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Research Article

OPTIMIZATION OF PHYSICO-CHEMICAL PARAMETERS FOR PIGMENT PRODUCTION IN BACTERIA ISOLATED FROM DIFFERENT EFFLUENT WATER SAMPLES

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Abstract: Prodigiosin and Carotenoid (Dihydroxy C50 Carotenoid) producing *Serratia marcescens* and *Micrococcus luteus* were isolated from different effluent samples and cultured in Nutrient Agar Medium. In the presnt study conducted maximum pigments (Prodigiosin and Carotenoid) production was observed at pH 7.5, temperatures at which the pigments showed optimum production was at room temperature (25ºC) and 16ºC, respectively, optimum concentration of carbon source was at 0.5% and the concentration of nitrogen source was at 0.5% and 2.0%, respectively. After incubation under optimised conditions, the broth was centrifuged and the supernatant was used as the pigment sample. The samples (pigments) were subjected to Thin Layer Chromatography (TLC), which showed spots on the TLC plate and the Rf value was calculated for different spots produced by the pigments. *Serratia marcescens* and *Micrococcus luteus* produced maximum amount of the pigments under these optimised conditions, which could be used to increase the production of Prodigiosin and Carotenoid pigments, respectively.
Key words: Prodigiosin, Carotenoid, *Serratia marcescens*, *Micrococcus luteus*, Thin Layer Chromatography

INTRODUCTION

Pigments have been extensively used in food production, fish industries, textile industries, paper production, agricultural practices and researches, water science and technology[1] and also having biological activities as antioxidants and anticancer agents. Microorganisms have been used for a long time for production of molecules as diverse as antibiotics, enzymes, vitamins, texturizing agents and so on[2]. Ingredients, such as colours, are considered natural when derived from biological sources like plants or microorganisms. Microorganisms produce various pigments like carotenoids, melanins, flavins, quinones, prodigiosins and more specifically monascins, violacein or indigo[3]

Prodigiosin, the bright red pigment produced by organisms of the genus, *Serratia*, is among the more conspicuous pigments extant in the microbial world. The chemical nature of Prodigiosin continues to be the subject of extensive study and it has been defined as a tri-pyrrylmethene. The Prodigiosin pigments have intrigued organic chemists and pharmacologists and may yet play roles in the treatment of deadly infectious diseases such as malaria and can also act as immunosuppressant drugs. There has always been a continuing curiosity in the Prodigiosin/ Serratia story and the major reason for this is the theory that these viscous, crimson bacterial colonies provide a naturalistic explaination[4]

Carotenoids are a group of coloured terpnoids with antioxidant properties which are widespread in the plant and animal kingdoms, as well in fungi and in photosynthetic and non-photosynthetic microorganisms[5]. Recently, carotenoids are used commercially as food colourants, animal feed supplement and, more recently, for nutraceuticals, cosmetic and Pharmaceutical purpose[6]. In addition, carotenoids have attracted superior attention as compared to synthetic pigments due to the beneficial role on human health. Carotenoids can inhibit various types of cancer and it enhances the immune response[7]. These pigments are capable of quenching photo sensitizers; interacting with singlet oxygen[8] and scavenging proxy radicals[9]. It also protects "life style – related" diseases such as cardiovascular disease and age related macular degeneration, due to their antioxidant activity and provitamin A function[10]. They play an important role in protection of macular region of the retina and hence prevents of cataracts and increases levels of iron absorption[11].

In the study that was conducted, an attempt was made to optimise the physico- chemical parameters for the production of pigments in *Serratia marcescens* and *Micrococcus luteus*, isolated from different effluent water samples.

MATERIALS AND METHODS

Sewage samples were collected from different industrial areas in Peenya, Bangalore, and screened for the presence of specific bacteria producing pigments.

