CHEMICAL ENGINEERING TRANSACTIONS Volume 20, 2010
Editors Enrico Bardone, Aurelio Viglia
 ISBN 978-88-95608-12-9 ISSN 1974-9791
DOI: 10.3303/CET1020022

Effect of pH and temperature on pigment production from an isolated bacterium

Gargi Goswami, Surabhi Chaudhuri and Debjani Dutta*
Department of Biotechnology, National Institute of Technology, Durgapur
Durgapur - 713209, West Bengal, India

Various synthetic coloring agents have the potential of carcinogenicity and/or teratogenicity. Natural colors, extracted from fruits, vegetables, seed roots and microorganisms and often called "biocolors" due to their biological origin have proved to be safe and edible coloring agents. An unidentified, yellow pigment producing bacterium was isolated from soil in areas around Durgapur, West Bengal, India. Preliminary morphological observations revealed that the colonies were circular, convex and yellow in color. Gram staining reactions showed that the bacterium was Gram-positive coccus. Colonies were grown in brain heart infusion medium with the aim to optimize temperature and pH for growth and pigment production. Pure colonies were grown at pH between 1 and 14 and temperature between 4°C and 45°C. Considerable growth and pigmentation was observed at temperatures between 15°C and 40°C and at pH between 6 and 10, with the maximum yield at pH 7 and 30°C. The pigment was extracted by solvent extraction method using methanol. The pigment extracts were analyzed by scanning the absorbance with a UV-Vis spectrophotometer and peaks were obtained at 437 nm, indicating that the pigment is a possible carotenoid.

Introduction

Colour is a vital constituent and is probably one of the first characteristics perceived by the senses. With the increasing awareness of toxicity of synthetic colours, demand for pigments from natural sources has increased (Babu and Shenolikar, 1995; Khanna and Singh, 1975). Natural colours are generally extracted from fruits, vegetables, roots and microorganisms and are often called "biocolours" because of their biological origin (Pattnaik et al., 1997). There is an increasing demand for natural colours in the food, pharmaceuticals, cosmetics, textile and in printing dye industry. Microbial pigments are a promising alternative to other colour additives extracted from vegetables or animals because they are considered natural, pose no seasonal production problems and show high productivity. Pigment producing microorganisms are yeast, fungi, bacteria, micro algae and are quite common in Nature.

Carotenoids are yellow to orange-red pigments present in a wide variety of bacteria, algae, fungi and plants (Goodwin and Briton, 1980) having the functions of food colorants, absorbers of light energy, oxygen transporters, provitamin A, scavengers of

Please cite this article as: Goswami G., Chaudhuri S. and Dutta D., (2010), Effect of ph and temperature on pigment production from an isolated bacterium, Chemical Engineering Transactions, 20, 127-132; DOI: 10.3303/CET1020022

active oxygen, antitumor and enhancers of in vitro antibody production (Krinsky, 1979; Mathews-Roch, 1979; Palozza et al., 1979; Tomita, 1983; Tee, 1992).
A Gram positive, yellow pigment producing coccus was isolated in the laboratory during routine screening of pigment producing microorganisms. The bacterium was cultured in brain heart infusion medium at varying temperature and pH. The pigment was extracted by solvent extraction using methanol and analyzed by spectrophotometer and High Performance Liquid Chromatography (HPLC). The aim of this study was to determine the optimum temperature and pH for growth and pigment production.

Materials and methods

Brain heart infusion broth, bacteriological agar and Gram staining kit were obtained from Himedia, India. Sodium chloride crystals and HPLC grade methanol were obtained from Merck, India.

Microorganism

The yellow pigment producing bacterium was isolated from soil in areas around Durgapur, West Bengal, India and maintained on brain heart infusion agar plates containing 27.5 g/l nutrient substrate (brain extract, heart extract and peptones), 2.0 g/l D(+)glucose, 5.0 g/l sodium chloride, 2.5 g/l di-sodium hydrogen phosphate and 15 g/l agar. Single colonies were transferred to a fresh plate every fortnight, incubated for 6 days and kept under refrigeration at 4°C.

