



INVESTIGATING PIGMENTS ISOLATED FROM SOIL BACTERIA FOR SUSTAINABLE FABRIC DYE

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Abstract: The textile industry's reliance on synthetic dyes has significant environmental and health impacts. This study explores the potential of bacterial pigments derived from soil as a sustainable alternative for fabric dyeing. Soil samples were collected and screened for pigment-producing bacteria, which were then isolated and identified. The extracted pigments were characterized and evaluated for their dyeing properties on fabrics. Results showed that the bacterial pigments exhibited fair color fastness and biodegradability, making them a promising sustainable option for fabric dyeing. This project work contributes to the development of eco-friendly dye production methods, reducing the industry's environmental footprint while promoting the use of renewable resources.

Keywords: Sustainable fabric dyeing, Bacterial pigments, soil microbiome, eco-friendly textiles, natural dyes.

1.INTRODUCTION

A pigment is a substance that, by wavelength-selective absorption, modifies the colour of light that is reflected or transmitted. The hue of the source light has a direct impact on how pigments appear. Unlike fluorescence, phosphorescence, and other types of luminescence, which involve a material emitting light, this physical process is not the same as luminescence. Many substances absorb light at specific wavelengths alone. The unique qualities of materials that humans have selected and created for use as pigments typically make them perfect for colouring other materials. Pigments are divided into two groups according to how they are made: inorganic pigments and organic pigments (Venil et al., 2020).

Chemical pigments, also known as synthetic dyes, have been used for generations in a variety of industries, including paper, textile, food, and cosmetics. Depending on the intended use, synthetic organic pigments can be tailored to have particular qualities and can be obtained with great purity, consistency, and stability. Their affordability and wide colour range have made them popular, but they also harm the environment because they are made of chemicals and have been shown to be carcinogenic. On the other hand, natural colours may be derived from bacteria, plants, and insects and are safe for the environment (Mishra et al., 2019).

Considering natural pigments are safer, more affordable, and biodegradable than chemically manufactured pigments, they have recently piqued the curiosity of researchers and the general public. Although plants and insects can be utilized as a source of natural colour, their limited availability and scarcity may be considered a disadvantage. However, because of their abundance and availability in nature, bacteria can be exploited. Previous studies have demonstrated that microbe-produced pigments contain antibacterial, anticancer, and other properties that make them an additional benefit when selecting microbial pigment. Astaxanthin (*Xanthophyllomyces dendrorhous*), Arpink Red (*Penicillium oxalicum*), β -carotene and lycopene (*Blakeslea trispora*), and riboflavin (*Ashbya gossypii*) are among the microbial pigments that the Food and Drug Administration has recently approved to be used in food industries (Mani et al., 2018).

1.1 Microorganisms in Industries

Recently, there has been an increased interest in microorganisms. Microorganisms have the ability to produce a wide range of commercially valuable goods, including wine, beer, antibiotics like streptomycin and penicillin, secondary metabolites (enzymes like amylase and pectinase), and many more (Singh et al., 2021).

In a short amount of time, microorganisms can be grown on a big scale under ideal conditions and with enough nutrients to yield the desired output. The ability to genetically modify the products as needed adds even another benefit to the use of microorganisms in industrial manufacturing (Kumar et al., 2017).

Nowadays, bacterial pigments are produced commercially in several industries, including the food, textile, and cosmetics sectors (Patel et al., 2019).

1.2 Distribution of microorganism in environment

Microbes are present everywhere. Microorganisms are unique among organisms in that they can endure in any environment, including low-oxygen environments, at any temperature and pH. They can be found in dry areas, Antarctic zones, marine water, thermal vents, and under rocks. Microbes can have helpful, negative, or undetectable effects on their surroundings that are not visible to humans (Madigan et al., 2021).

To give soil fertility and productivity, microbes are crucial to the development of soil aggregates and soil stability. There are various methods in which soil microorganisms contribute to these activities. For example, filamentous bacteria use an extensive network of hyphae to assemble clay particles into soil aggregates. Furthermore, some microorganisms create clay particle compaction or emit exopolysaccharides, which encourage soil aggregation. The native population of bacteria, fungus, algae, and protozoa is constantly abundant in the surface soil. This includes actinomycetes (Bardgett et al., 2019).

