# "IDENTIFICATION & CHARACTERIZATION OF PIGMENT PRODUCING BACTERIA FROM SOIL & STUDIES ON SOME INDUSTRIAL APPLICATIONS OF PIGMENT"

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**ABSTRACT**

The present study includes the extraction of pigment using ethyl acetate as solvent from bacteria isolated from soil collected from college campus. Three bacterial strains tentatively identified as red pigment producer as Serratia spp., lime yellow pigment producing organism as Micrococcus spp. and dark yellow pigment producing belongs to Staphylococcus spp. The absorption spectra of these three pigments showed absorption maxima 540 nm for red pigment, for lime yellow and dark yellow, maximum absorbance was 400nm and 440 nm respectively. The extracted pigments had antibacterial activity against Staphylococcus aureus and Proteus spp. The radical scavenging activity for the pigment was determined through DPPH which showed the maximum antioxidant activity at least concentrations. These pigments were also used for dyeing of textile and bioplastic material and in lip balm production with better outputs. Further, it is necessary to conduct purification and toxicology studies of these microbial pigments before their use as natural colorants in food and pharmaceutical products.

**Keywords:** Pigments, Antibacterial activity, Antioxidant, Lip balm, Textile dyeing

**INTRODUCTION**

Natural pigments are obtained from ores, insects, plants and microbes. Among microbes, bacteria have great potential to produce diverse bioproducts and one such product is pigments. The production and application of bacterial pigments as natural colorants has been investigated by various researchers1. Bacterial pigment production is now one of the emerging fields of research in microbiology and biotechnology to prove its potential for various industrial applications. Microorganism's like bacteria, algae and fungi have an ability to produce variety of pigments. These pigments from microbial sources have desirable properties like stability to light, temperature and pH. Microbial pigments also possesses anti-cancer properties and are a source of pro-Vitamin A. Microbial production has various benefits as their production is independent to weather condition, easy and fast growth of bacteria on different wastes, easy extraction of pigment. Hence, microbial production of pigments has many advantages over the other as they can be produced under controlled condition in a very less time2.

However, most of the research conducted at bacterial pigment production level is still at its beginning stage. Hence, more work on the bacterial pigments should be carried out to enhance its applicability for industry. There are many studies in the literature on bacterial pigments which focus mainly on production and application of specific pigment3. Pigment producing bacteria are ubiquitous and present in various ecological niches, such as rhizospheric soil, desert sand, fresh water and marine samples. They were reported in low and high temperature regions, can persist in salt regions and even as endophytes4. The use of bacteria for pigment production has several advantages

over fungi, such as short life cycle and ease for genetic modification. However, compared with fungal pigments, most of bacterial pigments are still at the research and development stage5.

The pigment production is more likely to be present in actinobacteria. Various genera such as Streptomyces, Nocardia, Micromonospora, Thermomonospora, Actinoplanes, Microbispora, Streptosporangium, Actinomadura, Rhodococcus, and Kitasatospora produce a wide variety of pigments6.

Red colored pigment from Comamonas testosterone growing on naphthalene was isolated from petroleum contaminated soil7.

Before the invention of synthetic pigments, natural pigments were widely used for many purposes such as the coloring of natural fibers (wool, cotton, silk), fur and leather, to color cosmetic products and to produce inks, watercolors and artist's paints. Since the introduction of synthetic dyes, many convenient and cheap synthetic pigments have appeared in the market and the use of natural dyes has decreased due to the relatively cheaper synthetic pigments8.

The bacterial pigments will offer good opportunities due to their enhanced environmental acceptability and superior performance characteristics, classical or conventional grades are expected to continue to dominate the organic market. Hence, in this study focus was made on isolation and characterization of pigment producing bacteria and evaluation of them for some industrial application.

## MATERIALS AND METHODS

### COLLECTION OF SOIL SAMPLE

### SOIL SAMPLES

Soil samples were collected from two places near waste dumping site and from Botanical garden of Yashwantrao Chavan College of Science Karad, Vidyanagar, Karad. Collected soil samples were serially diluted up to 10-9. 0.1 ml of each dilution were plated on sterile sterile 2% glycerol nutrient Agar plates and kept for incubation at 37oC for 48 hrs. The plates were observed for growth and pigment production after 48 hrs of incubation. The isolated pigment producing organisms were further streaked on sterile same plate to obtain pure culture and were further characterized for identification. Media and chemicals used in the present study were from HIMEDIA Biosciences.