Preparation of inoculum

A pure culture of the bacterium from the brain heart infusion agar plate was transferred into 250 ml Erlenmeyer flasks containing 100 ml of brain heart infusion broth and incubated with shaking. The 24 hour old culture, at the logarithmic stage of growth, having an optical density (660 nm) of 1.002, was used as the inoculum in all experiments.

Culture conditions

In one set of experiments, 250 ml Erlenmeyer flasks containing 50 ml of the growth medium were inoculated with a calculated amount of the seed culture and incubated at different temperatures ranging from 4°C to 45°C. In another set of experiments, the pH of the medium was varied from 1 to 14. In both the sets, the flasks were incubated with shaking for 6 days. All shake flask experiments were done in triplicate.

Determination of growth

Aliquots (1 ml) of the culture, diluted to 10 ml using 25% NaCl solution, were used to measure growth in terms of optical density (O.D.) at 660 nm with a spectrophotometer (U-2800, Hitachi) (Asker and Ohta, 1999). The experiment was carried out in triplicate.

Extraction and analysis of pigment

Extraction of the pigment was done following the method given by Asker and Ohta (1999) with modifications. 1 ml aliquots of cultures were centrifuged at 14000 xg for 45 min. The harvested cells were resuspended in distilled water for cell lyses to occur. The

pigment was then extracted with methanol by repeated centrifugation until the cell debris turned colorless. The pigment extracts were analyzed by scanning the absorbance in the wavelength region of 400-600 nm using the spectrophotometer. The maximum absorbance was determined at a wavelength of 437nm.

HPLC analysis of the pigment

The yellow pigment was analyzed by HPLC (Waters 600) equipped with a UV-Vis detector (Waters 2489). The pigment extracts were filtered through a 0.45 µm hydrophobic PTFE membrane (Waters). Chromatographic separation was performed on a reverse-phase column (C_{18}, 4.6x250 mm**,** Waters) where the temperature of the column was maintained at room temperature and the mobile phase was methanol at a flow rate of 1 ml/min. The pressure was 756 psi and the injection volume was 20 µl. The peaks were evaluated based on their absorbance at 437 nm.

Results and discussion

The isolated bacterium stained Gram positive. Preliminary morphological observations revealed that the colonies were circular, convex and yellow in color.

Figure 1 shows the HPLC chromatogram of the pigment produced by the isolated bacterium, when peaks were evaluated at 437 nm. The pigment probably belongs to the carotenoid family.

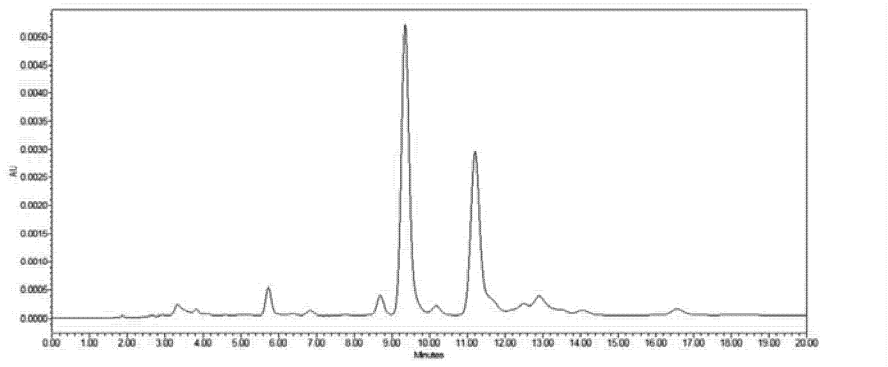

Figure 1: HPLC chromatogram of the yellow pigment

As shown in Figure 2, the bacterium has a moderate incubation period of 6 days, with a long log phase (about 2 days) characterized by an increase in growth. Growth was maximum on day 3, followed by a decrease from day 4.

