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Studies of melanin producing bacteria and extraction of bacterial melanin from sewage water

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Abstract
Melanin are the natural pigments which have their presence in animals, plants and in most of the microorganisms. The current study is aimed to isolate and identify melanin producing bacteria. The sewage water samples were randomly collected from two district Akola and Buldhana.
A nutrient agar medium containing tyrosine was used for isolation of melanin producing bacteria, which were examined for biochemical and morphological test. Isolates were then subculture on nutrient agar medium containing tyrosine for production of gas from glucose, growth in presence of Nacl, P^H tolerance, growth temperature and enzymatic activity. The melanin pigment was further characterized by FT-IR spectroscopy. The FT-IR result confirms that it was melanin pigment. Therefore, this study proved that sewage water sample can be used for the production of melanin and it has resistant anti-bacterial activity. In present study different bacterial species of *Azotobacter spp. Pseudomonas spp.* and *Bacillus spp*. were identified.

Keywords: Melanin producing bacteria, melanin pigment

1. Introduction

1.1 What is Melanin

Melanin is the predominantly indolic polymer and the major pigment present in surface structures of vertebrates. The origin of the name is from melons (Greek dark) is usually attributed to the Swedish chemist Berzelius in 1840. The term “melanin” has been used fairly indiscriminately to mean any dark pigment. The synthesis of melanin is one of the most universal, but at the same time enigmatic adaptations of living organisms to the variable conditions of the Earth. Melanin is widely dispersed in the animal and plant kingdoms and also synthesized from microorganisms (Riley, 1997) [1].

1.1.1 Classes of melanin-

- Based on colour and structural classes primarily there are three types of melanin i.e. eumelanins, pheomelanins and allomelanins.
- Eumelanins are black to brown colour pigments produced by melanisation by classic Mason-Rapper pathway, which produce tyrosine intermediates or metabolites by the action of tyrosinases.
- Pheomelanins are brown, red or yellow colour pigments which are produced in course of oxidation of tyrosine and/or phenylalanine to dihydroxyphenylalanine (DOPA) and dopaquinone. Pheomelanin results from cysteinylation of DOPA and these are sulphur containing compounds.
- Allomelanins include nitrogen free heterogeneous group of polymers formed from catechol precursors.

1.1.2 Properties of melanin-

The photochemical properties of melanin make it an excellent, photoprotectant. It absorbs harmful UV-radiation and transforms the energy into harmless heat through a process called "ultrafast internal conversion". This property enables melanin to dissipate more than 99.9% of the absorbed UV radiation as heat. This prevents the indirect DNA damage that is responsible for the formation of malignant melanoma and other skin cancers. (Meredith, 2004) [3].

Some individual animals and humans have very little or no melanin in their bodies, a condition known as albinism. Both pheomelanin and eumelanin are found in human skin and hair, but eumelanin is the most abundant melanin found in humans (Agar and Young, 2005) [4].

The eumelanins and pheomelanins commonly occur in animal species, while allomelanins can be seen in microorganisms and plants. Some of the funguses known to produce melanin are *Cryptococcus neoformans*, *Sporothrix schenckii*, *Sepia officinalis*, *Aspergillus niger*, *Penicillium marneffei*, *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *C. neoforman*. Coming to bacteria, some species of *Aeromonas salmonicida, Azotobacter*, *Mycobacterium*, *Micrococcus*, *Bacillus*, *Legionella*, *Streptomyces*, *Rhizobium*, *Vibrio*, *Proteus*, *Azospirillum*, *Pseudomonas aeruginosa*, *Hypomonas* sp, *Burkholderia cepacia*, *E. coli* etc. There are many microorganism which produce melanin like bacteria, fungi, algae, yeast, actinomycetes. In present study is based on melanin producing bacteria only.

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.

2. Material and Methods

2.1 Collection of sewage water samples

Sewage water samples were collected from different regions from Akola and Buldana districts. Sewage water samples were collected in plastic bottles. Then sewage water samples carried to laboratory for further investigation.

