

BIOCOLOUR FORMATION FROM PIGMENT PRODUCING BACTERIA AND ITS APPLICATION IN TEXTILE INDUSTRY.

Biological Science

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ABSTRACT

In present study, two bacterial strains PG 1 and PG 2 isolated from fruit and vegetable waste soil sample are potential source for pigment production. On the basis of biochemical characterization, strains were belonged to *Micrococcus* species. The orange pigment produced by PG1 showed Rf value 0.76 and λ_{max} was 350nm which was similar to torulene compound and yellow pigment produced by PG2 showed Rf value 0.69 and λ_{max} was 400nm which was similar to β -carotene compound. Germination assay showed that these pigments are nontoxic to plant. PG 2 species allowed the maximum seed germination percentage of 90% and PG 1 showed 85%. The two colours were used for dyeing of fibres with lime as mordant and their wash performance showed that colours are stable. In future, present study can be implemented to replace synthetic colorants used in textile and dyeing industries.

KEYWORDS

Bio colour, Germination, Textile, Pigment

Introduction:

Colours are the beauty of world that gives beautiful appearance to commercial products such as food, textile, cosmetics and pharmaceutical products. The demand for natural colours and synthetic colours are increasing in everyday life in these fields (Tibor, 2007). The artificial synthetic colours which are being used in foodstuff, dyestuff, cosmetic and pharmaceutical manufacturing processes produce hazardous effects such as allergies, tumour, cancer and severe damages to the vital organs (Duran et al., 2002) and the waste of synthetic colours also not favourable for eco-system. To overcome these hazardous effects of synthetic colorants, there is worldwide interest in process development for the production of pigments from natural sources, there is an increasing interest in the development of colours from natural sources (Babu et al., 1995).

The microbial pigments are getting a lot of attention owing to the stability of the pigments produced (Raisainen et al., 2002) and the availability of cultivation technology (Kim et al., 1999 and Parekh et al., 2000). Microbes show easy and fast growth in the low-cost culture medium (Dufosse, 2009) and independent from seasons and geographical conditions. Pigments like anthocyanin, prodigiosin and violacein are widely used to treat disease in pharmaceutical industry. Some microbial pigments are also being used in textile industry (Kumar et al., 2015).

Textile industries utilize huge amount of synthetic dye and produce effluents. Due to hazardous effects of synthetic dye on eco-system, there is a requirement of alternative natural source which have less toxic on environment and give a product that is eco-friendly. As a consequence, there is a revived interest in the use of natural pigments and dyes, which could be subjected to biodegradation in the environment.

Materials and Methods:

2.1. Isolation, screening and characterization of Pigment Producing Bacteria

Soil sample was collected from the dumping site of fruits and vegetables waste, Indore, Madhya Pradesh. All samples were serially diluted by serial dilution range of 10^{-1} to 10^{-6} . The samples (0.1 ml each) from each dilution were mounted by spread plate method on sterilized petri plates containing solidified nutrient agar media for isolation of bacterial colonies. Plates were incubated for 48 hours at $37 \pm 2^\circ\text{C}$ for pigment production. After the incubation, the pigment producing colonies were selected and purified using streak plate technique. The isolates were primarily examined according to their colony morphology and pigment colour. In order to determine the morphological and biochemical characterization of the selected bacterial isolate following biochemical tests were performed (Holt et al., 1994).

2.2. Extraction and analysis of pigment

The fresh biomass of pigment producing bacterial isolates were harvested on nutrient agar plates and dried at temperature 60°C for 24 hrs. Dried biomass was grounded to powdered form and weighted to know the yield and dissolved in methanol. The pigments were then analysed in a U.V.-Visible spectrophotometer for detecting their absorption maxima (λ_{max}). The wavelength range were selected 300-600 nm.

20 μ l of sample were spotted on the TLC plate. TLC plates were placed in a pre-saturated TLC chamber containing mobile phase (methanol: acetone, 30:20 v/v). TLC plate was kept for 5 minutes for drying of the spot and the retention factor (Rf) value was calculated.

$R_f = \text{Distance travelled by the compound} / \text{Distance travelled by solvent by the solvent front}$

2.3. Germination Assay

Moong (*Vignaradiata*) seeds were used to test the assay. Seeds were placed in soil in a pot and 5 ml of pigment (diluted in distilled water, 5 ml of pigment in 95 ml of distilled water) was added accordingly to the soil in pot. tap water was taken as control. After 10 days radical and plumule length of seeds were measured and germination percentage was calculated.

$\text{Germination \%} = \text{seed germinated} / \text{seeds taken} * 100$

3.4. Application of the Extracted Bicolour

(a) Dyeing of Textile Material

Pigments in methanol were used as a stock solution for orange and yellow pigment. Attempts were made to dye white cotton, polyester and nylon fibres with the extracted microbial bio colourants. The dyeing of all fibres was carried out at 100°C for 60 minutes with 500 μ l of pigments and dyed fibres were air dried. Thereafter fibres treated with lime as a mordant for increasing the binding capacity of pigment to fibres (Shirata et al., 2000).

