Isolation, Characterization of Melanin Producing Organism and Extraction of Melanin

Kshitija R. Deshmukh

Abstract - Melanin producing organism was isolated from a soil sample of Ahmednagar district, Maharashtra using Tyrosine casein media. The isolated organism's morphology and spore arrangement was studied. The isolated organism was suspected to belong to the genus Streptomyces. Parameter optimization of the Tyrosine casein media with respect to carbon and nitrogen source was done. Starch and L-glycine were optimized as carbon and nitrogen source respectively. Melanin pigment was produced and confirmed using L-DOPA (as substrate. Melanin was successfully extracted in a crude form which can further be purified and used in various applications such as cosmetics, treatments of several diseases and also in preparation of an organic photovoltaic cell.

Index terms – Melanin, Tyrosine-casein agar, L-DOPA (3, 4- Dihydroxyphenylalanine), Streptomyces, peptone - iron media, Melanin extraction, photoprotection

1 Introduction

Melanin is a diffusible, dark pigment which is water soluble. It is found in most organisms including human beings, animals as well as microorganisms.[5] The most common form of biological melanin is eumelanin. The other forms are pheomelanin and neuromelanin. [5][12] Melanin displays some unique properties like photoprotection, electrical conductivity, photoc1onductivity, threshold/memory switching, paramagnetic property, ion-exchange property and is a good sound absorber. It is seen as a potent pigment which can be used to make an organic photovoltaic cell, useful in preparation of bioplastics, in making UV absorbing optical lenses and also can play an important role in bioremediation.

2 Materials and Methods

Note: Sterile glassware and distilled water were used during the experimental studies.

To isolate melanin pigment producing organism, soil sample was added to distilled water and serial dilutions were performed. All tubes were mixed thoroughly. Dilutions prepared were inoculated into 100ml tyrosine-casein broth. The broth was incubated at 30°C for 3-4 weeks. Then, two loopful of broth culture was streaked on tyrosine-casein agar plates (L-tyrosine – 0.1g, Casein hydrolysate – 2.5g, Sodium nitrate – 1g, Agar – 2.5g, Distilled water – 100ml, pH – 7). The plates were incubated at 30°C for 5-6 days. Colonies showing diffusible pigment were selected from the mixed culture plate. The selected colonies were grown on fresh media to obtain pure culture. The pure culture obtained was stored at 4°C [3]. To perform morphological studies, pure culture was streaked on tyrosine-casein agar media plate and incubated at 30°C for 5-6 days. After incubation, the colony characteristics such as size, shape, color, gram character, pigment color were observed. To study spore arrangement, pure culture was streaked on tyrosine-casein agar block and the slide culture assembly plate was incubated at 30°C for 5-6 days. After incubation, the slide was observed under microscope at 45X. Catalase test was performed by inoculating 2 loopful of growth from pure culture slants into a test tube containing 3% solution of H2O2. In the melanin formation test, L-DOPA was directly added to the filtrate of tyrosine-casein and peptone-iron media (Peptone – 2g, K2HPO4 – 0.1g, Ferric ammonium citrate – 0.05g, Sodium thiosulphate – 0.08g, Distilled water – 100ml, pH – 7) broth culture each[11]. To produce the pigment, pure culture of isolated organism was inoculated in both the media and they were incubated at 30°C for 5-6 days. To study the effect of various carbon sources on the isolated organism's growth, pure culture was inoculated in four test tubes containing basal media (NaNO3 – 2g, K2HPO4 – 1g, MgSO4.7H2O – 0.5g, KCl – 0.5g, FeSO4.7H2O – 0.01g, Distilled water – 1000ml, pH – 7.2) with 1% starch, glycerol, glucose and sucrose as a carbon source respectively. These tubes were incubated at 30°C for 5-6 days. To study effect of various nitrogen sources, pure culture was inoculated in three test tubes containing basal media with 1% L-asparagine, L-proline and L-glycine as nitrogen sources using 1% glycerol as a carbon source. These tubes were incubated at 30°C for 5-6 days. To study effect of pH on growth and pigment production, pure culture was inoculated in three test tubes containing tyrosine-casein broth media with pH adjusted to 4, 7 and 10 respectively in each tube and then tubes were incubated at 30°C for 5-6 days. Effect of incubation temperature on growth pigment production was studied by inoculating pure culture in three test tubes containing tyrosine-casein broth media and incubating the tubes at 15°C, 30°C and 55°C respectively for 5-6 days. The melanin pigment was extracted by the following method [6]: Filtrate of tyrosine-casein and peptone-iron broth culture (50ml each) were adjusted to pH-7. Then, 0.5g of potassium persulphate was add-

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ed to each of the filtrate. They were allowed to stand for two hours at room temperature with intermittent shaking. After 2 hours, 50ml methanol was added to each of the filtrate. Then, they were allowed to stand for 3 days at room temperature. After 3 days, both the filtrates were centrifuged at 10,000rpm for 10 minutes. Supernatant was discarded and pellet was collected, dried and weighed.