2.2 Isolation of melanin producing bacteria

Melanin producing bacteria were primarily isolated from collected water samples by four way streaking technique using media composed of by Nutrient agar supplemented with L-tyrosine. The media and the glassware was autoclaved at 15 psi (121 ^{0}C) for 15 min prior to the experiment; these agar plates with media were incubated at 37 ^{0}C for 4 days. Selective colonies were separated out for sub culturing and characterization.

2.3 Morphological and biochemical characteristics of isolated bacteria

All the isolated colonies on the nutrient agar media were screened for colony character and gram character. Then colonies were subjected for the biochemical analysis for all the bacteria like IMViC test, sugar fermentation test, oxidase, catalase, urease.

2.4 Perform Antibiotic Susceptibility test-

All isolates of melanin producing bacteria were subjected to in vitro antibacterial testing method on nutrient agar supplemented with L-tyrosine. In this method nutrient agar plates were prepared.

1. Then bacterial culture were swabbed on the surface of sterile nutrient agar plates.Then antibiotic discs were aseptically placed over nutrient agar plates sufficiently separated from each other to avoid overlapping of zone of inhibition.
2. The plates were incubated at 37 ^{0}C for 48 hrs and diameter of the inhibition zone were measured in mm and the drug resistant pattern was studied by using the interpretation chart supplied by the antibiotic disc manufactures (HI MEDIA, Mumbai).

2.5 Antibacterial activity of melanin pigment-

Antibacterial activity was tested by well diffusion method. Pathogens like *Staphylococcus aures, Salmonella typhi, Klebsiella pneumonia, Proteus vulgarise* and *Escherichia coli* were swabbed on a Muller-Hinton agar and 3 µl of pigment extracted was placed in the well and incubated at 37℃ for 24 hrs.

2.6 Pigment Production-

1. Nutrient broth supplemented with tyrosine was used for inoculums preparation and pigment production. Bacterial cultures were added to 200 ml nutrient broth in 500ml flasks. This medium was then incubated at 40℃ in incubator.
2. After 10-15 days incubation until the liquid medium become darkly pigmented and nearly opaque. All the media used for the study were sterilized by autoclaving unless elsewhere stated.
3. After the incubation time, the medium was centrifuged at 5000 rpm for 15 min to separate the supernatant (broth) and the cells. The solid pellet of cells was separated and suspended in distilled water. These cells again centrifuged to collect the supernatant.

2.7 Extraction and Purification of Bacterial Melanin

1. Melanin was extracted from the overall supernatant by acidification with 3N HCl top ^{H}-2 and allowed to stand for 48 hrs initially at room temperature. This process was repeated for 3 more days until no precipitation found.
2. The supernatant were centrifuged at 5000 rpm for 30 min, equal volume of chloroform, ethyl acetate and methanol were added with cell free supernatant and mixed well. This step was repeated 2-3 times then supernatant were centrifuged at 5000 rpm for 15 min.
3. Then the obtained suspension was boiled for 20 min to prevent the formation of melanoidins. As a final point, crude pigment pellet was collected after centrifugation at 5000 rpm for 15 min and this purified pigment was used for further analysis.

2.8 FT-IR Spectroscopy studies-

Fourier transform infrared spectroscopy (FTIR) is most useful for identifying the types of chemical bonds (functional groups) and therefore, can be used to elucidate. The FT-IR analysis of pigment was carried out after mixing with KBr using FT-IR spectrophotometer.

3. Result and Discussion

Melanin producing bacteria were isolated from sewage water samples. Cultural characteristics Biochemical and enzyme test were done for all isolated melanin producing bacteria. (Holt *et al*., 1994) [17]. Antibiotic sensitivity test also had been done which was found resistant for all organisms. (Goswani and Bhowal, 2014) [8]. Antibacterial activity of melanin pigment against all tested pathogens and the zone of clearance against pathogen were observed.

