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Original Research Article

Production of food grade pigments from *Pseudomonas aeroginosa*

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Abstract

Pseudomonads are well known for their degradative abilities and play an important role in the environmental cleanup and they are well known for their opportunistic pathogenic properties. The characteristic feature of *Pseudomonas aeruginosa* is the production of soluble pigments like Pyocyanin, which is an secondary metabolite that is produced in both solid and liquid culture media. The initial pyocyanin pigment concentration at 6 h was 3.50 mg/ml whereas the initial concentration of pyorubrin was 2.50 mg/ml**.** The rate of increase in pigment concentration was nearly equal for both the pigments. In pyocyanin, all the concentration showed good, pleasant colouration. The maximum intensity was observed in 25 mg/ml. and the least colouration was observed in 5 mg/ml. with pyocyanin.

Keywords: *Pseudomonas aeruginosa, Pigments, pyocyanin, pyorubrin.*

Introduction

Bacterial pigment production is now one of the emerging fieldsof research to demonstrate its potential for various industrial applications (Venil and Lakshmanaperumalsamy, 2009). Most of the bacterial pigment production is still atthe R&D stage. Hence, work on the bacterial pigments should beintensified especially in finding cheap and suitable growth mediumwhich can reduce the cost and increase its applicability for indus-trial production (Ahmad et al., 2012)

P. aeruginosa produces a variety of extracellular pigments, of which phenazines comprise a significant portion. Phenazine compounds produced by fluorescent *Pseudomonas* species are biologically active metabolites that function in microbial competitiveness (Mazolla et al., 1992). Pyocyanin is the main phenazine pigment associated with this particular organism. 90 to 95 % of *P. aeruginosa* strains produce pyocyanin (Smirnov *et al*., 1990).

Materials and Methods

Isolation of *P. aeruginosa*

Sediment samples were collected from mangrove environment from Marakkanam, Tamil Nadu, India using sterile spatula and aseptically transferred in to sterile polythene bag. The sediment samples were serially diluted and plated on Zobell Marine agar and incubated at 35^{o}C for 48 h. All the blue green pigmented colonies were transferred to *Pseudomonas* isolation agar and Cetrimide Agar to ensure the results of blue green pigment production and stored in slants with same medium at 4^{o}C until use.

Identification of *P. aeruginosa* was done based on morphological, cultural, biochemical and physiological characteristics as suggested by Schaad et al. (2001) and the results were crosschecked with Bergey's Manual of Determinative Bacteriology (Buchanan et al., 1974).

P. aeruginosa was grown in the medium described by Frank and DeMoss (1959), (DL-Alanine- 10g; Glycerol - 20 ml; K_2HPO_4 - 0.139 g; $MgCl_2.6H_2O$ - 4.06 g; Ferric Citrate- 0.1 g; 50% seawater - 1000 ml). 200 ml of the medium was prepared in 500 ml of culture flask and the strain was inoculated as 1% concentration and incubated at 35^{o}C for 72 h.

Two volumes of chloroform were added to one volume of cell free culture supernatant and shaken well. The pyocyanin was then extracted from the chloroform into 0.2 N HCl to this deep red acid solution 0.4 M borate-NaOH buffer (pH 10) was added until the colour changed to blue and the blue coloured pyocyanin was again extracted into chloroform. This step was repeated 2 or 3 times, resulting in a clear blue solution of pyocyanin in chloroform and pyocyanin powder was collected by evaporating the chloroform (Frank and DeMoss, 1959). After the extraction of pyocyanin in chloroform phase, pyorubrin in the aqueous phase was separated and lyophilized (Palumbo, 1972).

A standard pyocyanin pigment graph was prepared by plotting known concentration of pyocyanin pigment and the OD was measured at 520 nm**.** Pyocyanin was extracted from the culture broth collected at different time intervals and estimated by measuring the OD at 520 nm and the concentration was calculated from the standard graph (MacDonald, 1967).

