Production of pigment producing bacteria and its applications

RAJESHWARI SIDDHARTH SHINDE
MGM college of Agriculture biotechnology, Aurangabad, Maharashtra, India

Abstract— Bacterial pigments are natural colorants produced by various types of bacteria. these pigments have gained attention due to their potential applications in various industries. Bacterial pigments are known for their vibrant colors and diverse chemical structure, making it attractive alternative for synthetic pigments. Our present study is aimed at isolating the bacterial pigments from different soil samples making dye out of it and use it in applications.

I. INTRODUCTION

Pigments produce color due to the presence of chromatophores , specifically molecules that capture sunlight energy and cause electron excitation . the modern definitions of pigments refers to substrate

Made of small particles that are insoluble in the medium they are applied to , used for their colorant , protective or other properties pigments have various applications in industries like food industry where pigments can be used as food color or sometimes flavor enhancers, antioxidants [1].

Distribution and Habitats: Pigmented bacteria are found in various environments, including terrestrial and marine habitats. They can be isolated from different geographical regions, ranging from polar regions to tropical environments. Some pigmented bacteria, like Stenotrophomonas and Rhodotorula, can adapt from terrestrial to marine environments through discharges from hospitals and domestic sewages [17]
Ecological Importance: Pigmented bacteria play a significant role in the ecosystem. They are known to tolerate harsh conditions by producing pigments. Marine pigmented microbes are gaining attention due to their varied bioactive pigment compounds, making them potential sources of novel chromogenic compounds for biotechnological and industrial applications .[5]

Color Variations: Pigmented bacteria can exhibit a wide range of colors, including green and blue, which are considered rare colors produced by microorganisms. The occurrence of pigmented bacteria is noticed in various niches such as air-water interfaces, glaciers, sea surfaces, salt lakes, deep-sea hydrothermal vents, and marine sediments .

Applications: Pigmented bacteria have applications beyond their colorful appearance. They are of interest for their antimicrobial activities, cytotoxic activities, and potential as natural food colorants[11]. The pigments produced by these bacteria have industrial applications in areas such as textiles, cosmetics, pharmaceuticals, and food .[1]

In summary, pigmented bacteria are diverse microorganisms that produce pigments with various colors and have ecological, industrial, and potential biomedical applications. Their ability to thrive in different environments and produce bio active compounds makes them valuable subjects of study in microbiology and biotechnology.[16]

II. MATERIALS AND METHOD

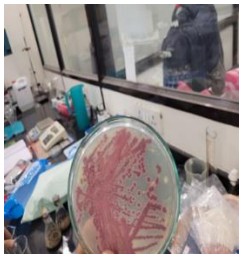
1] Isolation and enrichment of pigment producing bacteria :
Soil samples were collected from diverse environmental niches, followed by enrichment of pigment-producing organisms in nutrient broth at 37°C for 48 hours.
The spread plate method was employed for the isolation of pigment-producing bacteria from the enriched samples.
Serial dilutions of the sample suspensions were plated on nutrient agar plates and then incubated at 37°C for 24 to 48 hours.
Morphologically distinct bacterial colonies observed on the plates were selectively isolated on sterile nutrient agar plates to obtain pure cultures.

Pure cultures were sub-cultured on sterile nutrient agar slants and maintained as stock cultures for subsequent investigations.

- Introduction to stress conditions for pigment :

Stress conditions were induced using various salts, including magnesium sulfate, sodium chloride, calcium chloride, potassium chloride, sodium nitrate, dipotassium hydrogen phosphate, and potassium hydrogen phosphate.

Nutrient agar and agar were utilized as growth media to provide essential nutrients for bacterial growth and pigment production.