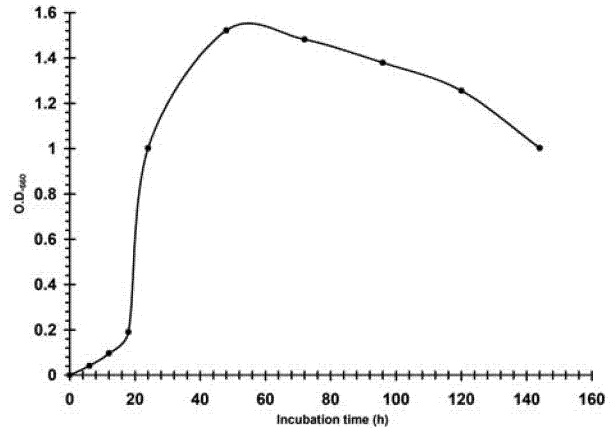

Figure 2: Growth curve of the isolated bacterium

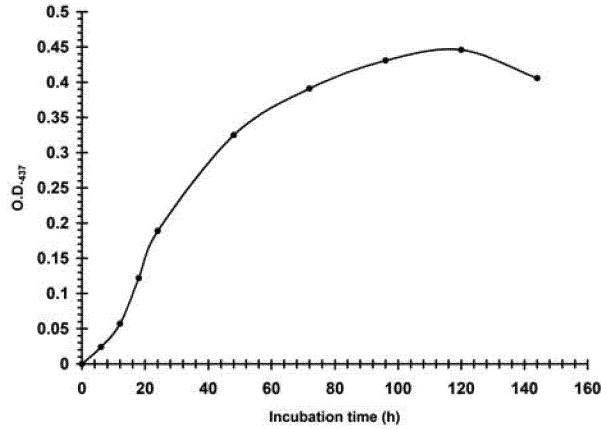

Figure 3: Pigmentation by the isolated bacterium

Considerable pigment production by the strain was observed after about 24 hours of incubation (Figure 3), as the culture became yellow in color. Pigment production increased to a maximum after 5 days of incubation, followed by a decrease on day 6.
The bacterium attains the stationary phase of its growth after about 72 hours of incubation. The bacterium produced the yellow pigment just after 24 hours of incubation which increased till 120 hours of incubation. The production decreased with further incubation.

Effect of temperature on growth and pigment production by the bacterium
Figure 4 demonstrates maximum growth and pigment production at 30°C. There was a gradual and uniform decrease in growth and pigment production with the increase in

temperature from 30°C to 40°C. At temperatures above 40°C and below 10°C, minimum growth and pigment content was observed.

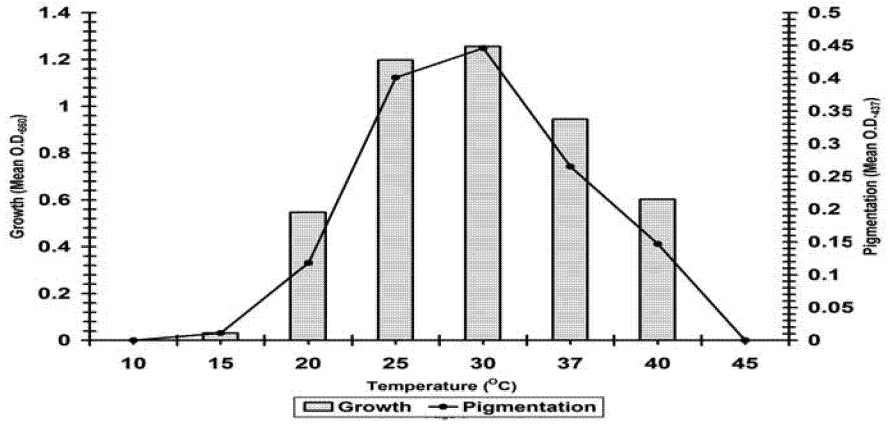

Figure 4: Effect of temperature on growth and pigmentation by the isolated bacterium

Effect of pH on growth and pigment production by the bacterium

It is observed from Figure 5 that maximum growth and pigment production was obtained at pH 7. There was a gradual and uniform decrease in growth and pigment production with the increase in pH from 7 to 10. No growth or pigmentation was observed below pH 6 and above pH 10.

