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Synthesis and Screening of Melanin from Bacterial Strain EP83

Biotechnology

KEYWORDS : Melanin, EP83, Tyrosine medium, UV- visible spectroscopy

Edwin A. Pithawala — Department of Life Sciences, School of Sciences, GujaratUniversity, Navrangpura, Ahmedabad 380009.

N. K. Jain — Department of Life Sciences, School of Sciences, GujaratUniversity, Navrangpura, Ahmedabad 380009.

ABSTRACT

Production of melanin is one of the most universal, but at the same time it is in explicable alterations of living organisms to the capricious environments of the Earth. Melanin producing organism (EP83) was isolated from a soil sample of Gujarat University Campus, Gujarat using Tyrosine media. Much less is known about melanogenic synthesis in microorganisms. The presence of melanins in representatives of almost every large taxon suggests an evolutionary importance of melanogenesis. Isolate EP83 is known to produce 4.3 µg melanin/ ml in 3 days with a required condition of pH range 6.0 to 8.5, optimum temperature 28-48^0 C, in aerobic condition. The pigment produced is extracellular and water soluble and confirmed as melanin with spectroscopic analysis (280 nm peak). This melanin is known to have large number of implication in terms of human requirements and therefore, this study proved that EP83 can be used for the industrial yield and production of melanin.

INTRODUCTION:

Melanin pigment is ubiquitous in nature, being found in most organisms. In animals melanins are derivatives of the amino acid tyrosine. The most common form of biological melanin is eumelanin, a polymer of dihydroxyindole carboxylic acids, and their reduced forms. Technically all melanins are derivatives of polyacetylene. The presence of melanin in the archaea and bacteria kingdoms is an issue of ongoing debate amongst researchers in the field.

Melanins are dark-brown to black pigments of macromolecules formed by oxidative polymerization of phenol and/or indolic compounds, which widely exist in animals, plants and microorganisms. They showed a broad spectrum of biological roles, including antioxidant (de Cássia et al. 2005, Hung, Y.C. et al. 2002), antitumor activity (El-Obeid, A. et al. 2006), antivenin activity (Hung, Y. C. et al. 2004), anti-virus (Montefiori, D. C. et al. 1991), liver protecting activity (Hung, Y. C. et al. 2003, Sava, V. M. et al. 2003) and radio protective (Dadachova, E. et al. 2007) etc. They are widely used in medicine, pharmacology, cosmetics and other fields.

Future prospects for the purpose of the study may include various Potential Applications in Dermatology and cosmetics including specific zones of abnormally high pigmentation such as moles and birthmarks may be de-pigmented to match to the surrounding skin. Conversely, in cases of vitiligo, unaffected skin may be lightened to achieve a more uniform appearance. Other therapeutic use of bio-synthetic melanin can be faceable to treat melanin deficiencies as cosmetic elegances of skin and hair. However complete skin depigmentation is simply a futile process.

As a part of these studies on the formation and stability of pigments excreted from EP83 the aim is to identify and optimize media composition to achieve more efficient production of pigment. The components of media such as carbon source, nitrogen source and their different concentrations, pH and incubation period are experimented to improve the production.

MATERIALS AND METHODS:

Microbial Strain, Inoculum and Media

The isolated strain (EP83) was screened from soil of Gujarat University campus. Almost 3 culture strains were screened to produce black-brown pigment. The production of melanin was ascertained qualitatively by performing biochemical tests. The media ingredients containing (g/l) 5 yeast extract, 5 peptone, 2 glucose, 2 $NaNO_3$, 1K_2HPO_4, 0.5 KCl, 0.5 $MgSO_4.7H_2O$, supplemented with 0.1% tyrosine to support the growth of melanin was used. 1 ml inoculum suspension of EP83 strainfor an initial optical density 0.35at 640 nmis inoculated into 50 ml of the brown pigment production medium (Petinate et al., 1999) and incubated for 3 days at optimum temperatures.

Identification of culture pigment

The culture was subjected to preliminary examination of the presence of melanin ghosts (Susana, F., C. 2006) formed within chemically defined minimal medium with L-tyrosine at 37^{0}C in a shaking incubator for 3 days. The pigment was extracted and identified using nine different tests as described by Thomas (1955). Melanin ghosts were isolated from the spent medium by boiling in HCl and ethanol extraction, as described by Eisenman et al (2007). Ghosts were fixed on glass slides by gently heating for 1-2 second and viewed under compound microscope (45X) without staining to confirm the presence of melanin pigment.

Extraction of Black-brown Pigment

The pigment was extracted by the method of Wei, Chen (2005). The culture broth was mixed with a four volume of methanol and mixed vigorously. The resulting mixture was centrifuged at 10,000 rpm for 10 min. The organic portion was collected and filtered through a 0.22 µpore sized filter paper. The filtrate was concentrated using a rotary evaporator and subsequently extracted with 3.0 M chloroform. The chloroform phase was collected and concentrated to obtain the resultant product as black-brown pigment.