Because of the stress brought on by dehydration, bacteria find the atmosphere to be an unfriendly environment. As a result, the amount of time that microorganisms can be active is restricted. Nevertheless, certain microbes develop resistance to these stresses by means of specific mechanisms that encourage the cessation of their biological activity (Potts et al., 2018).

Planktons are a type of photoautotrophic microbial community that includes prokaryotes (cyanobacteria) and eukaryotes (algae), as well as a heterotrophic community of bacteria (bacterioplankton) and protozoans (zooplankton). Because they can fix CO₂ into organic matter through photosynthesis, phytoplankton are the main producers in the food chain. Microbes that live in freshwater, brackish water, and marine water are found in the three microenvironments that make up the aquatic environment (Falkowski et al., 2020).

1.3 Pigments

Pigments are coloured substances that are used in food, clothing, paper goods, and other items. Pigments can be chemically or naturally occurring; they can be categorized as synthetic or natural depending on where they come from (Paliwal et al., n.d.). Petrochemicals and coal tars are two examples of the chemicals used to make synthetic pigments, commonly referred to as inorganic colour. Artificial colouring is thought to be more dependable and cost-effective. One of the primary effects of these man-made chemicals is oxidative cell damage, which can potentially lead to immunosuppression in humans and, in the worst situation, even cancer (Bhat et al., 2018).

Natural colorants are now driving the market demand over time as the trend moves towards natural sources for eco-friendly components. Microorganisms, namely bacteria, can produce an extensive variety of secondary metabolites and colours. There are numerous commercial uses for pigments derived from bacteria (Joshi et al., 2003; Venil & Lakshmanaperumalsamy, 2009; Ahmad et al., 2012).

It is estimated that the dyes and pigments sector wastes around 15% of its global production along the production chain up to the application, resulting in an environmental impact of 128 tons per day. Due to their poor biodegradability and tendency to bioaccumulate, a significant portion of pigments, namely inorganic pigments, end up as environmental contaminants. Discarded dyes and pigments in rivers and seawater, even at low concentrations, alter the water's pH, decreasing light penetration in the aqueous phase and thus harming an entire marine food chain that depends on photosynthetic species. When light-stable but nonbiodegradable colour compounds from the textile industries are taken into account, the issue is made clear. Furthermore, even in amounts that are not disclosed, heavy metals can still be dumped and end up in the water (Kumar et al., 2020).

1.3.1 Microbial Pigments

Microbial pigments are pigments that are made by microorganisms, including algae, fungi, and bacteria.

Table 1.3.1 Microbial pigments in diverse settings

Pigment	Colour	Microorganisms
Phycocyanin	Green, Blue	<i>Pseudomonas aeruginosa</i>
Riboflavins	Yellow	<i>Ashbya gossypii</i>
Prodigiosin	Red	<i>Serratia marcescens</i>
Anthraquinone	Red	<i>Penicillium oxalicum</i>
Violacein	Purple	<i>Janthinobacterium lividum</i>
Xanthomonadin	Yellow	<i>Xanthomonas oryzae</i>
Carotenoids	Orange	<i>Brevibacterium</i>

1.6 Production of pigments

Pigment production is not as difficult as that of synthetic pigments. Large bio fermenters are used to grow the pigment-producing bacteria first on a smaller scale before being moved to a larger one (Smith et al., 2024). For a microorganism to produce more pigment, different ideal conditions must be met such as ideal temperature, pH, aeration, media formulation, and humidity (Doe et al., 2023). Small-scale manufacture can be carried out in a microbiology lab to analyse and evaluate the pigment of our choice (Johnson et al., 2022). The pigment-producing microorganisms are moved to a pilot-scale fermentor upon completion of the need, and they are subsequently moved to a larger fermentor known as a bioreactor (Smith et al., 2024).

3. MATERIALS AND METHODS

3.1 MATERIALS REQUIRED

- Soil samples – Four soil samples were collected from four different places S -01 (kitchen waste dump region), S-02 (car parking region), S-03(garbage dump region), S-04(soil around a tree)
- Media used: Nutrient agar, Peptone water, MR-Vp broth, Simmon citrate agar, Triple sugar iron agar, Nitrate broth
- Chemicals used: Ethanol, Kovac's reagent, Methyl red indicator, Barritt's reagent A and Barritt's reagent B, Nitrate reagent A and Nitrate reagent B.