I. Isolation of Microorganism

1 ml of sewage water was mixed with 9 ml of distilled water in a test tube (10^{-1} dilution) and mixed properly using a cyclo-mixer. 1 ml of 10^{-1} dilution solution was taken and mixed with 9 ml of distilled water to make 10^{-2} dilution. Similarly, serial dilution was carried out up to 10^{-6} dilution. Nutrient agar plates were prepared and autoclaved. Then they were inoculated by spreading 0.1 ml of serially diluted sample (10^{-3}& 10^{-6}) and kept for incubation for 24-48 hours. The morphological and the biochemical tests were carried

out in order to confirm the characteristics of the culture species.

II. Extraction of the pigment from the microorganism

2 ml of 24 hours grown culture broth was taken in a test tube and centrifuged at 8000 rpm for 10 minutes in a cooling centrifuge. Supernatant was discarded, and 2 ml of methanol was added to the pellet and mixed properly using a cyclo mixer until the cells, containing the pigment get colourless. The sample was centrifuged again at 8000 rpm for 10 minutes to pellet out the pigment extracted cells. Supernatant was transferred in to another test tube. Absorbance was read at various wavelengths using a spectrophotometer.

III. Effect of temperature on pigment production

The pigments were inoculated in five different nutrient broth containing test tubes and they were kept at different temperatures (16ºC, 25ºC, 30ºC, 37ºC, 40ºC) for 24 hours incubation. The production of the pigments were estimated after incubation. The maximum temperature, at which the maximum production of prodigiosin and carotenoid takes place, were observed, chosen and maintained for following studies.

IV. Effect of pH on pigment production

The pigments were inoculated in five different nutrient broth containing test tubes and they were kept at different pH (6.0, 6.5, 7.0, 7.5, 8.0) for 24 hours incubation. The production of the pigments were estimated after incubation. The maximum pH, at which the maximum production of prodigiosin and carotenoid takes place, were observed, chosen and maintained for following studies.

V. Effect of different concentrations of carbon source on pigment production

The pigments were inoculated in five different Nutrient broth containing test tubes with different carbon (dextrose) concentrations (0.5%, 1.0%, 1.5%, 2.0%, 2.5%) and they were kept for 24 hours incubation. The production of pigments were estimated after incubation. The maximum concentration of carbon source at which the maximum production of prodigiosin and carotenoid takes place, were observed, chosen and maintained for following studies.

VI. Effect of different concentrations of nitrogen source on pigment production

The pigments were inoculated in five different Nutrient broth containing test tubes with different nitrogen (peptone) concentrations (0.5%, 1.0%, 1.5%, 2.0%, 2.5%) and they were kept for 24 hours incubation. The production of pigments wee estimated after incubation. The maximum concentration of nitrogen source at which the maximum production of prodigiosin and carotenoid takes place, were observed, chosen and maintained for following studies.

VII. Mass production of the pigments

After the optimisation of the above mentioned physico-chemical parameters, the mass production of the pigments were carried out.

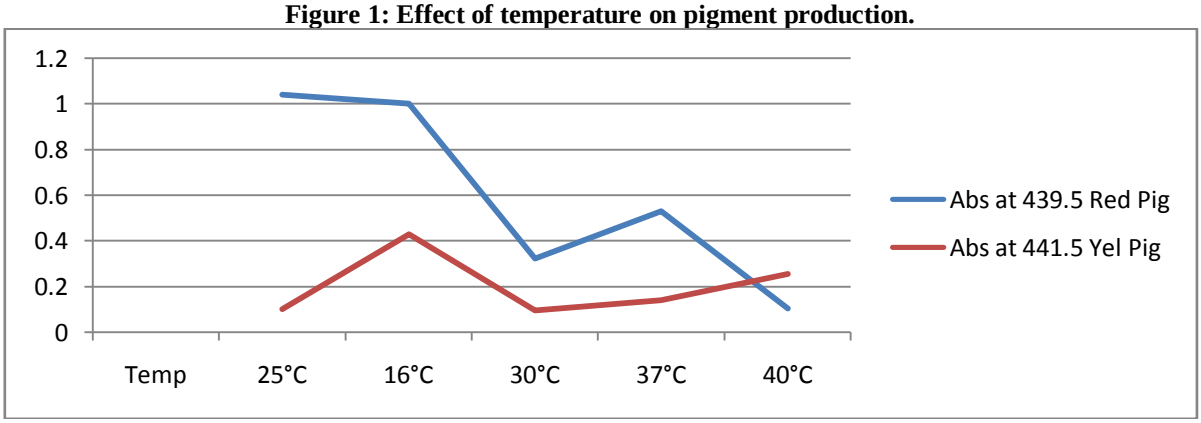
VIII. Separation of pigments by Thin Layer Chromatography (TLC)

