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Isolation, Characterization and Screening of Microbial Melanin for its role in Protection of Plant Growth Promoting Bacteria

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Abstract

Plants are susceptible to vulnerable impacts caused by presence of heavy metals, viruses, bacteria, fungi, insects and pests. Along with diseases, availability of nutrients, water, temperature and the role of protecting the role plant growth promoting bacteria (PGPB) is also a matter of concern. PGPB may promote plant growth directly usually by either facilitating resource acquisition or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various heavy metals on plant growth and development. Although a significant increase in the use of PGPB in agriculture was observed in the last two decades, there is a dearth of long-term studies addressing the effects of PGPB on existing microbial community structure. Melanin is a negative charge hydrophobic complex pigment that is a substance made of small particles virtually insoluble in the environment and is usually used for its color, protective or other characteristics. Melanin, its ability to chelate metals by which the toxicity of metals can be reduced. In this direction, soil and vegetable waste samples were collected and enriched. A total of fourteen isolates were obtained and these isolates were screened for melanin production. Black brownish colonies were melanin positive colonies. These isolates were subjected to production of melanin. Melanin was extracted and subjected to FTIR (Fourier Transform Infrared Spectroscopy). On the basis of FTIR results, melanin was confirmed and then tested for its metal chelating ability followed by its application as protectant for plant growth promotion. Significant growth was observed when compared with control proves its vital role in plant growth promotion.

Keywords: PGPB, Melanin, FTIR, Growth promotion

Introduction

Melanin is an ancient pigment that occurred very early in all living organisms. Melanin is typically known for its unique ability to absorb a wide range of radiations [5]. Melanin is a bioactive-complex pigment which helps to protect the microorganisms from environmental stresses. Pigmented compounds from microbes are provisionally classified as melanin on the basis of their physical and chemical properties [6].
Melanin pigments were extracted from a wide variety of microorganisms including bacteria and fungi. In the present study isolation, identification and characterization of melanin [7]. Melanin is a common name for a group

of biopolymers with the dominance of potential applications in medical sciences, cosmeceutical, bioremediation, and bioelectronic applications [8]. Actinomycetes are characterized by the production of various pigments on natural or synthetic media [9]. Actinomycetes often show themselves to have outstanding antimicrobial activity [10]. Actinomycete are biotechnologically important microorganism and they produce many pigments with industrial applications is a diffusible, dark pigment which is water soluble.[7] Actinomycetes also synthesizes and excrete dark pigments, melanin or melanoid, which are considered to be a useful criterion for taxonomic studies.[8]
Melanin compounds are irregular, dark brown polymers that are produced by various microorganisms by the fermentative oxidation, and have the radioprotective and antioxidant properties that can effectively protect the living organisms from ultraviolet radiation [11]. Melanin's are used as the adsorbents of radionuclides and heavy metal ions [12], show strong biological activities in the clearance of free radicals and protection against radiation [13], improve the survival rate under serious environmental stressors, such as extreme temperatures, drought, irradiation and metals exposure [14], and can substitute for synthetic pigments [15]. These pigments are usually described in terms of various shades of blue, violet, red rose, yellow, green, brown and black. The pigments may be dissolved into the medium or it may be retained in the mycelium [16]. HPLC is used for quantification of melanin, particularly when it is necessary to distinguish between eumelanin and pheomelanin[17].

Actinomycetes are known to produce various kinds of antibiotics and moreover these antibiotics include many pigments. Production of pigments by actinomycetes has been utilized as an important cultural characteristic in describing the organisms [9]. Melanogenesis in nature is initiated through the activation of tyrosinase, laccase and other polyphenol oxidases in response to external stimuli such as chemical stresses (metal ions, ROS, oxidizing agents), UV exposure, and pathogenic attack (particularly in insects) [18].

Actinomycetes are Gram-positive bacteria showing a filamentous growth. They are a group of organisms widespread in nature, and play a significant role in the future of biotechnology, because of their importance as producers of vitamins, enzymes, antitumor agents, immune modifying agents and mainly antibiotic compounds [19].
The current work focuses on application of melanin for improvement of plant growth promotion.