3.2 METHODOLOGY

3.2.1 Collection of sample

Location 1: Kitchen waste dump area

Location 2: Car parking areas

Location 3: Garbage dump area

Location 4: Rhizospheric soil

3.2.2 Serial dilution of sample

9 ml of distilled water was taken in 7 sterile test tubes for each sample. The test tubes were marked as 10^1 to 10^6 . 1 gm of soil was weighed and added to the 9 ml of distilled water and it was serially diluted. 1 ml from the last test tube was discarded after serial dilution, and it was repeated for each sample (Gurung et al., 2020).

3.2.3 Standard plating technique

The serially diluted samples were plated using the standard plating technique.

- **Spread Plate Technique:**

1. Sterile nutrient agar plates were taken and marked respectively.
2. 0.1 ml of the samples from the dilutions of 10^{-2} and 10^{-3} were added on respective solid nutrient agar plates using sterile pipettes.
3. The glass L rod was sterilized by using ethanol and heat.
4. The L rod was cooled and the sample was spread in a clockwise and anticlockwise direction using the rod.
5. After inoculation, the plates were inverted and kept for incubation at 37°C for 24–48 hours (Rathore et al., 2019).

3.2.4 Isolation and Identification of Colonies

3.2.4.2 Gram's Staining:

The pigmented colonies were picked using a heat-sterilized loop and gram staining was done using standard technique (Singh et al., 2021).

3.2.4.3 Biochemical Tests:

- Indole test
- Methyl red test
- Voges-Proskauer test
- Citrate test
- Nitrate test
- Catalase test

were performed by inoculating a loopful of each pigmented colony in the respective test media and left for incubation at 37°C for 24 to 48 hours (Rathore et al., 2019).

3.2.5 Pigment production

50 ml of nutrient broth was used to inoculate the isolated pigmented colonies using a sterile inoculation loop, and it was then incubated at 37°C for 24 to 48 hours (Singh et al., 2021).

4. RESULTS

4.1 Isolation of Pigmented bacteria

From the different soil samples processed all the samples consisted of pigmented bacteria.

The majority of the bacteria that produced colour were identified from kitchen waste dumps and garbage dumping sites, with the least amount coming from rhizospheric regions.

Table 4.1: Species identification

Pigment colour	Microorganism
Yellow	<i>Micrococcus luteus</i>
Pink	<i>Exiguobacterium aurantiacum</i>
Light pink	<i>Rhodococcus ruber</i>
Medium yellow	<i>Domibacillus enclensis</i>
Orange	<i>Exiguobacterium aurantiacum</i>
Light orange	<i>Lactobacillus paralimenlarius</i>