Thin Layer Chromatography of the pigments were carried out with the help of a sheet of plastic, which is coated with a thin layer of silica gel, that acts as the stationary phase. The pigment sample (mobile phase) was then loaded onto the plate, which is drawn up the plate via the capillary action. Separation of the pigments takes place at the end of the process.

RESULTS AND DISCUSSION

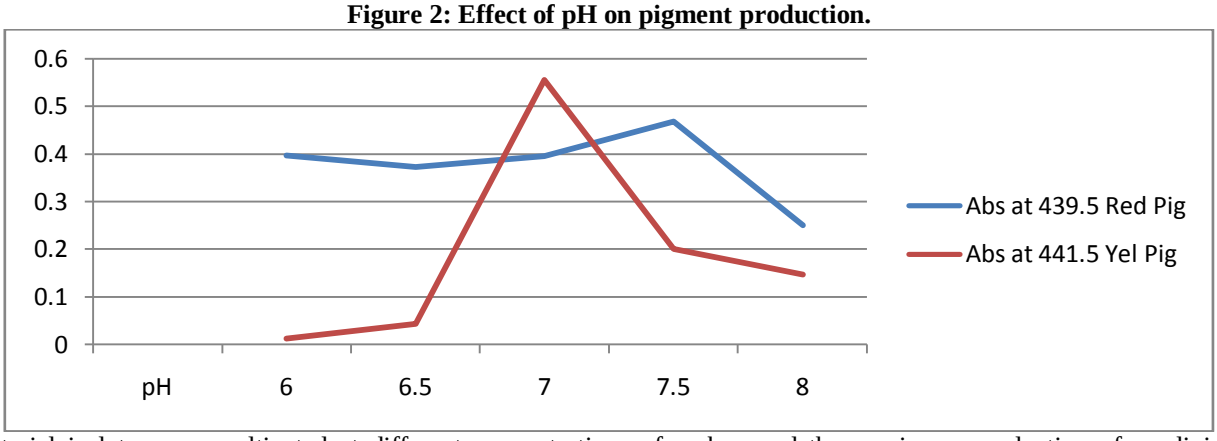
Two sewage samples were collected from different industrial areas in Peenya, Bangalore and screened for prodigiosin and carotenoid producing microorganism. The isolated colonies were identified based on their morphological and biochemical characteristics. The isolates were confirmed as *Serratia marcescens* and *Micrococcus luteus*. The amount of prodigiosin and carotenoid produced by the bacterial isolates were estimated. These isolates were further taken for cultivation under submerged fermentation. The maximum amount of prodigiosin and carotenoid produced, were recorded at an absorbance of 439.5 and 441.4, respectively. The bacterial isolates were cultivated at different temperatures and the maximum production of prodigiosin and carotenoid were obtained at a temperature of 25ºC and 16ºC. A graph was plotted with temperature on the x-axis and absorbance on the y-axis.

Figure 1: Effect of temperature on pigment production.