### Identification of the Pigment producing Strain

The preliminary identification of the strain was confirmed by Morphological characteristics such as Gram Staining, Cell size, shape, arrangement etc., Cultural characteristics and biochemical properties.

### Studies on pigment production

### Biomass production for pigment

Mass cultivation was carried out in 500 ml broth taken in 1 liter capacity Erlenmeyer flask inoculated with inoculum with cell density adjusted by hemocytometer to 106 cells / ml and incubated on Rotary Shaker for 48 - 72 hours.

### Extraction of Pigments

The Ethyl Acetate was used for extraction of pigment but a modified procedure for the isolation of pigment was carried out where 72 hour old culture of broth mixed with Ethyl Acetate and vortexed vigorously and then centrifuged at 10,000 rpm 0oC for 10 minute. The resulting supernatant was collected and filtered through Whatman filter paper. The water layer is discarded and pigment extract was then reconcentrated using evaporating dish until minimal volume was obtained. Cell pellet were used for obtain intracellular pigments. Intracellular pigments obtained by using ultrasonicator and ethyl acetate and allowed to evaporate water. This ethyl acetate extract was used to check the applications of pigment.

This dried pigment was also suspended in DMSO (Dimethyl sulfoxide) solvent to evaluate its antimicrobial activity against laboratory pathogens9.

**UV-Vis. Spectral analysis**

Spectral analysis of extracted pigment was done by using UV- Visible spectrophotometer (Systronics model 119). The extract was scanned in the range of 300 to 700 nm to find out the maximum absorption wavelength. Ethyl acetate was used as a blank.

**Studies on Applications of bacterial pigments**

**Antimicrobial activity of pigment**

Antibacterial activity of pigment was studied by agar well diffusion method. Test organism was inoculated into sterile cooled molten nutrient agar plates, test organism used were Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus sp., Proteus sp.

Nutrient agar plates were used for well diffusion method. The plate was inoculated with pure culture and wells were bored in the plates. Then the wells were filled with appropriate amount of pigment and it was kept in refrigerator for half an hour for diffusion. After that it was incubated at 37oC for 24hrs and the result was observed by measuring zone of inhibition. DMSO was taken as a control.

**Antioxidant activity**

The 2, 2-diphenyl-1-picrylhydrazylradical scavenging activity (DPPH) was carried out by the method10. For the examination of DPPH radical scavenging activity, crude extracts were prepared at different concentrations.

Percentage scavenging = [(Ac-At)/Ac] x100.

Where At is absorbance of sample and Ac absorbance of control.

**Preparation of lip balm**

The bio lip balm was prepared using shaded Bee wax along with coconut oil and glycerol as source of lanolin. All the contents were mixed in the ratio 2:4:1 (w/v/w) in bowl. The container was kept in water bath and heated until the wax melts completely and all the ingredients are homogeneously mixed. The 0.1 gm pigment was added to it to color the mixture. Then the mixture was poured into container and allowed to cool.

The formulation was developed and evaluated for color, odor, appearance and spread ability over a minimum of two days at room temperature (28$^{o}$C) and oven temperature (55 $^{o}$C).

**Textile Application**

The extracted pigment was used for dyeing the absorbent cotton, and thread. The samples were pre - mordant with 5% of ferrous sulphate and copper sulphate separately. Finally the absorbent cotton and thread were dyed in 50 ml of colored filtrate. Dyeing time was 45 min and incubated at 70-80oC. The samples were washed with cold water after dyeing.

**Biocoloration of Bio-Plastic**

15 gram of cornstarch was taken into the 100 ml water in conical flask. 10 ml of vinegar and glycerin were added into it followed by 1 ml of extracted pigment. Mixture was heated with constant stirring until it become clear, gel-like. Solution was then poured in to metal pan and dried for a couple of days.

**Results and Discussion**

Two Soil samples were collected from campus of Yashwantrao Chavan College of Science Karad, Maharashtra, India. Soil was used for isolation of pigment producing bacteria on 2% glycerol nutrient agar medium. Total three pigment producing bacteria were identified and characterized which were red, lime yellow and dark yellow (Fig. 1, 2, 3).

These bacteria were then identified and characterized with the help of morphological characteristics and biochemical tests (Table 1, Table 2 and Table 3). Their identification at genus level was done with the help of Bergey's Manual of Determinative Bacteriology.