Test org. / Sample	E. coli.	S. aureus	Salmonella typhi	Proteus vulgaris	Klebsiella
Pseudomonas	8mm	11mm	10mm	21mm	12mm
Azotobacter	29mm	20mm	17mm	15mm	22mm
Bacillus subtilis	12mm	17mm	14mm	19mm	15mm

For pigment production nutrient broth supplemented with tyrosine had been used for inoculum preparation and pigment production. After incubation period the medium had been centrifuged at 5000 rpm for 15 mins, then melanin was extracted from overall supernatant and dried at room temperature. At last FTIR spectroscopy was performed for further characterization of the pigment providing information on functional groups and detailed structural analysis of melanin. The IR spectrum of the three bacterial melanin pigment showed a broad absorption at 3278.99 cm-¹,3030.17 cm-¹,3278.99 cm-¹ revealed the presence of the –OH group. Absorption at 2877.79 cm-¹, 2879.72 cm-¹,2887.44 cm-¹ which indicates as –CH. Absorption at 1867.09 cm-¹,1745.58 cm-¹,1743.65 cm-¹ was attributed to aromatic ring C=C stretching. These characteristics properties of the IR spectrum of this pigment were similar to earlier reports. The spectroscopic properties of the melanin pigment correlated with those of melanin produced by various microorganisms as reported previously.

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6. References

1. Riley P. Molecules in focus Melanin. Int J Biochem Cell Biol. 1997; 29:1235-1239.
2. Tarangini K, Mishra S. Production, characterization and Analysis of Melanin from Isolated Marine *Pseudomonas sp*. using Vegitable waste. 2013; 2(5):40-46.
3. Paul Meridith, Jennifer Risez. Radiative Relaxation Quantum Yields for Synthetic Eumelanin. 2004; 79:211-216.
4. Agar N, Young AR. Melanogenesis: a photoprotective response to DNA damage? Mutat. Res. 2005; 571:121-132.
5. Pithawala A. Edwin Synthesis and Screening of Melanin from Bacterial Strain EP83, 2013; 2(7). ISSN No 2277-8179.
6. Sajjan. Purification and Physiochemical Characterization of Melanin Pigment from *Klebsiella sp*. GSK. J Microbiol. Biotechnol. 2010; 20(11):1513-1520.
7. Vasantthabharathi V. Melanin production from marine *Streptomyces,* African Journal of Biotechnology. 2011; 10(54):11224-11234.
8. Goswami. Bhowal, Identification and Characterization of Extracellular Red Pigment Producing Bacteria Isolated from Soil. International Journal of Current Microbiology and Applied Sciences, ISSN: 2319-7706 2014; 3(9):169-176.
9. Selvakumar P, Rajasekar S, Periasamy K, Raaman N. Isolation and characterization of melanin pigment from Pleurotus cystidios (telomorph of Antromyocopsis macrocapa).World J Microbiol. Biotechnol. 2008; 24:2125-2131.
10. Hoti L, Balaraman K. Formation of melanin pigment by a mutant of Bacillus thuringiensis H-14. J Gen. Microbiol. 1993; 139:2365-2369.
11. Eisenman HC, Mues M, Weber SE, Frases S, Chaskes S, Gerfen G, *et al*. Cryptococcus neoformans laccase catalyses melanin synthesis from both D- and L-DOPA. Microbiology 2007; 153:3954-3962.
12. Fava F, Gioia DD, Merchetti L. Characterization of a pigment produced by Pseudomonas fluorescens during 3-chlorobenzoate co-metabolism. Chemosphere 1993; 27:825-835.
13. Wenlin Y, Stephen HB, Jeffrey OD. Melanin biosynthesis by Frankia strain CeI5. Physiol. Plant. 2007; 131:180-190.
14. Gómez-Marín AM, Sánchez CI. Thermal and mass spectroscopic characterization of a sulphur-containing bacterial melanin from *Bacillus subtilis*. J Non-Cryst. Solids. 2010; 356:1576-1580.
15. Harki E, Talou T, Dargent R. Purification, characterisation and analysis of melanin extracted from *Tuber melanosporum* Vitt, Food Chem., 1997; 58:69-73.
16. Youngchim S, Morris-Jones R, Hay RJ, Hamilton AJ. Production of melanin by Aspergillus fumigatus, J Med Microbiol. 2004; 53:175-181.
17. Holt JN, Krieg P. Stanely and William, R. Enterobacteriaceae In: Bergey's manual of Determinative Bacteriology 9th Ed. Baltimore; Williams and Walk in Co. 1994, 87-175.