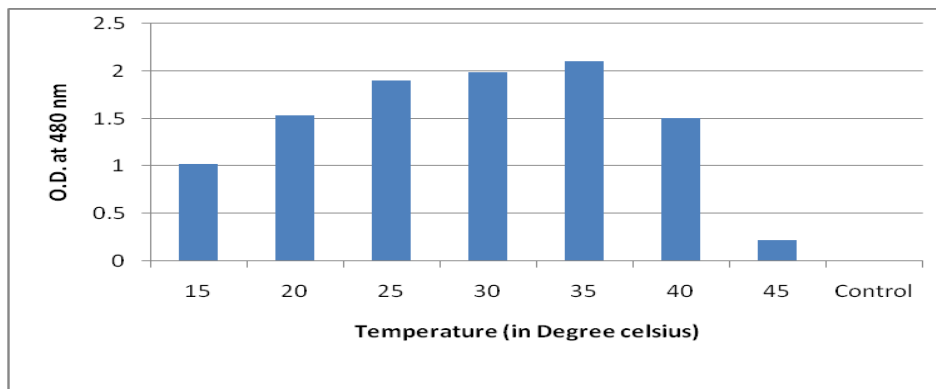


Fig.4 Effect of different pH on growth of *Streptomyces flavofuscus* ARITM02

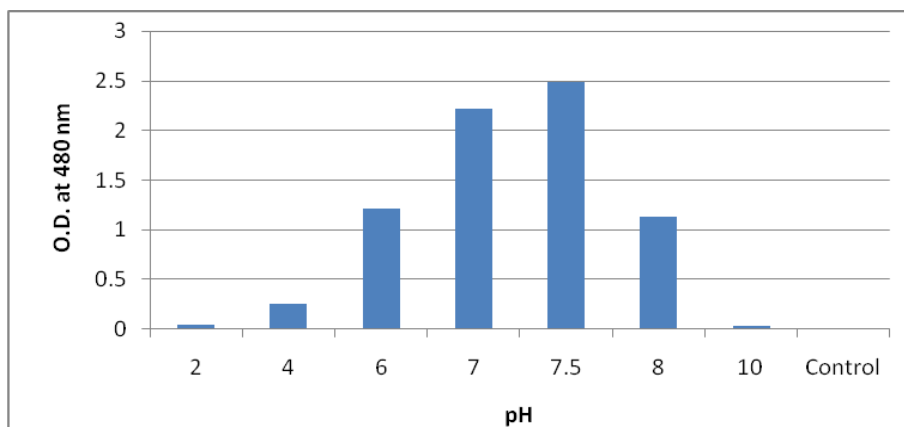


Fig.5 Effect of different carbon sources on growth of *Streptomyces flavofuscus* ARITM02

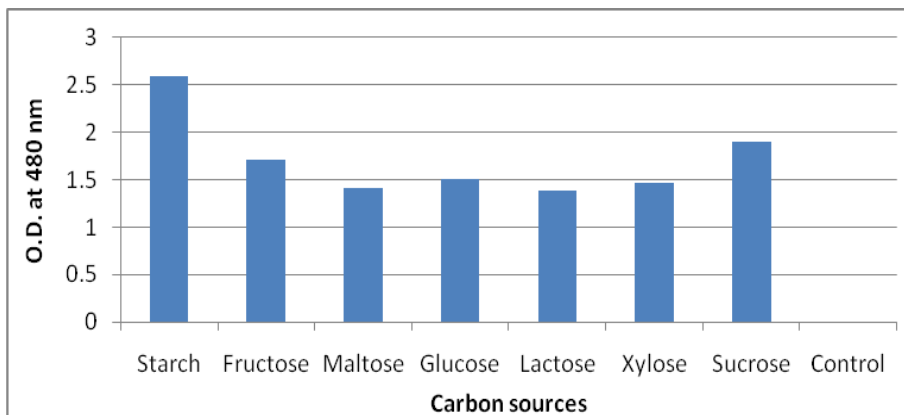
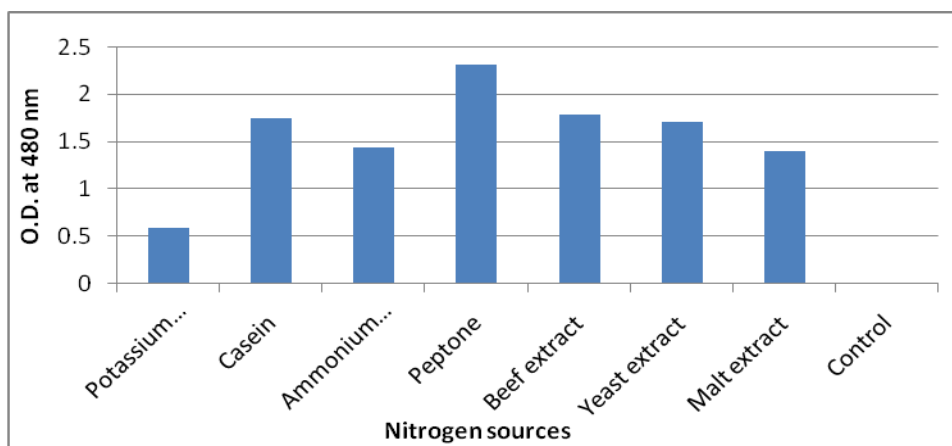


Fig.6 Effect of different nitrogen sources on growth of *Streptomyces flavofuscus* ARITM02



In conclusion, the pigment production was carried out using *Streptomyces flavofuscus* ARITM02 for the maximum growth and

production of pigment the suitable factors were optimized experimentally by using starch casein broth. The temperature 35⁰C,

pH 7.5, incubation period 96 hrs, carbon source Starch, nitrogen source peptone were found best cultural conditions for maximum production of pigment. This pigment could be used in many industries as a natural pigment.

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