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Isolation and Screening of Melanin Producing Microorganism

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Abstract: Melanin is negatively charge compound composed of multi-functional polymers and polyphenolic compounds that are produced by various microorganisms by fermentation oxidation. Melanins are frequently used in medicines, pharmacology and cosmetic preparation. In present study, total 25 bacterial isolates were isolated from soil sample of various sugarcane and rice farm in vapi and valsad region. 19 strains among 25 bacterial isolates were capable of producing dark brown melanin pigment and isolate a-19 found to be highest producer of melanin which was confirmed by l- dopa as substrate.
Keywords: melanin, l-dopa

I. INTRODUCTION

The term "Melanin" originates from a Greek word "melanos" which means black. It is a pigment which is ubiquitous in nature. Melanin pigment is found in most organisms including human beings, animals as well as microorganisms.Melanins are negatively charged compounds composed of multifunctional polymers and polyphenolic compounds that are produced by various microorganisms by fermentation oxidation. The different species of *Streptomyces* like *Streptoverticillium rubrireticuli, Streptomyces echinoruber* are capable of producing melanin pigment (Mukesh Sharma et al 2014).In humans, melanin is the primary determinant of skin color. In the human skin, it is produced by melanocytes which are found in the basal layer of epidermis.The ability of melanin to scavenge reactive oxygen species (ROS),such as singlet oxygen, hydroxyl radical and superoxide anion, has been firmly established in model systems, suggesting that melanin could protect pigment cells against oxidative stress that may accompany the formation of ROS in these cells.This antioxidant ability makes melanin a good food additive, which can help in reducing the risk for chronic diseases including cancer and heart disease (Pham-Huy et al., 2008). L-tyrosine is used as precursor through the action of tyrosinase to produce melanin. There are various types of melanin: Eumelanin (Brown-black), Pheomelanin (yellow-red), Allomelanin, and Neuromelanin.They have also radio protective and antioxidant properties that can effectively protect the living organisms from ultraviolet radiation. Melanin have to ability to undergo polymerization is interesting in industry for its nanotechnology uses in bio plastics and biopolymers (Nakato, 2006).

II. METHODS AND MATERIALS

A. Media and Reagents

L-tyrosine (HPLC Mumbai), Casein hydrolysate (HPLC Mumbai), Sodium nitrate (Rankem Mumbai), Agar-agar (Saffron life science Gujarat), Peptone (Hi-Media Mumbai), di Potassiumorthophosphate (K_2HPO_4) (Rankem Mumbai), Ferric ammonium citrate (Rankem New Delhi), Sodium thiosulphate ($NA_2S_2O_3.5H_2O$) (Rankem Mumbai) were used of analytical grade. All this reagents and media were prepared in distilled water.

B. Screening and Isolation of Melanin Producing Microorganism from Soil Sample

For the isolation of melanin producing microorganism, soil samples from rice field, sugarcane industry, sugarcane field and garden were collected of different localities from Vapi and Valsad, Gujarat-396001, India. Tyrosine casein agar medium was used for screening of melanin producing species (gm/litter), [L-tyrosine -1gm, Casein hydrolysate – 25gm, Sodium nitrate -10gm, Agar-agar -32gm, and pH 7].(Kshtija R. Deshmukh, 2012)Serial dilution was performed till 10^{-7} dilution and last three dilutions were spread on sterile tyrosine casein agar plates. Plates were incubated at 37°c for 7-8 days. After incubation typically black pigmented colonies were selected from mixed plate culture and maintained on fresh medium to get pure cultures and were preserved. Gram's staining was performed for the morphological characterisation.

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C. Inoculum Preparation

From the preserved culture plate, an isolated colony was transferred into 50ml Erlenmeyer flask containing peptone iron broth. Static condition of flask was maintained at 30°c for 7 days. To inoculate in production medium containing peptone iron broth, the freshly grown 7 days old culture with 1.0 O.D at 600nm was used as inoculum. (Shreya et al 2016)

D. Production Medium

Melanin production was carried out in 250ml Erlenmeyer flask containing 100ml of peptone iron broth through submerged fermentation. Peptone iron medium was inoculated with 5% inoculum with 1 O.D of selected strain and then incubated at 30°c for 5-6 days. The culture suspension was centrifuged at 5000 rpm for 20 minute. The cell free supernatant was used as a source of melanin for estimation.

E. Assay for Melanin Production

Melanin pigment was estimated by taking 2ml of the supernatant and 1ml of substrate solution (L-DOPA). The reaction mixture was incubated at 37°c for 5 minutes and read spectrophotometrically at 480 nm (Shreya et.al 2016).

F. Effect of Incubation Time on Melanin Production

Time course of melanin production was studied in the melanin production medium using static flask cultures. Optimum incubation time for maximum melanin production was determined by incubating the inoculated media for a total of 192 hours and analysing the samples at a regular interval of 24 hours(0 hour, 24 hours, 48 hours, 72 hours,96 hours, 120 hours, 144 hours, 168 hours, 192 hours) for melanin production at 30° C under static condition. At each time interval of 24 hours was withdrawn and centrifuged at 5000 rpm for 20 minutes. The cell free supernatant was used as a source of melanin for melanin estimation.