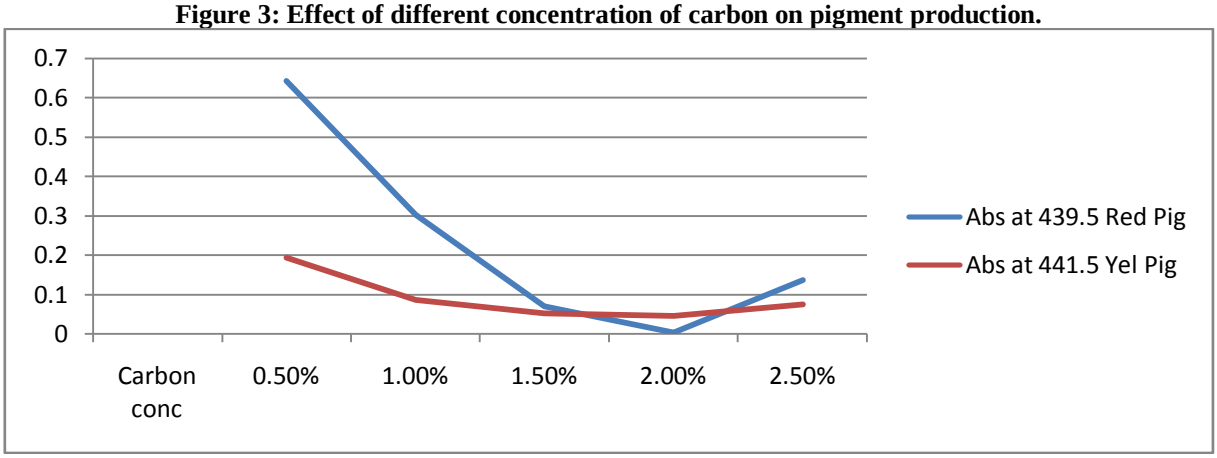
The bacterial isolates were cultivated at different pH and the maximum production of prodigiosin and carotenoid were obtained at a pH of 7.5%. A graph was plotted with pH on the x-axis and absorbance on the y-axis.

Figure 2: Effect of pH on pigment production.



The bacterial isolates were cultivated at different concentrations of carbon and the maximum production of prodigiosin and carotenoid were obtained at 0.5%. A graph was plotted with the concentration of carbon on the x-axis and the absorbance on the y-axis.

Figure 3: Effect of different concentration of carbon on pigment production.



The bacterial isolates were cultivated at different concentrations of nitrogen and the maximum production of prodigiosin and carotenoid were obtained at 0.5% and 2.0%, respectively.

Figure 4: Effect of different concentration of nitrogen on pigment production.

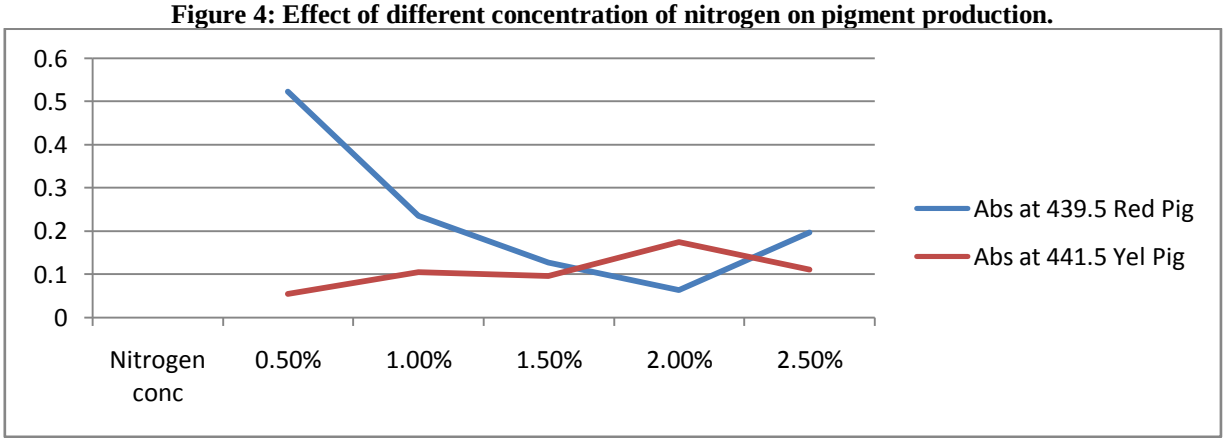


Table 1: Separation of Pigments by TLC.

Distance travel by the solvent (cm)	No: of Spots	Distance Travel By The Sample (cm)	
		Red Pigment	Yellow Pigment
10.5	1	**4.7**	**4.8**
	2	**7.0**	**8.0**
	3	**8.0**	**8.8**

Table 2: Rf values of the samples.