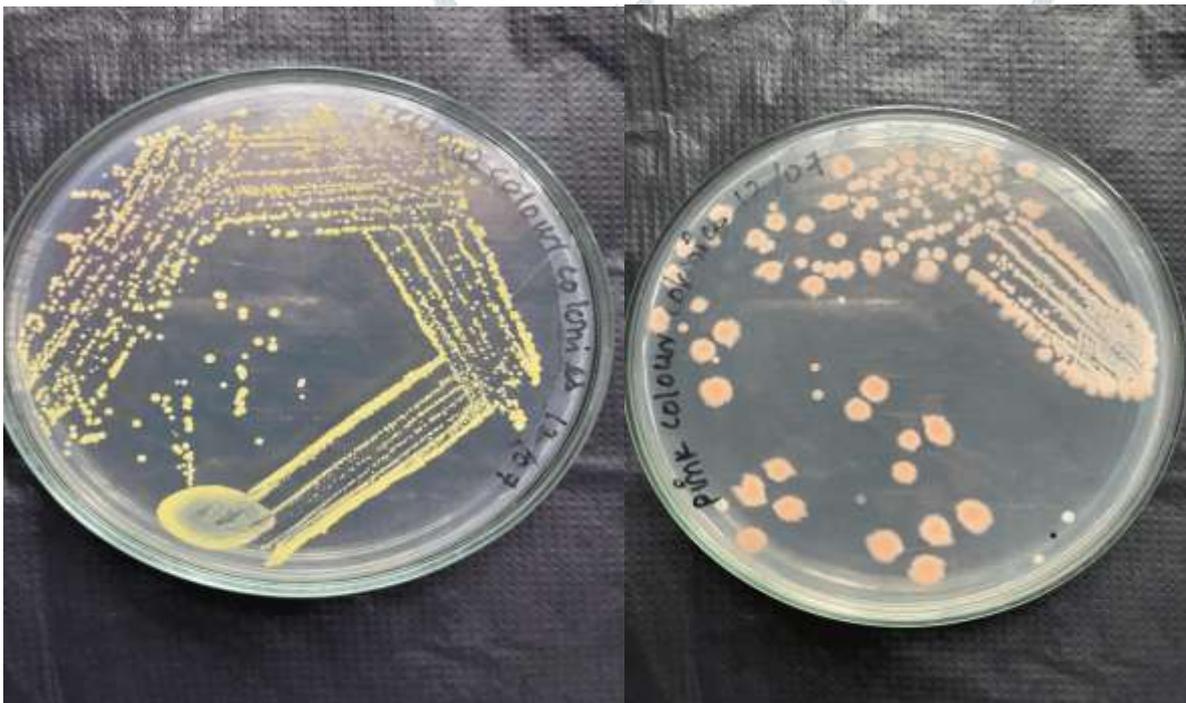




Plate 4.2 Pigmented colonies

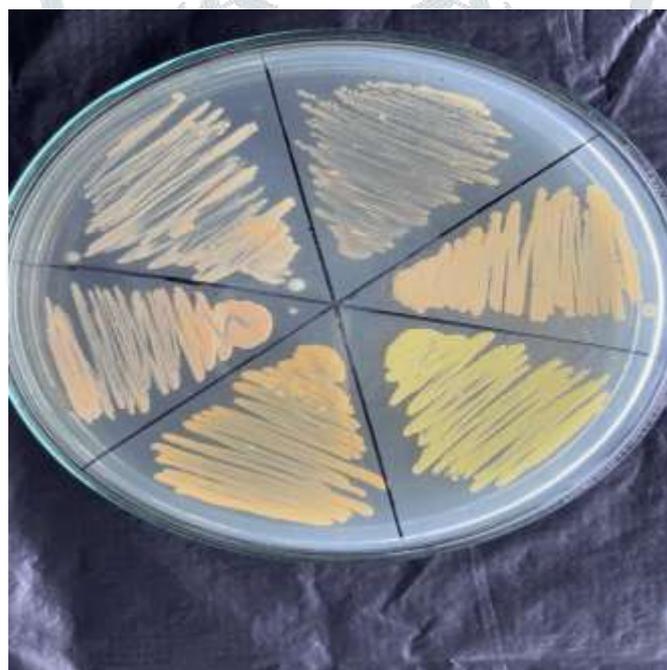


Plate 4.2.1 Subculture of pigmented bacteria

4.2 Identification of bacteria

Biochemical testing (Table-4.2.2) and colony morphology (table- 4.2.1) were used to identify and describe the isolated colonies. Using the MALDI-TOF technology, their identification at the species level were confirmed.

Table 4.2.1: Colony morphology

Colour	Margin	Shape	Opacity	Consistency	Elevation
Yellow	entire	circular	opaque	smooth	convex
Pink	entire	circular	opaque	smooth	convex
Light pink	irregular	crateriform	opaque	wrinkled	raised in center
Medium yellow	entire	circular	opaque	smooth	convex
Orange	entire	circular	opaque	smooth	convex
Light orange	entire	circular	opaque	sticky	flat

Table 4.2.2: Biochemical test

Pigment	Indole	Methyl red	Voges proskauer	Citrate test	Nitrate test	TSI test	Gram's staining	Motility
Yellow	-	-	-	+	-	A/A	Gram positive cocci	-
Pink	-	-	-	-	+	A/A	Gram positive rod	-
Light pink	-	-	-	+	+	A/A	Gram positive rod	-
Medium yellow	-	-	-	+	+	K/A	Gram positive rod	-
Orange	-	-	-	-	+	A/A	Gram positive rod	-
Light orange	-	-	-	-	-	A/A	Gram positive rod	-

(- sign indicates negative results, + indicates positive results A indicates acid production and K indicates alkaline)