III. RESULT AND DISCUSSION

A. Morphological Studies

Some colony characteristics of the isolated organism were observed such as- shape was circular, colour of the colony observed was white, its gram character was gram negative , pigment produced by the isolated organism was diffusible and brown-black in colour.

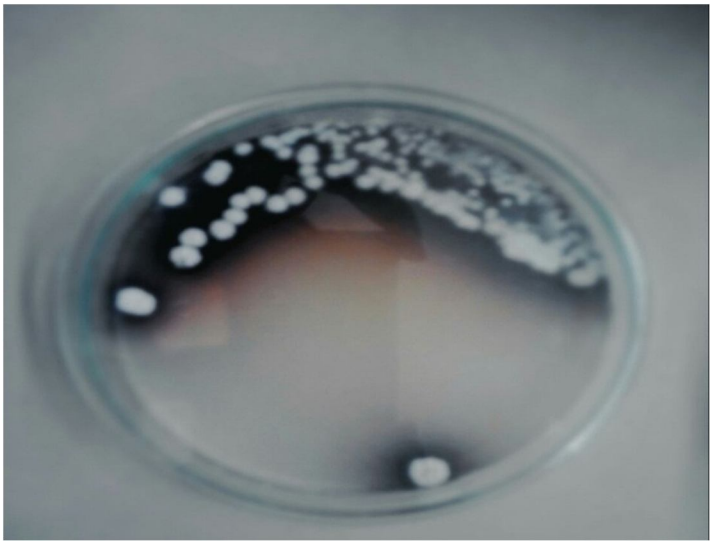

Figure 1: Melanin producing isolate A-19

B. Screening of Melanin Producing Bacteria

Melanin formation test: Red coloration was observed and brown-black diffusible pigment produced by the isolated organism was confirmed as melanin. Out of all the isolate obtained, A-19 isolate gave 12.5 mg/ml maximum melanin production, followed by isolates A-6,A-16, A-7 giving 10.3mg/ml, 9.83mg/ml and 9.18mg/ml. Whereas isolates A-18,A-13,A-1 giving minimum melanin production which was found to be 4.5mg/ml, 4.7mg/ml and 5mg/ml respectively.

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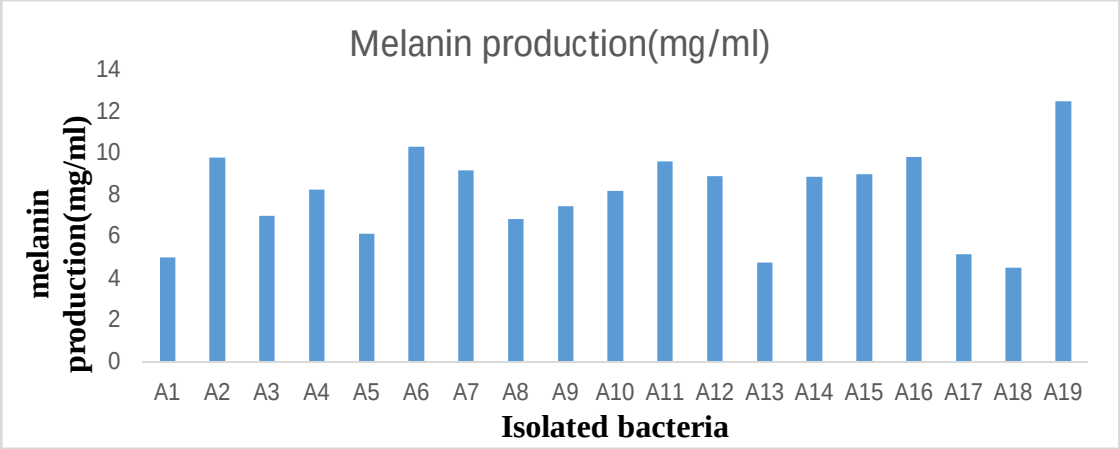

.Figure 2: graphical presentation of isolates showing melanin (mg/ml) production

Figure 3: Melanin production by A-19 isolate

C. Effect of Incubation Time on Melanin Production

The pigment production was effected by incubation time and gradual increase in pigment production was achieved with increasing the incubation period. Maximum melanin production (24.2mg/ml) was observed at 168thhours of incubation. However, further incubation, in decreased melanin production.