	Red pigment	Yellow pigment
Rf value	0.466	0.457
	0.666	0.761
	0.761	0.838
	0.852	---

The TLC values were calculated for the pigments. Both the pigments underwent the Thin Layer Chromatography (TLC) technique (Table 1, in order to determine the purity of the substance and the Retention factor (Rf) was calculated for both the pigments and the values were recorded. The pigments (Red and Yellow), were used for the TLC procedure and the TLC plates (TLC plate 1 and TLC plate 2), were observed under UV-Hand Illuminator.

Conclusion

The effluent water samples, that usually pollute the environment, can be used for the large-scale production of isolated microbial pigments that have proven to be extremely advantageous for use as colorants in food stuffs and cosmetics, used as dyes in textile industry, use as an antioxidants etc.

The aim of the study was to produce the pigments; Prodigiosin and Carotenoid, from *Serratia marcescens* and *Micrococcus luteus*, respectively and to optimise the conditions; Temperature, pH, Carbon source and Nitrogen source for the production of pigments.

Prodigiosin and **Carotenoid** (Dihydroxy C50 Carotenoid) producing *Serratia marcescens* and *Micrococcus luteus* was isolated from effluent samples and cultured in Nutrient Agar Medium. Maximum pigments (Prodigiosin and Carotenoid) production was observed at pH 7.5, temperatures at which the pigments, Prodigiosin and Carotenoid showed optimum production was at room temperature (25^oC) and 16^oC, respectively, concentration of carbon source at which the pigments, Prodigiosin and Carotenoid showed optimum production was at 0.5% and the concentration of nitrogen source at which the pigments, Prodigiosin and Carotenoid showed optimum production was at 0.5% and 2.0%, respectively.

After incubation under optimised conditions, the broth was centrifuged and the supernatant was used as the pigment sample. The samples (pigments) were subjected to Thin Layer Chromatography (TLC), which showed spots on the TLC plate and the R_f value was calculated for different spots produced by the pigments. *Serratia marcescens* and *Micrococcusluteus* produced maximum amount of the pigments under these optimised conditions, which could be used to increase the production of Prodigiosin and Carotenoid pigments, respectively.

References

1. Khanafari A, Assadi MM, Fakhr FA. Review of prodigiosin, pigmentation in Serratiamarcescens. **2006**; 1-13.
2. Dufosse L. Microbial Pigments. *Encyclopedia Microbiol*. **2009**; 4: 457-471.
3. Chidambaram Kulandaisamy Venil, Perumalsamy Lakshmanaperumalsamy. An Insightful Overview on Microbial Pigment, Prodigiosin. *Journal of Biology*, **2009**; 5(3): 49-61.
4. Phadwal K. Carotenoid biosynthetic pathway: molecular phylogenies and evolutionary behavior of crt genes in eubacteria. *Gene*, 2005; 345: 35-43.
5. Klein-Marcuschamer D, Ajikumar PK and Stephanopoulos G. Engineering microbial cell factories for biosynthesis of isopernoid molecules: beyond lycopene. *Trends in Biotechnol*, **2007**; 25(9): 417-424.
6. Krinsky NI. The Biological properties of carotenoids. *Pure Appl. Chem*, **1994**; 66: 1003-1010.
7. Guerin M, Mark EH. and Olaizola M. HaematococcusAstaxanthin: Applications for human health and nutrition. *Trends in Biotechnol*, **2003**; 21: 210-216.
8. Conn PF, Lambert C, Land EJ, Schalch W and Truscott TG. Carotene-oxygen radical interactions. FreeRadic. Res. Commun, **1992**; 16: 401–408.
9. Mares-Perlman JA, Millen AE, Ficek TL and Hankinson SE. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. Overview.J. Nutr, **2002**; 132: 5185–5245.
10. Young M et al. Genome sequence of the Fleming strain of Micrococcus luteus, a simple free-living action bacterium. *J Bacteriol*, **2010**; 192: 841-60.
11. Semba et al. The Role of Carotenoids as Functional Foods in Disease Prevention and Treatment. *The Natural Therapist*, **2007**; 25(2): 143.