The *P. aeruginosa* was cultured in mineral salt media (Frank and DeMoss 1959). 10 ml culture sample was collected at every 6 hour interval until 72 hours in 30 ml screw cap tubes which was sterilized previously in an autoclave. The pigment pyocyanin was extracted from the culture media by adopting the method described by MacDonald (1967) as mentioned above. OD of the samples were measured at 520 nm and its concentration was estimated by plotting the values in the standard graph.

Pyorubrin extracted from the culture broth collected at different time intervals was estimated by measuring the OD at 520nm and the concentration was calculated from the standard graph Palumbo, 1972). A standard pyorubrin pigment graph was prepared by plotting known concentration of the pigments.

Broth culture sample was collected at different intervals and centrifuged at 10,000 rpm for 15 min. The pellet was dried in hot air oven at 80^{o}C for a period of 24 h and the biomass quoted in terms of mg/ml (dry weight).

The pigments were extracted and estimated. Various concentrations of the pigment was prepared (5, 10, 15, 20 and 25 mg/ml). 15 ml of different concentrations were taken and 3% agar was added to it. It was then heated to boiling and then cooled to solidification. This to check whether the pigments give an appealing colour in food materials.

Results and Discussion

Out of the 25 different colonies isolated in Zobell marine agar medium, only two strains showed growth in *Pseudomonas* isolation medium and also produced blue green diffusible pigment. These two isolates were subjected to morphological, cultural, biochemical and physiological characterization. Based on the results the isolates were identified as *Pseudomonas aeruginosa* (Table 1). Out of the two strains producing pigments, one strain was chosen for the present study as it showed comparatively intense pigment production.

Pigment production in mineral salt medium revealed that the appearance of pigment starts at 6th h. The pyocyanin and pyorubrin was estimated at six hour interval. Pigments showed steady increase in concentration up to 60 h of incubation and decreased afterwards (Fig 1.). The initial pyocyanin production was comparatively more than pyorubrin. The initial pyocyanin pigment concentration at 6 h was 3.50 mg/ml whereas the initial concentration of pyorubrin was 2.50 mg/ml**.** The rate of increase in pigment concentration was nearly equal for both the pigments. There is a correlation observed between the biomass and pigment production up to 66 h after which decrease in biomass was observed (Figs 2&3).

Table 1. Physiological and biochemical characteristics of *P. aeruginosa*

Test/characteristics	Results
Colony	Circular, raised, and lobed colony
Pigment on nutrient agar	Diffusible green pigment turning to blue green in 48 hours.
Gram reaction	Negative
Motility	Motile
Shape, size and arrangement	Small rods, mostly single
Growth at 48°C	+
Citrate utilization	+
Catalase	+
Oxidase	+
Methyl red	-
Vogus proskuaer	-
Indole production	-
H_2S production	+
Starch hydrolysis	-
Lipid hydrolysis	+
Gelatin liquefaction	+

In pyocyanin, all the concentration showed good, pleasant colouration. The maximum intensity was observed in 25 mg/ml. and the least colouration was observed in 5 mg/ml. with pyocyanin (Fig.4). Even in lower concentration the colour obtained which was pleasant to see. Like wise pyorubrin showed a chocolate brown colour and other things holds good with that of pyocyanin.