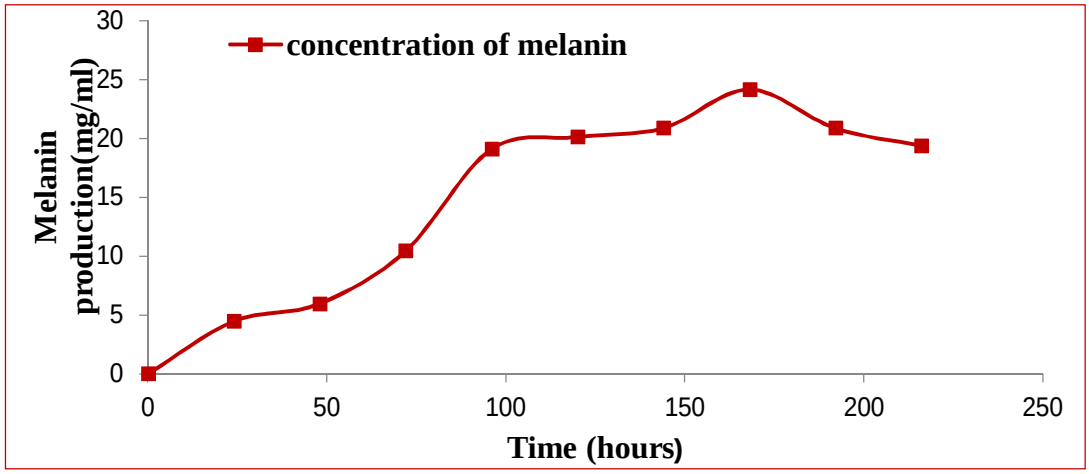

Figure 4: Effect of melanin production on melanin production

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IV. CONCLUSION

The melanin producing organism was isolated from soil using tyrosine casein media. 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 and isolate A19 shows maximum production.in time coarse study it was found that maximum production of melanin was observed at 168th hour after that time reduction in the melanin production was observed.

V. ACKNOWLEDGEMENT

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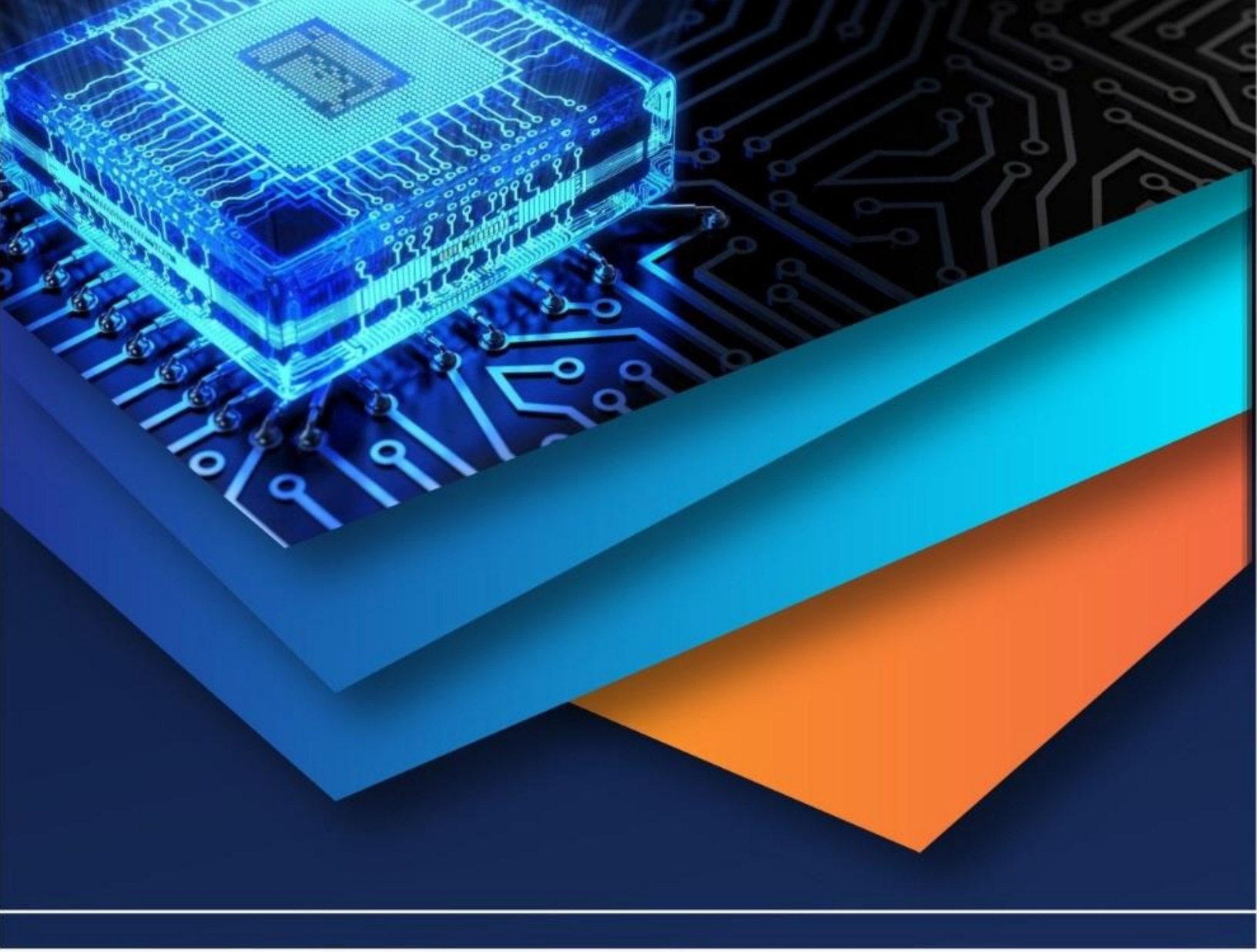

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