Melanin Determination

Melanin Determination was estimated maximum as 4.3 µg /ml at 3 days, pigment production was evaluated by measuring O.D. of the filtrate at 400 nm (Sanchez-Amat et al., 1998). All determinations were performed in a Systronics UV-Visible spectrophotometer177. Determination of melanin content using standard curve was calculated and considered for all experimental assay. The determination assays of melanin for each optimization parameters were carried out for 5 times to minimize the percentage of error and thereby the use of statistical analysis can be achieved for consistently significant and consensus data.

RESULTS AND DISCUSSIONS:

Cultural and Microscopic Characteristics

Based upon the cultural characteristics EP83 grows in pH range 6.0 to 8.5, optimum temperature 28-48^0 C, in aerobic condition with urease, catalase, oxidase Positive and indole, MR, H2S Negative producing both acid and gas from sugar. Gelatine is not liquefied and it utilizes citrate, hydrolysis casein and starch. The culture is resistant to KCN. The colony is pink on Mac-Conkey Agar, yellow on N. Agar and Black-brown with dispersed, diffused pigmentation in tyrosine medium (Fig 1; [I]). Microscopically they are Gram Negative short rods.

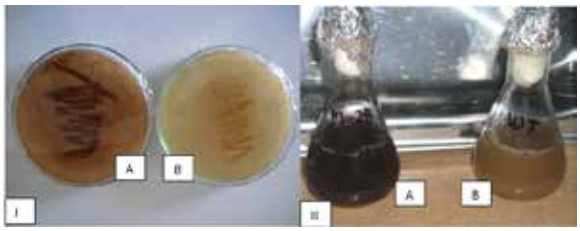

Figure 1 [I] Growth and pigment of EP83 in medium supported with Tyrosine (A) and without tyrosine (B) [II] Pigment production in medium supported with Tyrosine (A) and without tyrosine (B) (Incubation Duration 3 days)

The structure of melanin polymers is little understood and an accurate definition of melanin does not exist. However, the following results and criteria are indicative of melanins: black-brown colour, solubility in water and insoluble in organic solvents, resistance to degradation with precipitates by hot or cold concentrated acids, decolorizes H_2O_2 and soluble in hot alkali solutions. It gives brown precipitates with poly-phenolic agent $FeCl_3$ and silver precipitates with $AgNO_3$ Thomas (1955). These characteristics and the spectral properties (Fig 3) of the melanin pigment isolated in the present study revealed that melanin was synthesized by the EP83 (Butler and Day 1988, Casadevall et al. 2000).

Melanin Ghost

EP83 culture was grown in the presence of L-tyrosine and melanin ghosts were recovered by acid treatments and removal of unwanted cellular materials. The resulting particles were visualized under microscope (Fig. 2). The isolated melanin ghosts were similar to those in previous reports (Susana, F., C. 2006).

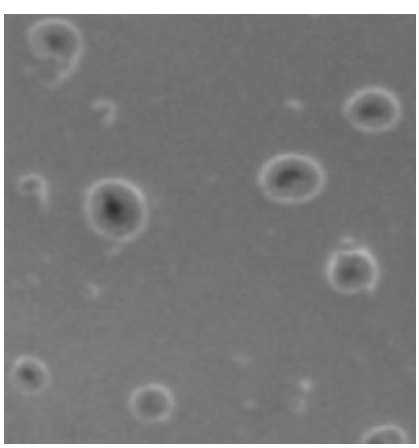

Figure 2: Microscopy of melanin ghosts without staining (45x)

Spectroscopic Analysis of the Pigment:

Graph representing the characteristic nature of melanin a black pigment as produced from EP83. Characteristic absorption peak confirming the presence of melanin was showed at 280 nm as shown in Figure 3. This is due to the presence of many complex conjugated structures in the melanin molecule (Cockell and Knowland 1999). The decrease in the absorption with increasing wavelength is almost linear in the case of melanins.

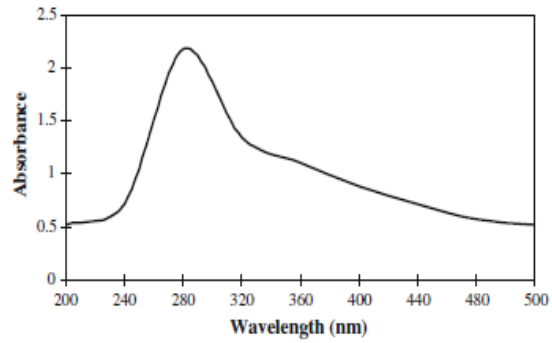

Figure 3: UV-Visible Spectroscopic curve of the Pigment as produced by Isolate EP83

CONCLUSION:

In conclusion, melanin pigment from EP83 is very similar to that of a typical melanin, as per the literature surveyed during the study, which constitutes a diverse group of aromatic polymers with many different potential applications in cosmetic and pharmaceutical industries. Bacterial synthesis of melanin pigment is an alternative option for commercial-scale production. Based upon results production of melanin can be yield from EP83 a bacterial origin. EP83 strain produced a high amount of melanin pigment as compared with other reported strains, and this culture produced melanin in 3 days extracellular with solubility in the growth medium along with supplemented L-tyrosine. This may shed new light on the evolution of melanogenesis in higher organisms as well.

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