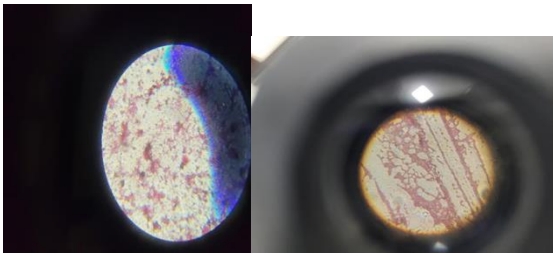
Streaking plate Chemicals used in pigmented dye

Slant of pigmented bacteria streaking process

- Characterization of isolated pigment producing bacteria:

Gram staining was performed to study the gram characteristics of the bacterial isolates.

Morphological colony characteristics such as size, shape, color, texture, were noted for each isolate.[2]



This are gram negative cocci bacteria

- Dyeing process for Fabric evaluation:

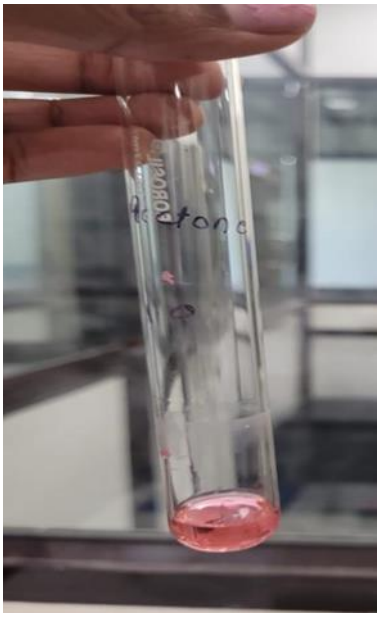
Cotton fabrics procured from local markets in Mumbai were dyed using different dye baths with pH ranging from 3 to 11.

The dyeing process was conducted at 80°C for 60 minutes with a material-to-liquor ratio of 1:20.

Control experiments were performed at the pH of the extract without altering it, with acetone used for color comparison.

Various buffers such as HCl/KCl, citric acid, Tris, sodium phosphate, ammonium chloride, and sodium hydrogen phosphate were employed to adjust the pH levels.

Instruments including a spectrophotometer, laminar air flow cabinet, autoclave, magnetic stirrer, water bath, and incubator were utilized for the dyeing process.
[6] [10].
Buffers used for testing are as followed :
1] HCL / KCL = pH 1
2] Citric Acid = pH 3
3] Tris = pH 5
4] Sodium phosphate = pH 7
5] Ammonium chloride = pH 9
6] Sodium hydrogen phosphate = 11



acetone

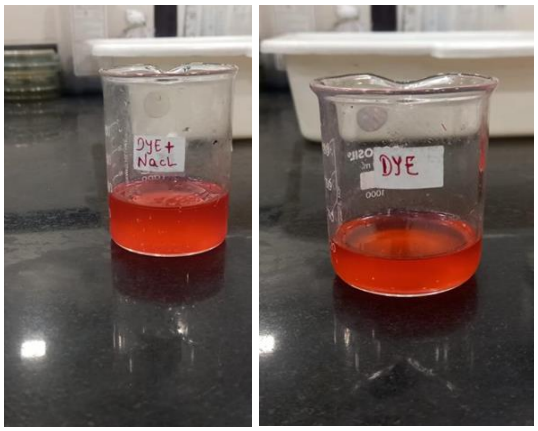
Dye dissolved in different buffers for pH testing

- Effect of concentration of microbial colourant on the dye ability of fabric :

On obtaining the pH value for dyeing different concentrations of the extract ranging from 10 - 40 ml were taken keeping MLR as 1:20 at 80 degree celcius for 60 minutes effect of temperature and dyeing time was studied by keeping MLR 1:20 .

