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Microbial production of textile grade pigments

A. Sadasivan Nair[1]*, B. Prakash Kumar[1] and J. Anu Geo[2]

[1]St .Thomas College, Palai, Kottayam, Kerala, India.
[2]Karunya University, Coimbatore, Tamil Nadu, India.

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Biological pigments or biochromes are substances produced by living organisms and have a color resulting from selective color absorption. A total of 37 isolates of *Actinomycetes* and 2 bacteria were isolated from 26 different soil samples collected from different habitats of Kerala. The isolates were screened for pigment production. The effect of pigment production by the strains on various carbon and nitrogen sources were analyzed. The test microorganisms gave maximum pigmentation on glycerol as carbon source and histidine as nitrogen source when they were taken in an optimum concentration of 1:1. For the extraction of microbial pigment, both the organisms were grown on cotton saturated basal medium. The use of these pigments as colouring agents for textile fabrics were demonstrated. Out of the seven different cloth materials (silk, jute, synthetic fiber, satin, shiffon, cotton and polyester) used, silk, jute and synthetic fiber had uptake of the colour of the pigment.

Key words: *Actinomycetes*, pigments, glycerol, histidine, cotton.

INTRODUCTION

Pigments from natural sources have been obtained since long time ago, and their interest has increased due to the toxicity problems caused by those of synthetic origin (Amal et al., 2011). To counteract the harmful effect of synthetic dyes, the pigments from microbial sources are found to be a good alternative (Cañizares-Villanueva et al., 1998; Kramar et al., 2014). In nature, color rich and pigment producing microorganisms (fungi, yeasts and bacteria) are quite common (Dufosse, 2009). Microbial pigments have meaningful advantages over artificial and inorganic colors. Obtaining natural pigments from microbial sources viz. bacteria (Shirata et al., 2000), fungi (Sharmaa et al., 2012), Actinomycetes (Conn, 1943) and algae is the most rewarding perspective (Yeliseev and Kaplan, 1997). Microbial pigments are non-toxic, non-carcinogenic, pharmacological and biodegradable in nature (Venil et al., 2013) and production is one of the evolving area of research and have various industrial applications (Mansi and Gaurav, 2016).

The microbial pigments (except, the photosynthesis pigments) are secondary metabolites being synthesized in idiophase (Barkovich and Liao, 2001). The production of microbial pigments are influenced by pH, temperature, aeration carbon and nitrogen (Amal et al., 2011). Microbial pigment production can be increased in geometric proportions through genetic engineering, as compared to the scaling up methods of chemists (Joshi et al., 2003). Microorganisms produce a large variety of

*Corresponding author. E-mail: anukoickal09@gmail.com. Tel: +919446104525.

stable pigments and the fermentation has higher yields in pigments and lower residues as compared to the use of plants and animals (Duran et al., 2002). Thus, biosynthesis of dyes and pigments via fermentation processes (El-Fouly et al., 2015) have attracted more attention in recent years (Duran, et al., 2002; Hobson et al., 1998). The aim of this study was to isolate pigment-producing microorganisms, extract of the pigments from them and to use them as colouring agents for fabrics in textile industry.

MATERIAL AND METHODS

Isolation of microorganisms from soil

Various pre treatment procedures and selective media were used to assess the optimal conditions for the isolation of pigmented microorganisms, especially actinomycetes. 26 soil samples were collected from different habitats like hills, forests, agricultural fields, rhizosphere, non rhizosphere soil, rubber plantations, river basins, paddy fields, coir pith soils, rock soils, metal sediments, thicket, etc. Pre-treatment procedures for soil samples included air drying, heating at 70°C for 15 min and treating with SDS (0.05%) at 40°C for 20 min. Pre-treated soil samples were serially diluted and plated onto Actinomycetes Isolation Agar, starch casein nitrate agar, nutrient agar and glycerol asparagine agar and the plates were incubated at 25°C for 2 to 3 weeks. After incubation, typically pigmented colonies were selected and maintained on fresh medium of starch casein nitrate (SCN) agar.

Characterisation of the pigment producing microorganisms

Morphological analysis

Morphological identification of isolated strains of microorganisms was done by microscopic observation and standard staining techniques.

Microscopic observation of the isolated strains was done by wet mount and cover slip culture technique and staining techniques used are Gram staining, spore staining and acid fast staining.

Physiological and biochemical test

With minor modifications, these tests were performed by the methods of Gordon et al. (1996). The biochemical test included in the study are decomposition of casein, tyrosine, xanthine, hypoxanthine, urea, esculin and utilization of carbohydrates viz. glucose fructose, lactose, xylose, and sucrose. The inoculums were tested for NaCl resistance and gelatin liquefaction.

The pigmented strains from starch casein nitrate agar were introduced into basal medium in order to standardize the substrate for pigment production

Effect of carbon and nitrogen source on pigment production

The different carbon and nitrogen sources that influence the pigment production in the isolated strains were also investigated using protocol as described in Dastager (2006).