Materials and Methods

Sample collection, enrichment of samples and isolation of microorganisms

Soil samples were collected from the local garden area, vegetable dumping area and Karnala region, Panvel, Navi Mumbai. One gram of the collected samples were inoculated into an enrichment medium namely sterile tyrosine casein broth. Enrichment broth was incubated at room temperature for 48 – 72 hours followed by isolation of microorganisms into sterile tyrosine casein agar plates. Plates were incubated at room temperature for 48 – 72 hours

Screening of melanin producing isolates

Melanin producing microorganisms were identified by the presence of microbial colonies with brown black color (diffusible) in agar plates. Selective colonies were separated out and streaked on tyrosine casein agar slants, sterile tyrosine casein agar plates for further characterization.

Production and extraction of melanin

Melanin production was carried out using sterile tyrosine casein broth by inoculating the isolates followed by incubation at room temperature for five days. Filtrate of tyrosine casein broth culture adjusted to pH-7. 0.5g of potassium persulphate was added to each of the filtrates. Allow to stand for two hours. at room temperature. After two hours, methanol was added to each filtrate. Allow to stand for three days at room temperature. Both filtrates were centrifuged at 10,000 rpm for 10 minutes. Pellet was dried and subjected to characterization by FTIR.

Characterization of melanin using Fourier Transform Infrared Spectroscopy

Sample was mixed with KBr powder and then compressed and then the sample was subjected to FTIR.

Application of melanin for plant growth promotion

Rice seeds (*Oryza sativa*) were mixed with extracted melanin and growth parameters like seed germination percentage, root length and shoot height were measured.

Germination Percentage (GP) = D/E x 100%
D is the number of germinated seeds on the seventh day,
E is the number of total seeds investigated

Results and Discussion

Sample collection, enrichment of samples and isolation of microorganisms

Enriched sample was serially diluted followed by isolation on to Tyrosine agar. The black brownish colonies were observed. These colonies were selected and isolated on a tyrosine agar plate and were kept for 48 hours at room temperature. Melanin producing microorganisms was identified by the presence microbial colonies with brown black color (diffusible) in agar plates

Selective colonies were separated out and streaked on Tyrosine agar slants, sterile Tyrosine agar plates for further characterization. A total of fourteen isolates were obtained after 48 of incubation. The colonies were screened for black brownish coloration. Those colonies which are black brownish in nature were selected for further research. As everyone knows the pigment producing microbes were found abundant in soil and vegetable waste.

Maintenance of pigment producing organism:

It is essential to maintain the obtained isolates throughout the research. For their maintenance, tyrosine agar was used. Single colony was streaked on slant and incubated at room temperature for 48 hours and then slants were wrapped with paper to reduce the chances of contamination. Slants were stored at 10°C. After 20-25 days culture was re-streaked for maintaining the fresh culture.

Production and extraction of melanin:

To characterize and determine the application of melanin, it was produced in bulk using the isolates and production media. Production was carried out in liquid media i.e., tyrosine broth. Optical density of 0.1 was adjusted using saline as blank and was inoculated into the production media. The broth was kept on shaker for 5 days and growth was monitored and also melanin production was checked after 5 days. Within 3 days a faint red color was observed in the inoculated broth. After 5 days the intensity of color increases and it changes to the darkish red and brown color and extraction of melanin was carried out as mentioned previously.

Characterization of melanin using Fourier-Transform Infrared spectroscopy

Peaks observed near 3300 cm-1 may be due to characteristic O-H stretching or N-H stretching vibrations of the carboxylic acid, and phenolic groups in melanin. Melanin form isolates K7, V1(8), V4(9) confirmed by FT-IR studies. The results of FTIR are shown in figure no. 1, 2, 3.