1] We took cotton fabric from local market:

2] We took dye in one beaker and 40 ml dye + 0.3 gm Nacl in other beaker because salt act as fixator in this so dye can stick on clothes



3] We soaked cloth in both of this dyes and then kept it on high temperatures on water bath for observing whether color fades or stays

At 50 °C

At 70°C

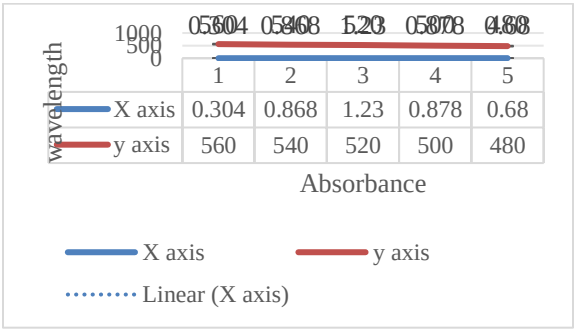
At 80.1 °C

4] Dye was applied on clothes

As we can see the cloth which was kept for soaking in dye + sodium chloride was dyed properly and the one which was just soaked in dye got patches .

We observed the wavelength of Bacteria pigments dye as followed :

The absorbance values obtained at various wavelengths signify the pigment's ability to absorb light at specific wavelengths, reflecting its unique spectral signature. The observed absorption peaks correspond to the pigment's molecular structure and chromophoric groups, offering valuable information about its chemical composition and potential applications. The wavelength-dependent absorbance data can be analyzed to determine the pigment's color characteristics, stability, and potential interactions with other compounds.



Absorbance	Wavelength
0.304	560
0.868	540
1.230	520
0.878	500
0.68	480

Antibacterial test

Klebsiella pneumoniae was selected as the test organism for evaluating the antimicrobial activity of the bacterial pigment.

Petri plates containing agar and nutrient agar were inoculated with *Klebsiella pneumoniae* to assess the antibacterial efficacy of the pigment.

Wells were carefully created in the agar plates, with one well containing the bacterial pigment solution and another well containing acetone as a control, as the pigment was soluble in acetone.

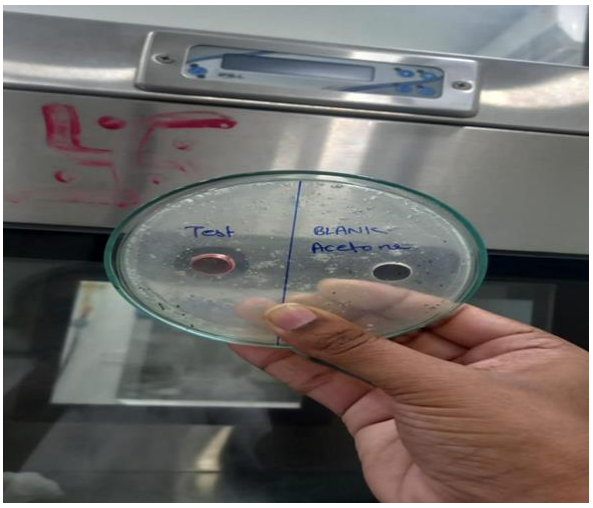
A second set of petri plates was prepared with wells containing ethanol and the pigment dissolved in ethanol to further evaluate the antimicrobial properties.

The diffusion assay was conducted by measuring the zones of inhibition around the wells to determine the effectiveness of the bacterial pigment against *Klebsiella pneumoniae.*

The results were analyzed quantitatively to ascertain the antimicrobial potential of the pigment against the test organism.

Statistical analysis, including the calculation of mean zones of inhibition and standard deviations, was performed to validate the antimicrobial activity of the bacterial pigment.

The experimental setup was replicated to ensure the reliability and reproducibility of the antibacterial test results.[4][12]



Catalase test: We spreaded pigmented bacteria on slants and put few drops of Hydrogen peroxide on it by dropper we observed Bubble formation on it.

So bacteria is positive for catalase test in conclusion this bacteria produce Catalase enzyme.[3][15]

Applications of pigmented bacteria

Pharmaceutical industry - Microbes have properties like anticancer , antibiotic and anti immuno suppressive properties . Astaxanthins and Xanthophylls which act as neutraceuticals this xanthophylls by the process of antioxidation antifree radical or oher mechanisms helps to preven Carcinogenecity.