**Extraction of Pigments**

Initially the pigment was extracted by

different solvents with their different concentrations. Acetone, Ethyl acetate and Chloroform were used. There was no pigment extraction observed in Chloroform and acetone solvent. The ethyl acetate solvent has capacity to extract the pigment from the cell. The pigments extracted were red, lime yellow and dark yellow in color.

In UV-Vis spectroscopy, maximum absorbance of Red pigment was obtained as 540 nm, for lime yellow and dark yellow maximum absorbance was 400 and 440 nm respectively (Figure 4) which was close to the absorption spectrum of beta carotene (450 nm) studied by Kaiser11.

Similar studies have been carried out in Serratia marcescens where the maximum absorbance of red pigment was found to be at 534.76 nm using UV–Vis analysis spectra2. Studies carried by Hines12, showed the maximum absorbance for prodigiosin pigment at 535 nm.

**Antimicrobial activity of pigment**

The extracted pigments were dissolved with solvent DMSO to evaluate the antimicrobial activity against laboratory isolates of pathogen by well diffusion method. The zone of inhibition was measured to evaluate antimicrobial activity. All the pigments isolated showed antibacterial activity against the test pathogens with yellow pigment being less effective as compare to red pigment. For red pigment, maximum zone of inhibition was observed against Staphylococcus aureus (35mm) and minimum against Salmonella typhi (18mm). However lime yellow pigment fails to inhibit any of isolate whereas dark yellow pigment found most effective against Proteus sp. (32mm) and least effective against E. coli (16mm).

Based on the results, it was obvious that red pigment extract showed excellent anti-bacterial activity against Staphylococcus aureus while dark yellow effective against Proteus sp

Yellow colored pigment was found to become very faint after 2 to 3 days and hence was not used for further applications.

**Antioxidant Activity**

Antioxidant potential is the capability of a substance to scavenge free radicals available in its surroundings. The free radical-scavenging activity of pigment along with standard ascorbic acid was determined by the DPPH assay. Ethyl acetate extract of red pigment of isolate IS 1 showed 85% DPPH activity which was considerable good when compared with the standard ascorbic acid showing 95% activity at same concentration (Figure 4). In addition, radical scavenging activity performed with different concentration of ethyl acetate extract showed concentration dependent scavenging activity. Similar results were reported by Mani13, while more antioxidant activity when compared with results of Srinivasan14.

**Preparation Of Lip Balm**

Red lip balm was formulated have suitable characteristics such as color, odor, and uniformity. It was noticed that there were no water formation, cracks or bleeding of color and blooming even after 15 days of observation at room temperature 28□C (Figure 5).

**Textile Application**

The pigment extracted from biological source was used as an alternative to the synthetic colorants and also are safe and cost effective. A piece of cotton and cotton thread were used as textile materials to observe the coloring capacity of Ethyl Acetate extracted red pigment (Figure 6). Usually any sort of dye required fixative which helps in the attachment of dye to the material.

In our study we observe that extracted pigment did not require any fixative to incorporate the color texture to the textile material. The pink color was retentive enough to withstand a consecutive 3 normal water wash treatment.

**Biocoloration Of Bio-plastic**

The coloration of bioplastic is becoming increasingly important and is achieved today almost exclusively with the help of polymeric carrier materials and suitable colorants. However there is a problem of the biodegradability of the colorant so the only option is to search for microbial pigment having ability to color bioplastic material. In the present study red pigment was used to color bioplastic. However further studies on its stability and strength, need for additives and proper dispersion need to be done. This study opens new area of preparing ecofriendly biocolor for coloring biodegradable materials to improve its market value.

**Conclusion**

The organisms in the soil are an important source for the search of novel bioactive molecules with biotechnological importance such as microbial pigments. With this view the present study was undertaken. In this present study, from 2 soil samples 3 pigment producing isolates namely IS1, IS2 and IS3 with red, lime yellow and dark yellow were collected. Then according to Bergey's Manual of Systematic bacteriology, red pigment producing bacterium IS1 was tentatively identified as Serratia spp., lime yellow pigment producing organism IS2 was Micrococcus spp.and dark yellow pigment producing IS3 belongs to Staphylococcus spp. Furthermore pigments were tested for antimicrobial activity which showed that extracted red pigment inhibited growth of Staphylococcus aureus and dark yellow pigment showed activity against Proteus sp. Also present study focused on its antioxidant activity in which the red pigment was used in the DPPH radical scavenging activity showed better activity when compare to standard. Thus pigment can be taken as source of natural colorant in medicinal, food and pharmaceutical fields.