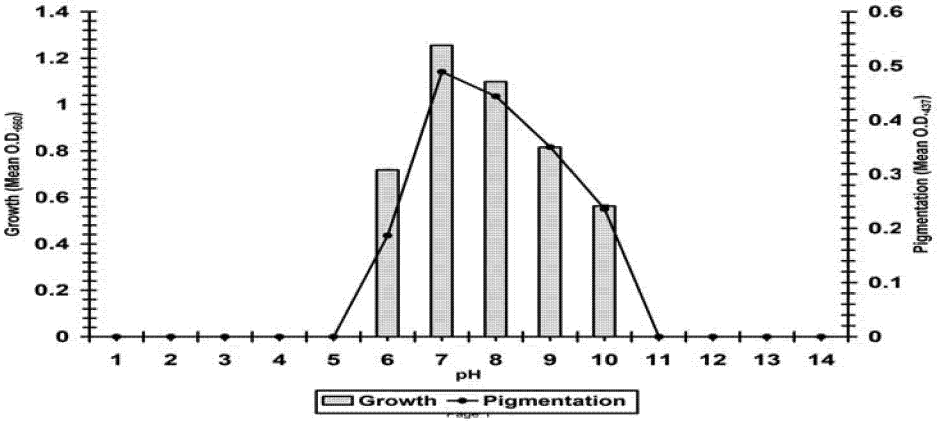

Figure 5: Effect of pH on growth and pigmentation by the isolated bacterium

The bacterium is a strict aerobe since both growth and pigment production increased with shaking. Maximum growth and pigment production occurred in 100 ml medium per 250 ml Erlenmeyer flask. Increasing the volume of the medium above 100 ml in a 250 ml Erlenmeyer flask decreased the amount of dissolved oxygen; therefore, growth of and pigment production by the cells decreased under these conditions. Asker and

Ohta (1999) have reported similar observations. Yokoyama and Miki (1995) reported that varying the medium volume controlled the growth of and carotenoid production by *Agrobacterium auranticum.*

Isolation of a new biological strain producing natural colorant is important in both food and feed industries, but it also has to facilitate its industrial application (Asker and Ohta, 1999).

In summary, the optimum growth and pigment production of the isolated bacterium was realized when inoculated with 2% seed culture and cultured at 30°C and pH 7 with shaking at 180 rpm for 6 d. Maximum peak was obtained at 437 nm when the methanolic extracts of the pigment were analyzed by scanning the absorbance with a UV-Vis spectrophotometer. The present study indicates that pigment production is influenced by physical factors such as temperature and pH of the culture medium. There should be many other factors, affecting pigmentation by the bacterium such as shaker speed, source and concentration of nutrient components. A thorough understanding of the regulation and pathway of pigment production will allow us to develop defined bioprocess for the enhanced production of the desired pigment.

References

Asker D. and Ohta Y., 1999, Production of canthaxanthin by extremely halophilic bacteria, J. Biosci. Bioeng. 88(6), 617-621.

Babu S. and Shenolikar I.S., 1995, Health and nutritional implications of food colors, Indian J. Med. Res., 102, 245-249.

Goodwin T.W. and Briton G., Ed., Goodwin T.W., 1980, Distribution and analysis of carotenoids. Plant pigments. Academic press, London, United Kingdom.

Khanna S.K. and Singh G.B., 1975, Toxicity of commonly used food colors: A review, J. Sci. Indian Res., 34, 631-635.

Krinsky N.I., 1979, Carotenoid protection against oxidation, Pure Appl. Chem. 51, 649-660.

Mathews-Roch M.M., Ed., Bauernfeind J.C., 1979, Carotenoids in medical applications. Carotenoids as colorants and vitamins A precursors. Academic Press, New York.

Palozza P., Luberto C., Ricci P. and Sgarlata E., 1979, Effect of β-carotene and canthaxanthin on tert-butyl hydroperoxide induced lipid per oxidation in murine normal and tumor thymocytes, Arch. Biochem. Biophys., 297, 291-295.

Pattnaik P., Roy U. and Jain P., 1997, Biocolours: New Generation Additives for Food, Indian Food Industry, 16(5), 21-32.

Tee E.S., 1992, Carotenoids and retinoid in human nutrition, Crit. Rev. Food Sci. Nutr., 31, 103-163.

Tomita Y., 1983, Immunological role of vitamin A and its related substances in prevention of cancer, Nutr. Cancer 5, 187-194.

Yokoyama A. and Miki W., 1995, Composition and presumed biosynthetic pathway of carotenoids in the astaxanthin-producing bacterium *Agrobacterium aurantiacum*, FEMS Microbial. Lett., 128, 139-144.