



"LAB SCALE APPLICATIONS OF MELANIN PIGMENT OF *STREPTOMYCES* FOR PRINTING AND DYEING OF WOOL FABRICS."

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ABSTRACT

Actinomycetes are characterized by production of various pigments on natural or synthetic media. These pigments usually described in terms of various shades of blue, red, rose, yellow, green, brown and black. *Streptomyces* are major group in *Actinomycetes* as blackish brown (melanin) pigment producer in soil. The melanin formation in the *Streptomyces* species is the key feature for the classification of the *Streptomyces* group. Isolation of *Streptomyces* was carried out on sterile Glycerol Asparagin Agar medium and isolates were preserved on same media for further study. A total 25 *Streptomyces* were isolated from soil of Shirala region out of these 4 *Streptomyces* were produce diffusible dark brown pigment on peptone-yeast extract iron agar. Screened isolates were grown in liquid media such as 1% sugar cane waste. Pigment obtained after growth were simply tested in lab scale manner for printing and dyeing of wool fabrics. The antibacterial activity of extracted pigment has been investigated.



KEYWORDS : Melanin, pigment, *Streptomyces*, Printing.

INTRODUCTION

Actinomycetes are characterized by production of various pigments on natural or synthetic media. These pigments usually described in terms of various shades of blue, red, rose, yellow, green, brown and black. *Actinomycetes* had known to be produced various kinds of antibiotics and moreover these antibiotics include many pigments (Miyaura *et.al.*, 1960). Production of pigments by *Actinomycetes* has been utilized as an important cultural characteristic in describing the organism. Nevertheless, very little is known about the exact chemical nature of pigments, because the formation of pigment is influenced by the pH of the medium, aeration, temperature of the growth and carbon and nitrogen sources. *Actinomycetes* also synthesized and excrete dark pigments melanin or melanoid which considered being useful criterion for taxonomical studies (Zonova, 1965). The textile industry produces and uses approximately 1.3 million tones of dyes pigments and dye precursors valued at around 23 \$ billion, almost all of which is manufactured synthetically. However synthetic dyes have some limitations primarily. Their production process requires hazardous chemicals; creating workers safety concerns. They may generate hazardous wastes. These dyes are not environment friendly.

Concerning the toxic effects of synthetic dyes there has been a renewed effort to study and implement the various natural dyes in the dyestuff industry toxicity problems caused by those of synthetic origin. The production and evaluation of microbial pigment as textile colorant is currently being investigated. The research were explores method where natural dyes are produced also melanin is frequently used in medicines, pharmacology and cosmetics preparation. *Streptomyces* strain which is capable of producing

brown colored pigment used for printing, dyeing of wool fabrics being studied during the work. Antibacterial activity of extracted pigment against bacteria has been studied. Fermentative production of extracellular pigment from *Streptomyces coelicolor* MSIS1 (Mohanasrinivasan *et al.*, 2013). Isolation, Characterization of Melanin Producing Organism and Extraction of Melanin (Deshmukh, 2012)

This pigment had many applications in different sectors such as printing, dyeing, pharmaceuticals and food industries. Shirala region comes under heavy rain fall. Blackish brown (melanin) pigment had studied throughout the world by different groups of scientists but no one had reported from Shirala region. Natural dyes can provide the much needed alternative to the complex world of the chemical dyes (Francalanci *et al.*, 2001). So this work is undertaken.

MATERIAL AND METHODS:

Collection and treatment of soil sample:

A total 3 soil samples were collected in sterile polythene bags from Shirala agricultural fields and brought them in laboratory. Collected soil samples were treated with calcium carbonate for stimulation of actinomycetes and then phenol to reduce number of non-streptomyces actinomycetes.

Enrichment of Actinomycetes:

Each pretreated soil samples were separately inoculated in sterile Bennett's broth for enrichment of actinomycetes.

Isolation:

Enriched soil samples were serially diluted in water and grown on solidified Glycerol asparagin agar medium for isolation of actinomycetes (Cochrane, 1961). The plates were incubated at ambient temperature for 3 to 4 weeks. After incubation dry, powdery colonies were selected from mixed plate culture and maintained on fresh medium to get pure cultures. The pure cultures were stored at 4°C until further examination.

Characterization:

Streptomyces were identified by morphology characters, spore chain morphology, arrangement of spores in substrate mycelium and diffusible pigments.etc.

Melanin formation:

Melanin formation was tested on peptone yeast-extract iron agar.

Fermentation:

Melanin production was performed in a shake flask culture with 1000 ml of production media. The production media contained the following parameters; pH 7.0, temperature 35°C, salinity 15 ppm, yeast extract- 0.2%, soya-bean meal and 1.0% of sugarcane waste concentration, for 7 days incubation.

Extraction:

After the incubation, pH of the fermented broth was adjusted to 12 using 2N NaOH and centrifuged at 4000 rpm for 15 minutes. Supernatant was collected and pH of the supernatant was again adjusted to 2 using 2N HCl. Recentrifugation was done at 4000 rpm for 15 minutes. The obtained pellet was collected and suspended in methanol. The pigment was extracted from methanol by using rotary vacuum evaporator and the amaranth colored pellet obtained was dried and stored at 5°C.

Antibacterial activity of Melanin:

Antibacterial activity of melanin was carried out on Muller-Hinton agar medium. Extracted melanin pigment of *Streptomyces* was tested on already identified test microorganisms by using agar diffusion method.

Lab scale applications of melanin:

The extracted melanin powder was dissolved in water and such colored solution was tested for their coloring property against wool fabrics and same was used in an ink pen to writing a text.