3 Results and Discussion

Morphological and spore arrangement studies: Some colony characteristics of the isolated organism were observed such as - size was 0.2 cm in diameter, shape was circular, color of the colony observed was powdery white, its gram character was gram positive, spore arrangement was spiral, pigment produced by the isolated organism was diffusible and brown-black in color. Also, the catalase test was confirmed to be positive as effervescence was observed. Based on the above observations and reference to Bergey's manual of systematic bacteriology, Volume 4(Pg. No. 2452-2456), the brown-black diffusible pigment producing organism isolated from soil may be tentatively belonging to the genus Streptomyces[2][9]. Melanin formation test: Red coloration was observed and the brown-black diffusible pigment produced by the isolated organism was confirmed as 'Melanin' (Fig: 1 & 2). Effect of various parameters under study on growth and pigment production of isolated organism (Table1). Starch was observed to show maximum growth among all other carbon sources used while glycerol and sucrose showed little growth. No growth was observed while using glucose as a carbon source. Among all the nitrogen sources studied, L-glycine was found to be most efficient while L-proline showed less growth and L-asparagine did not show any growth. pH - 7 was observed to be optimum for growth and production of melanin for the isolated organism while at pH-10 it was less efficient. At 30°C incubation temperature, the isolated organism showed optimum growth and melanin production whereas at 55°C less growth and production was observed. At pH-4 and 15°C incubation temperature, no growth and pigment production was observed. Melanin pigment extraction(Table 2) & (Fig: 4). Both the media produce melanin pigment but the peptone-iron media was observed to produce slightly more amount of pigment as compared to tyrosine-casein media.

4 Conclusion

The Melanin producing organism was isolated from soil using tyrosine casein media. According to the colony morphology, spore arrangement studies and reference to Bergey's manual volume 4, it was observed that the isolated organism may be tentatively belonging to the genus Streptomyces. It produced a brown-black diffusible pigment. By performing the melanin formation test using L-DOPA as substrate, red coloration was observed and it was confirmed that the pigment produced by the isolated organism was melanin. Tyrosine-casein as well as Peptone-iron media showed production of melanin pigment. Starch and L-glycine were observed as the best carbon and nitrogen sources for growth of the organism among other sources under study. pH value at 7 and incubation temperature at 30°C was observed to be optimum for growth and production of melanin for the isolated organism. The amount of pigment extracted from peptone-iron media was slightly more as compared to that of tyrosine-casein media. The melanin pigment obtained by extraction was a brown-black melanin i.e. eumelanin.

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7 FIGURES AND TABLES

Figure 1

Figure 3

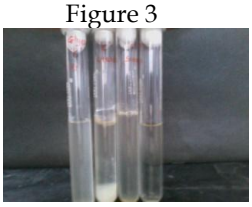

Figure 2

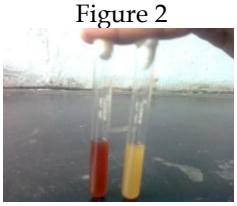

Figure 4

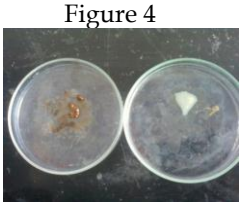

Table 1: Effect of various parameters on growth and pigment production

Parameters		Growth observed	Melanin Production
Carbon source	Starch	++	Not Observed
(Fig: 3)	Glycerol	+	
	Glucose	-	
	Sucrose	+	
Nitrogen source	L-asparagine	-	Not Observed
	L-proline	+	
	L-glycine	++	
pH value	4	-	-
	7	++	++
	10	+	+
Temperature	15°C	-	-
	30°C	++	++
	55°C	+	+

Key→ (++) = maximum growth
(+)= growth observed
(-)= no growth

Table 2: Melanin pigment extraction

Media used	Tyrosine-casein media	Peptone-iron media
Initial weight(of empty petriplate)	38.00g	42.10g
Total weight (petriplate+ pigment extracted)	40.20g	44.32g
Difference(weight of pig-ment)	2.20g	2.22g