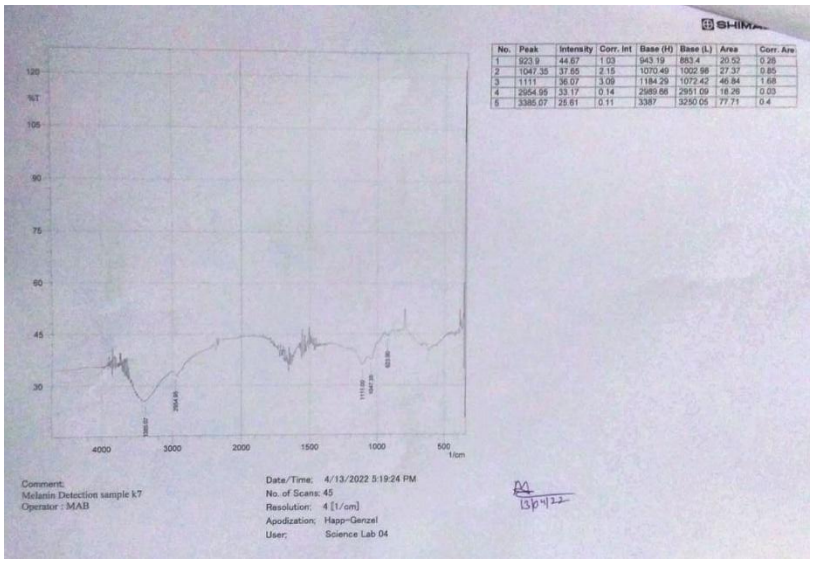

Figure No. 1*: FTIR result of melanin from isolate K7*

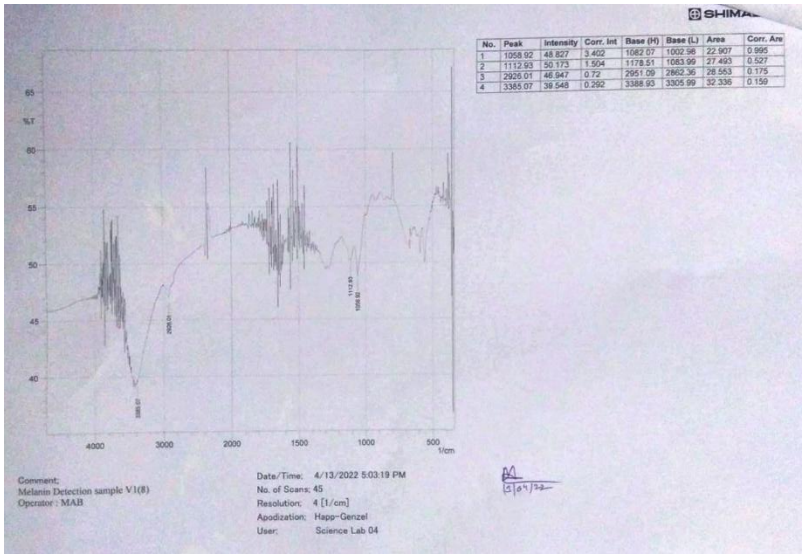

Figure No. 2: *FTIR result of melanin from isolate V1(8)*

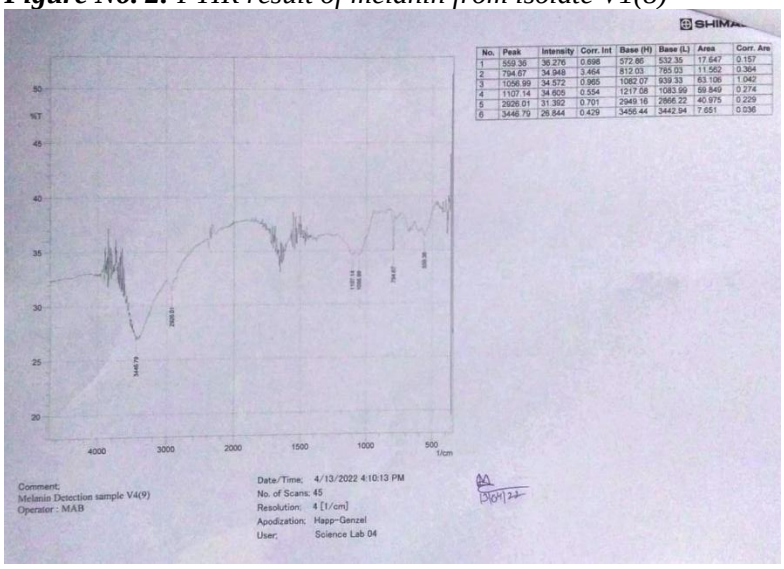

Figure No. 3*: FTIR result of melanin from isolate V4(9)*

Application of melanin for plant growth promotion

Seeds were mixed with melanin and germination percentage, root and shoot length were calculated. It was measured that melanin from isolate K7 was efficient in improving seed germination (82%) when compared with control (55%). Shoot length of treated plantlets showed 15.3cm while control showed 11.2cm and root length of treated plantlets measured as 13.1cm while control was 8.6cm. This indicates bacterial melanin helps in improvement of plant growth parameters.

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