Effect of carbon sources on pigment production: The basal medium of the following composition: 2.0 g of $NaNO_3$, 1.0 g of K_2HPO_4, 0.5 g of $MgSO_4.7H_2O$, 0.01 g of $FeSO_4$, 0.5 g of KCL in 1000 ml distilled water (pH 7.2) was used. The effect of carbon source with 1% *viz* L-glycerol, starch, dextrin, maltose, arabinose, lactose, lactose, galactose, raffinose, glucose on pigment production was studied. The flasks containing 200 ml basal medium along with 1% carbon source were taken and the cultures were inoculated and placed on a shaker for 7 to 10 days at room temperature.

Effect of nitrogen sources on pigment production: The effect of nitrogen sources *viz* L-lysine, L-arginine, L-histidine, L-valine, L-tyrosine, L-asparagine, L-proline, L-glycine L–alanine, on pigment production was studied with same basal medium using 1% L-glycerol as the carbon source.

The basal medium with 1% glycerol as carbon source and 1% aminoacids were taken in conical flasks and cultures were inoculated and placed on a shaker for 7 to 10days at room temperature for pigment production.

Extraction of pigments from broth

The cultures inoculated in a conical flask containing cotton saturated basal medium with 1% glycerol and 1% L-histidine were placed on a shaker at room temperature. The pigment was extracted from the cotton by squeezing. The solution was then centrifuged. The supernatant or the pellet, which retained the pigment, was taken and vaporized in a water bath. The pigment powder obtained was stored for textile coloring. Seven different cloth materials (cotton, polyester, shiffon, satin, silk, jute, and a synthetic Fiber) purchased from the local textile market were cut into squares and presoaked in water. The cloths were then soaked in pigment sample and kept in boiling water bath for 1-2 h and air-dried.

RESULTS AND DISCUSSION

A total of thirty nine isolates were collected from different habitats like hills, forests, agricultural fields, rhizosphere soils, non rhizosphere soils, rubber plantations, river basins, paddy fields, coir pith soils, rock soils, metal sediments, etc under controlled pH condition (set as 7.2). The eight isolates among 39 produced diffusible pigments in starch casein nitrate agar, nutrient agar and glycerol asparagine agar and were labelled as B1, C1, G1, O1, P1, S1, V1 and Y1 (Figure 1). Based on the morphological and biochemical analysis (Berd, 1973), strain B1 and V1 were identified as *Streptomyces* sp, C1 and G1 as *Actinomyces* sp., S1 as *Serratia*, O1 as *Rhodococcus* sp., P1 and Y1as *Nocardia* sp., (Tables 1 and 2). In additon to that, morphological characters like sporulation (Bystrykh et al.,1996), diffusible pigments (Shirling and Gottlieb, 1966) support the conclusion (Tables 1 and 2).

The results revealed that the pigment production by the strains was affected by carbon and nitrogen sources. The effect of carbon and nitrogen sources on pigment production in the broth was observed from the fourth day of inoculation and reached a maximum in seven days (Table 3). The pigment production started within 4 days in cotton saturated basal medium containing optimal concentrations of carbon (glycerol) and nitrogen

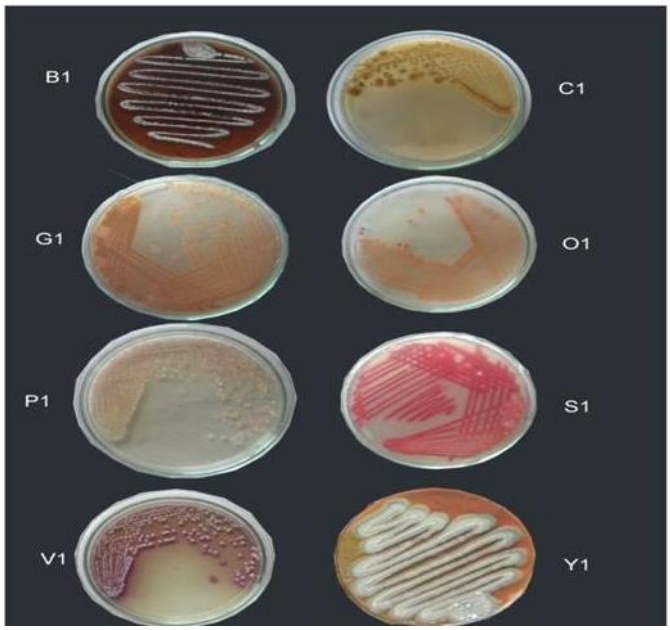

Figure 1. Pigment producing microorganisms.

Table 1. Morphological analysis.