A red pigment Astaxanthin is an important Carotenoid[7] . Prodiogiosin is also one of important pigment that can be used for treatment of diabetes also used in immune suppressive anti cancer properties

Textile industry -

This industry produces large amount of waste consisting of synthetic dye but synthetic dye have lot of drawbacks like toxicity, carcinogenecity , and mutagenecity.

Which are leading to various health problems like skin cancers , and some allergies therefore pigmented bacteria comes as better alternative for synthetic dyes as they are easily biodegradable balance ecology and has increasing market value .[13]

Cosmetic industry - Due to the usage of various colorings in cosmetics and its global market, efforts have been made to investigate the use of biopigments in cosmetics, particularly in skincare products . As people grow older, their skin becomes thinner and loses its elasticity and moisture capacity . Because the skin is exposed to chemicals and ultraviolet (UV) radiation, it might lose its antioxidant capability and age faster . Therefore, skincare is not only important for the skin's appearance and health, but also for the skin's barrier function .

Many cosmetic products are composed of synthetic chemicals, which may cause side effects in the body; for example, some pigments may cause damage to

cells and some UV filters may even cause tumor formation . Moreover, metals are widely used in cosmetics as pigments—for example, in eye shadow, lipstick, blush, and eyeliner. However, some metals, such as cadmium and chromium, are harmful to the human body and may even affect human metabolism. However, the toxicity of cosmetics was not taken into consideration in the early period, which led to a variety of negative adverse effects, including deformities, blindness, and even death. Although there are limitations to their use, these limitations are not entirely effective. The cosmetics that are absorbed by our body may act as carcinogens, reproductive toxins, endocrine disruptors, mutagens, and sources of neurotoxicity .[14]

Application of bacterial dye in cosmetics

Ingredients for making nourishment cream from dye :
1] Vitamin E - 1.5 ml
2] Olive oil - 2.8 ml
3] Bees wax - 4 gm
4] Liquid paraffin - 22 ml
5] Sodium benzoate - 0.05 gm
6] Borax - 0.4 gm
7] Natural bacterial pigment - as much as required

Procedure is as followed :

1] In a heat resistant container , combine beeswax and liquid paraffin . heat gently until the bees wax melts completely
2] Add Vitamin E , olive oil , and sodium benzoate to the melted mixture . stir well to combine .
3] In a separate container , dissolve borax in a small amount of water
4] Slowly add the borax solution to the wax oil mixture while stirring continuosly
5] Allow the mixture to cool slightly but not solidify completely
6] Add natural pigment for color . mix thoroughly to ensure even distribution
7] Pour the cream into sterilized container and allow it to solidify completely
8] Label the container with date of preparation and store in cool sterilized places away from sunlight .

Benefits of using this cream :

1] Moisturization : this cream can help to hydrate skin make it softer and smoother .

2] Antioxidant production : ingredients such as vitamin E are antioxidant that can protect skin from free radicals

3] Skin nourishment and anti inflammatory effects : it stops inflammation and redness of skin caused by irritation

4] antibacterial and potential healing properties : it repairs damaged skin and minor wound cuts .

5] this cream from bacterial dye gives pinkish appearance just like dye and there was no irritation after its appliance on skin.

Cream made from pigmented bacteria

III. DISCUSSION

Summary of findings: we studied that this bacteria is gram negative cocci .
Which give pinkish red pigments under stress conditions when salt is provided so as mechanism for its survival it gives pigments. This bacteria doesn’t multiply in solid substrate fermentation it only comes on combination of agar and nutrient agar . It dissolved in acetone and ethanol solution . DMF acted as decolorizer for this pigment . the bacteria was positive for catalase test , antibacterial test, and its ideal pH is 5 This bacterial pigment is ideal for dying process because its color doesn’t fade even at 80°c where most of dying process of cloth is done.