(b) Wash Performance of the Dyed textile material

I. Washing Fastness

Dyed fibres were added in preheated soap solution (detergent, at 60°C) and water was taken in ratio of 1: 50 (0.5 g/25 ml) for 30 minutes. Then specimen was removed and rinsed in cold water.

II. Rubbing Fastness

The rub fastness of the dyed fibres was carried out by rubbing the fibres manually and checking for fading of colour.

III. Light Fastness

The dyed fibres were exposed to sunlight for 24 h. The colour fastness to light was evaluated by comparison of colour change of the exposed

portion to the unexposed original material.

The rating for rubbing, light and washing fastness was determined to respect to staining on cotton, polyester and nylon showing rating between 1 to 5.

Results and Discussion:

3.1. Isolation, screening and characterization of Pigment Producing Bacteria

Total 2 bacterial isolates were found PG1 was orange colour producer and PG2 was yellow colour producer. According to Bergey's manual the PG 1 and PG 2 isolates were identified on the morphological and biochemical tests that showed orange and yellow strains as *Micrococcus* species

3.2. Extraction and analysis of pigment

The dry weight of bacterial biomass 0.07gm and 0.10gm were obtained for PG1 and PG2. The spectrophotometric analysis showed maximum absorption wavelength for PG 1 was 350nm and PG 2 was 400nm (Figure 1). Results of TLC analysis revealed the presence of two major pigments. The Rf value of orange color was noted as 0.76 which was similar to toluene compound. The Rf values of yellow color was 0.69 which was similar to β -carotene.

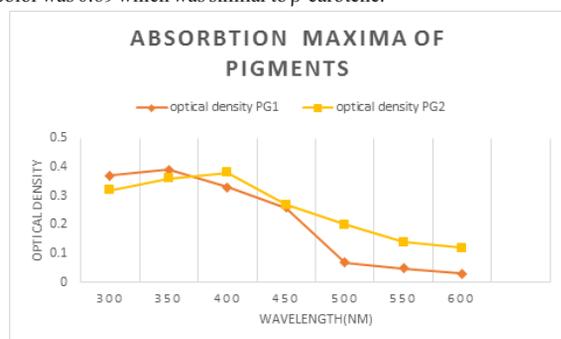


Figure:1 Spectrophotometric analysis of pigments.

3.3. Germination Effects

After 10 days, radical length and plumule length of seeds were measured. PG 2 culture allowed the maximum seed germination percentage of 90% and PG 1 showed germination percentage of 85%.

3.4. Application of the Extracted Bicolour

(a) Dyeing of Textile Material

The pigments produced from the 2 bacterial isolates were subject to application in fabrics. The fibres and cotton dyed with microbial bio colourants have shown uniformity and levelness (Figure2).



Figure2: Dyeing of pigments on fibres

(b) Wash Performance of Dyed Textile Material

The dyed fibres of cotton/ polyester/nylon were tested for assessment of fastness properties such as, washing fastness, rubbing fastness and light fastness and rubbing fastness (Table 1).

Fastness Properties	Orange	Yellow
Washing Fastness		
Cotton	5	5
Polyester	4	4
Nylon	2	3
Rubbing Fastness		
Cotton	4	5
Polyester	3	3
Nylon	2	3
Light Fastness		

Cotton	3	4
Polyester	2	3
Nylon	2	3

(Rating: 5 - Excellent, 4 – Very good, 3 – Good, 2 – Poor, 1 – Very poor)

Table1: Rating of fastness properties of dyed textile material

Conclusion

We concluded from the present investigations, that two bacterial strains viz., PG 1 and PG 2 isolated from soil sample are potential source for pigment production. The bacterial strains (PG 1 and PG 2) were identified as gram positive and coccus. On the basis of biochemical characterization, both strains (PG 1 and PG 2) belong to *Micrococcus*. The pigments were identified using a combination of UV-Visible spectrophotometer and Rf values of Thin Layer Chromatography. Polar organic solvents such as methanol and acetone have been selected for extraction of carotenoids from bacterial isolates. Carotenoids absorb maximally at 350 – 550nm. The orange pigment produced by PG1 showed Rf value 0.76 and λ_{max} was 350nm which was similar to toluene compound. The yellow pigment produced by PG2 showed Rf value 0.69 and λ_{max} was 400nm which was similar to β -carotene compound. The studies carried out on germination properties showed that these pigments are nontoxic to plant but rather influence their growth. PG 2 culture allowed the maximum seed germination percentage of 90% and PG 1 showed germination percentage of 85%. The two colours i.e., orange and yellow can be used for dyeing of fibres with lime as mordant. The dyed fibres also checked for their wash performance and results showed that colours are stable. Thus, the current study deals with an approach of developing new sources of biocolours from easily cultivated bacterial species that can be further exploited at larger scale. Therefore, due to hazardous effects of synthetic dye on eco-system, there is a requirement of alternative natural source which have less toxic on environment and give a product that is eco-friendly. As a consequence, there is a revived interest in the use of natural pigments and dyes, which could be subjected to biodegradation in the environment.

Acknowledgement:

We are thankful to Dr. Anil Kumar for providing research facilities in the School of Biotechnology, Devi Ahilya Vishwavidyalaya, Indore (M.P.).

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