Strain	Location	Gram staining	Spore staining	Acid fast staining	Morphology
B1	Agricultural land	+	+	+	Dry powdery aerial mycelium above, Spores in white and diffusible brown pigment beneath.
C1	Hill	+	+	-	Dry powdery aerial mycelium, septate hyphae, spores in white and diffusible cinnamon colored pigment.
G1	Spinney	+	+		Dry, powdery aerial mycelium, septate, spores in off-white color, golden yellow diffusible pigment beneath.
O1	Forest	-	-	-	Large, circular, mucoid, regular, raised, easily emulsifiable, non-diffusible orange pigmented colonies.
P1	Rice field	+	+	-	Dry, powdery, septate hyphae, spores in off white color, peach colored diffusible pigments beneath the plate.
S1	Rhizosphere soil	-	-	-	Large, circular, regular, raised, opaque, easily emulsifiable non-diffusible rose colored pigment producing colonies.
V1	Spinney	+	+	+	Dry, powdery, aerial mycelium, septate hyphae, grey colored spores with violet colored diffusible pigment.
Y1	Hill	+	+	+	Dry, powdery, septate hyphae, green colored spores with diffusible yellow pigment.

+Positive, -Negative.

(histidine) sources (1:1). The pigment was extracted from the media and the broth was vaporized in boiling water bath. The comparative efficiency of different cloth materials (silk, jute, synthetic fiber, satin, shiffon, cotton and polyester) to uptake the colour of the pigment was checked, in which silk, jute and synthetic fibers were

Table 2. Biochemical analysis.

Isolates	B1	C1	G1	O1	P1	S1	V1	Y1
Pigmentation in solid medium	Brown	Cinnamon	Golden yellow	Orange	Peach	Rose	Violet	Yellow
Solubility	Acetone	Water	Water	n	n	Water	Water	Water
Reduction of nitrate	+	+	+	+	+	+	+	+
Hydrolysis of								
Starch	+	+	+	+	+	+	+	+
Casein	+	+	+	+	+	+	+	+
Gelatin	-	-	-	-	-	+	-	-
Xanthine	+	-	-	+	+	+	+	+
Hypo xanthine	+	+	+	+	+	-	-	+
Tyrosine	+	+	-	+	+	+	+	+
Urea	+	-	+	-	-	-	-	+
Utilization of								
Glucose	+	+	+	+	-	+	+	+
Fructose	+	-	-	+	-	+	-	+
Lactose	-	-	-	-	-	+	-	+
Sucrose	-	-	-	-	-	+	-	+
Xylose	-	-	-	+	-	+	-	+
NaCl resistance	Up to 5%	Up to 5%	Up to 5%	Up to 5%	Up to 5%	Up to 5%	Up to 5%	Up to 5%

+ Positive, –Negative, n- not defined.

Table 3. Effect of carbon and nitrogen source on pigment production.

S/N	Carbon Source	Intensity of pigmentation	Nitrogen source	Intensity of pigmentation
1.	Glycerol	+++	Lysine	++
2.	Glucose	++	Arginine	++
3.	Dextrin	+	Histidine	+++
4.	Maltose	+	Valine	-
5.	Arabinose	++	Tyrosine	-
6.	Lactose	+	Asparagine	-
7.	Galactose	++	Glycine	+
8.	Raffinose	-	Alanine	-
9.	Starch	-	Proline	+

Intensity of pigmentation after incubation for 5 to 7 days was scored as + to +++ (maximal pigment).

found to be most effective in adsorbing the pigment colour (Figure 2). Similarly, Feng et al. (2015) also reported that the pigment production was significantly increased with the use of glycerol as the carbon source support the present work. Tallapragada (2013) suggested that the pigment yield from *Monascus purpureus LPB97* is directly propotional to glycerol concentration. However, other than carbon, nitrogen also play an important role in pigment production. The pigment production in *Bacillus subtilis* (Joshi et al., 2003) was enhanced by the addition of histidine in the medium (Hajjaj et al., 2012; Dufosse et al., 2005). Due to the lack of standard pigment samples, the intensity of the produced pigments were measured by visual observation. Dyeing process is a simple technique in which cloth materials are either dipped in the pigment extract or boiled with the bacterial cells. The difference in color was achieved due to the change in dipping time and temperature. Among the various white cloth materials

Figure 2. Extracted pigments on textiles.

used, silk, jute, synthetic fiber took up the color of the pigment (Figure 2). Others such as satin, shiffon, cotton and polyester showed a light adsorption of the pigments. Venil et al. (2013) suggetested that the dyeing performance vary based on the type of the fibre. Colorfastness of the dyed fabric needs to be ascertained before making any further comments on the nature of the isolated pigments as dyeing component for textile industry.

Conclusion

It is possible with the present study to isolate 8 pigments from microorganisms isolated from soil sample. It is also possible for identification of an appropriate media for pigment production by isolated microorganisms. Further, the use of these pigments as coloring agents for textile fabrics could be demonstrated. Standardization of fermentation conditions for large-scale production of these pigments is another area that needs further study. Extensive studies needs to be conducted to purify and characterize the pigment to identify its structure.

Conflict of Interests

The authors have not declared any conflict of interests.

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