These pigments were tested for textile dyeing applications and lip balm production which showed good results. Therefore the study concludes that pigments have excellent antimicrobial activity which could be very useful for pharmaceuticals and natural pigments could be manipulated as a prominent source to replace the synthetic chemicals for the preparation of textile dyes. Additional efforts on studies on use of pigments as biocoloring agent are also in consideration.

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Fig. 1 IS I

Fig. 2 IS 2

Fig. 3 IS

**Table 1:** Morphophological, Biochemical & Physiological Characterization of the Pigment Producing Microorganism **Colony Morphology**

| Code of Isolate | Size | Shape | Color | Opacity | Margin | Elevation | Consistency | Gram Nature | Motility |
|---|---|---|---|---|---|---|---|---|---|
| IS1 | 3mm | Circular | Red | Opaque | Entire | Convex | Sticky | Gram negative short rod | Motile |
| IS2 | <1mm | Circular | Lime yellow | Opaque | Entire | Dome shape | Moist | Gram positive cocci | Non Motile |
| IS3 | 2mm | Circular | Dark yellow | Opaque | Entire | Low convex | Moist | Gram positive cocci | Non Motile |

**Table 2:** Biochemical Test of Sugar Fermentation

| Code of Isolate | Glucose | Mannitol | Raffinose | Fructose | Sucrose | Arabinose | Cellobiose | Lactose |
|---|---|---|---|---|---|---|---|---|
| IS1 | + | + | + | - | - | + | + | + |
| IS2 | + | - | - | + | - | - | + | + |
| IS3 | + | - | - | + | - | - | + | + |

Key, + = Acid Production, - = No Acid Production,

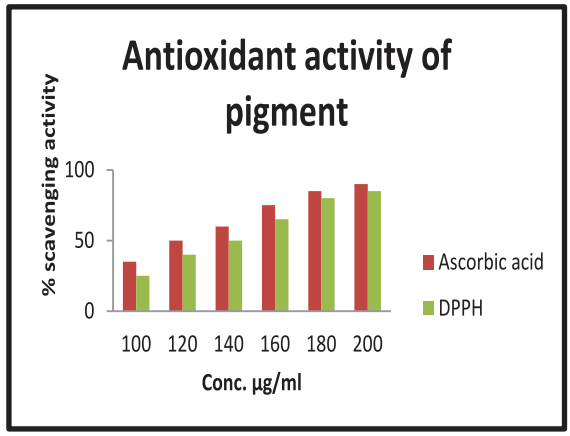


Figure 4 Determination of antioxidant potential of Pigment

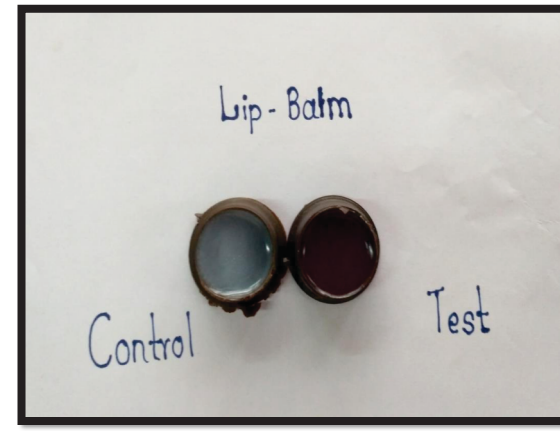


Fig. 5. Natural lip balm produced from extracted red pigment

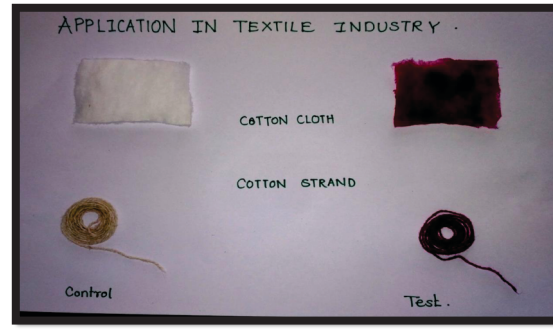


Fig. 6. Textile dyeing from extracted red pigment.

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