RESULTS AND DISCUSSION:

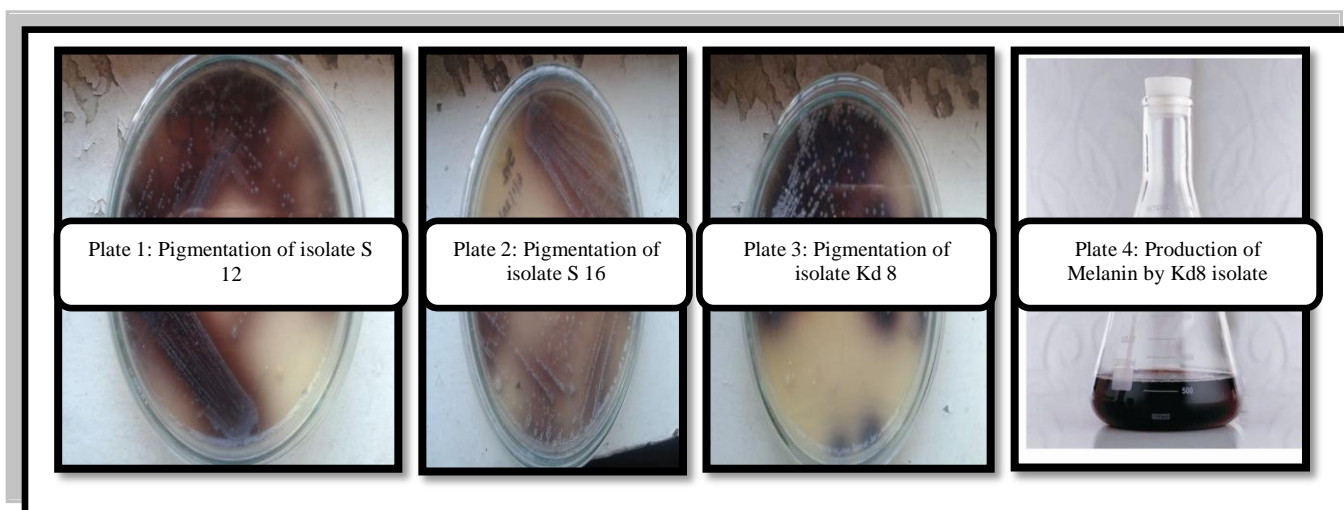
A total 25 actinomycete isolates were isolated from three different soil samples. Out of 25 actinomycete isolates, 4 isolates were ability to produce blackish brown pigmentation on peptone yeast extract iron agar medium and representative results were seen in plate 1 to 3. Those 4 Isolates producing diffusible dark brown pigment was further selected for studying their biochemical characteristics.

Table 1: Pigmentation and biochemical characterization of *Streptomyces* isolates

Sr. No.	Isolates	Pigmentation on Peptone yeast extract iron agar	Reduction of nitrate	Catalase	Hydrolysis of Starch, Casein, Gelatin and Glucose
1	S12	+	+	+	+
2	S16	+	+	+	+
3	Kd8	++	+	+	+
4	Kd5	+	+	+	+

Notations: - '+' = Positive biochemical test and '-' = Negative biochemical test

The isolates Kd8 showed highest blackish brown color intensity in pigmentation medium. Cultural and biochemical characteristics of isolates were noticed in table 1. It is noticed from table that four isolates showed nitrate reduction test positive. These four isolates showed catalase, amylase, caseinase and gelatinase activity positive. All four isolates utilize glucose as carbon source.



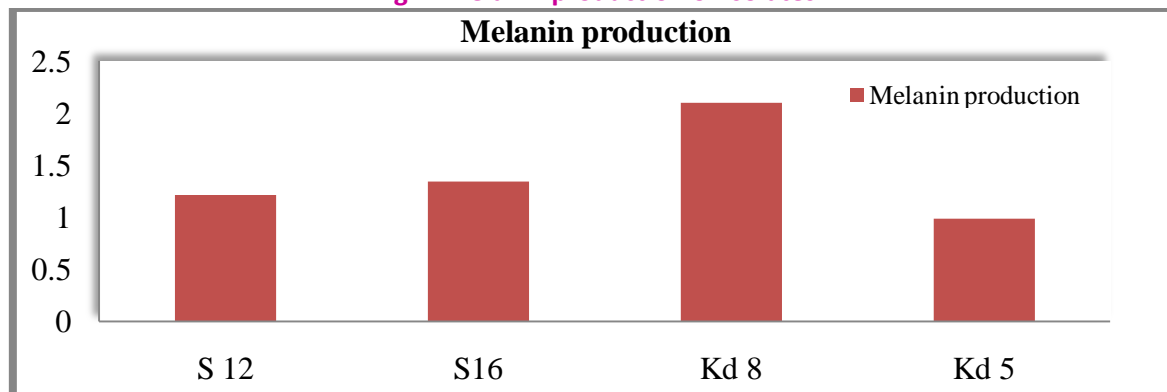
1

S12

1.212

2	S16	1.345
3	Kd8	2.101
4	Kd5	0.987

Fig. 1 Melanin production of isolates



Melanin produced by *Streptomyces* was showed antibacterial activity against *E. coli* and *K. pneumoniae*. Melanin of *Streptomyces* showed maximum zone of inhibition against *E. coli* than *K. pneumoniae*. The isolate Kd8 was isolated from soil sample collected from Kokrud region of Shirala tahsil. Kd 8 was found to be most potent producer of melanin pigment.

It was easily colored to the wool fabrics in a laboratory and used for writing a text document through the pigment produced by *Streptomyces*.

To actually test this pigment in printing and their alkaline and heat tolerating ability. The application of this pigment in dyeing of fabrics on industrial scale is an area of interest. The yield of potential isolate is increased by practices of media optimization.

CONCLUSION:

The isolate belonging to soil of Kokrud region being highest producer of blackish brown pigment. This pigment gave effective color to the wool fabrics and printing.

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