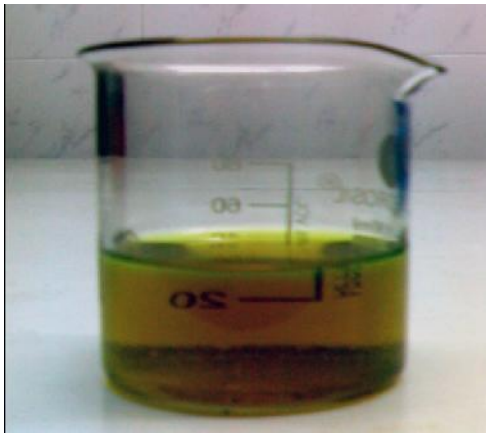

Fig1. Pigment production by P. *aeruginosa*

A. P. aeruginosa culture in mineral salt medium, B. Pyocyanin pigment and C. Pyorubrin pigment.

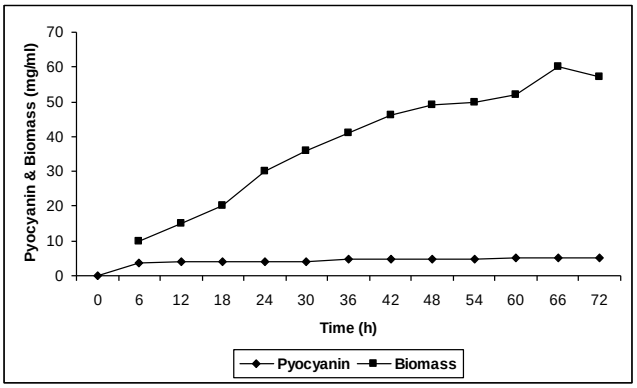

Fig 2. Pyocyanin pigment production

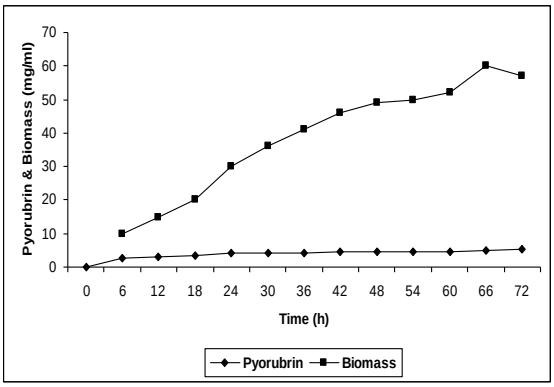

Fig 3. Pyorubrin pigment production

The pyocyanin and pyorubrin pigments showed a steady increase in concentration till 60 h after which no further production was noted and these results were comparable with that of Graham et al. (1979). The main factor involved in the accumulation of pyocyanin as shown by Burton et al. (1948) in Pseudomonad cultures is the concentration of iron and its availability to the organism. With both iron and sulfate pyorubrin is formed.

Pyocyanin pigment from *P. aeruginosa* produced good colouration when mixed with agar. It showed a light blue colouration which did not deterioated on boiling. The pigment did not make the agar to loose its property. The pigment seems to have a binding property with agar. Pyorubrin also had all these properties except that it gave chocolate brown colour on boiling. Many microbial pigments have already been commercialized: Astaxanthin from *Xanthophyllomyces dendrorhous*, Arpink red from *Penicillium oxalicum*, riboflavin from *Asbhya gossypii*. Like wise *P. aeruginosa* pigments also has the potential to use as colourant in beverages, cakes, confectionaries, puddings etc. the pigments may also be used to decorate and display the food items also.

Thus the results of the present study clearly indicated that the phenazine pigments, pyocyanin and pyorubrin of *P. aeruginosa* can be produced in large quantities by the strain used and no special environment is needed for the production. The results of antibacterial and hemolytic activity were also favourable.

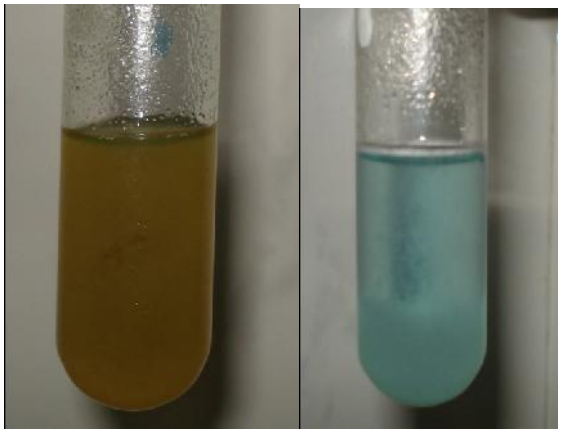

Fig. 4. A. Pyorubrin & B. Pyocyanin as food colourants in agar.

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