Pigmented bacteria play several important ecological roles, contributing to ecosystem dynamics, nutrient

cycling, and even influencing the behavior of other organisms. Here are some key ecological significance's of pigmented bacteria like

Bioremediation: Certain pigmented bacteria possess metabolic capabilities that allow them to degrade or detoxify pollutants in the environment. For example, some species of Pseudomonas can degrade hydrocarbons, while others can detoxify heavy metals. These bacteria play a crucial role in bioremediation efforts, helping to clean up contaminated sites and restore ecosystem health.

Biological Pigments: The pigments produced by bacteria serve various ecological functions. For instance, they can protect bacteria from harmful UV radiation, regulate their internal environment, or serve as signaling molecules. Additionally, bacterial pigments can influence the coloration of ecosystems, such as the pink hues seen in saline lakes due to the presence of pigmented halophilic bacteria.

Biosynthesis Pathways Many pigmented bacteria possess specialized biosynthetic pathways that enable them to produce pigments. These pathways involve a series of enzymatic reactions that convert precursor molecules into the final pigment molecules. The genes encoding the enzymes involved in pigment biosynthesis are often organized in clusters known as pigment biosynthetic gene clusters (BGCs).

Interpretation of results:

Sample Procurement: Soil samples were collected from various locations in Mumbai city, including garden areas, lakes, agricultural regions, and virgin lands mixed with marshy soil.

Isolation and Enrichment of Microorganisms: Pigment-producing organisms were enriched from the collected soil samples by incubating them in nutrient broth at 37 ° Celsius for 48 hours. The spread plate method was then employed to isolate pigment-producing bacteria after enrichment. Serial dilutions of the sample suspensions were spread on nutrient agar plates and incubated at 37 ° Celsius for 24 to 48 hours. These pure cultures were sub-cultured on sterile nutrient agar slants and maintained as stock cultures for subsequent studies.

Induction of Pigment Production Under Stress Conditions: To induce pigment production, stress conditions were imposed on the bacterial cultures using various salts, including magnesium sulfate, sodium chloride, calcium chloride, potassium chloride, sodium nitrate, dipotassium hydrogen phosphate, and potassium hydrogen phosphate. These stress conditions were applied to stimulate the bacteria to produce pigments as secondary metabolites.

Spectrophotometric Analysis: The absorbance spectrum of the bacterial pigments was measured using a spectrophotometer. The spectrophotometric analysis involved scanning the samples at different wavelengths to determine the absorbance values. The wavelength range scanned and the resolution settings of the spectrophotometer were optimized for accurate measurement of absorbance values.

Data Analysis and Interpretation: The absorbance data obtained from the spectrophotometric measurements were processed and analyzed to generate the absorbance spectrum of the bacterial pigments. The absorbance values at different wavelengths were plotted to characterize the spectral properties of the pigments. Statistical analysis, including mean absorbance values and standard deviations, was performed to interpret the spectral data and draw valid conclusions regarding the pigment composition and color properties.

Quality Control and Reproducibility: To ensure the reproducibility and reliability of the absorbance measurements, quality control checks were implemented during the spectrophotometric analysis. These measures included conducting replicate scans, calibrating the spectrophotometer, and validating the results to minimize experimental errors and ensure the integrity of the data.

CONCLUSION

Based on what was recorded throughout this process we saw the biological activity of bacterial pigments what stress conditions, temperatures , and pH must be given to them so bacteria will produce pigment as secondary metabolite and this biological compound has wide range of applications and solution to today's harmful problems that can cause harm to humanity and

environment. Study for massification of this bacterias are necessary to have its application in industry. This bacteria have applications in wide range of fields like therapeutic , cosmetic , various type of dyes, and food industries .

This bacteria themselves have varieties of strains and pigments that are so vibrant in color so they can have various applications.

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