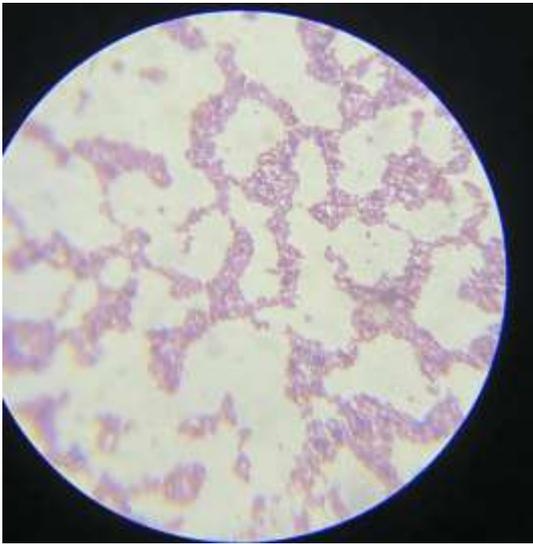


Fig 4.2.2 Gram's staining: gram positive cocci

Fig 4.2.3 Gram's staining: gram positive rods

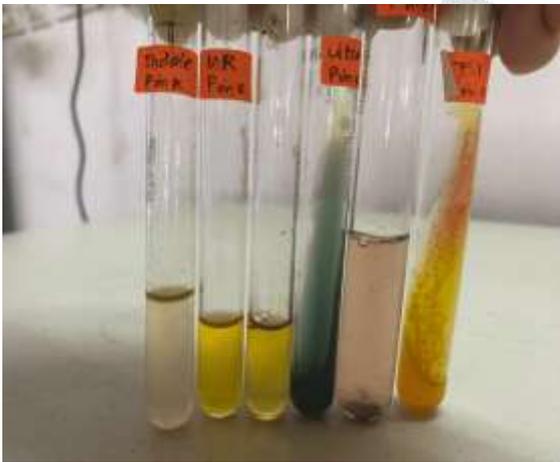


Fig 4.2.2 a Biochemical result: pink pigment

Fig 4.2.2 b Biochemical result: yellow pigment

4.3 Extraction of Pigment:

It was discovered that more pink and yellow pigment was produced when compared to other pigmented bacteria. Using centrifugation, filtration, and the addition of ethanol, the pigments were extracted.



Fig 4.3 Pigmented colonies in nutrient broth

4.4 Characterization of Pigment

4.4.1 Thin layer Chromatography

The technique known as thin layer chromatography, or TLC, is used to separate and examine compound mixtures.

Hexane and acetone were used as the solvent system for the thin layer chromatography (TLC) examination. The TLC plate showed several distinct spots, each of which corresponded to a different component of the bacterial pigment mixture, as revealed by the results.

The retention factor (Rf) values noted is give in table – 4.4.

Table 4.4 a) Yellow pigment TLC result

S.NO	RF Value
1.	0.23
2.	0.35
3.	0.40
4.	0.57
5.	0.61
6.	0.76
7.	0.85
8.	0.92

Table 4.4 b) Pink pigment TLC result

S.NO	RF Value
1.	0.25
2.	0.65
3.	0.57
4.	0.48
5.	0.40
6.	0.25

The broad range of Rf values suggests that the yellow bacterial pigment is made up of several different components. A distinct chemical with varied affinities for the mobile phase (hexane: acetone solvent system) and stationary phase (TLC plate) is represented by each Rf value.

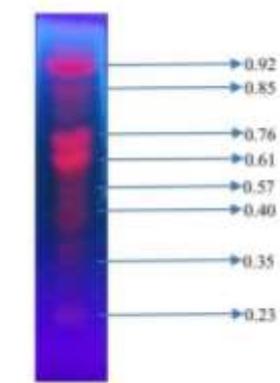


Fig 4.4 TLC of Yellow pigment

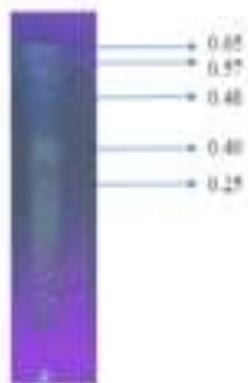


Fig 4.5 TLC of pink pigment

4.4.2 UV- Vis Spectroscopy

UV-Vis spectroscopy measures how much ultraviolet and visible light a sample absorbs or lets through.

The extracted pink and yellow pigment's maximum absorption spectrum, measured with an UV- visible spectrophotometer using methanol as a blank, showed one peak within the range of 200 to 300 nm.

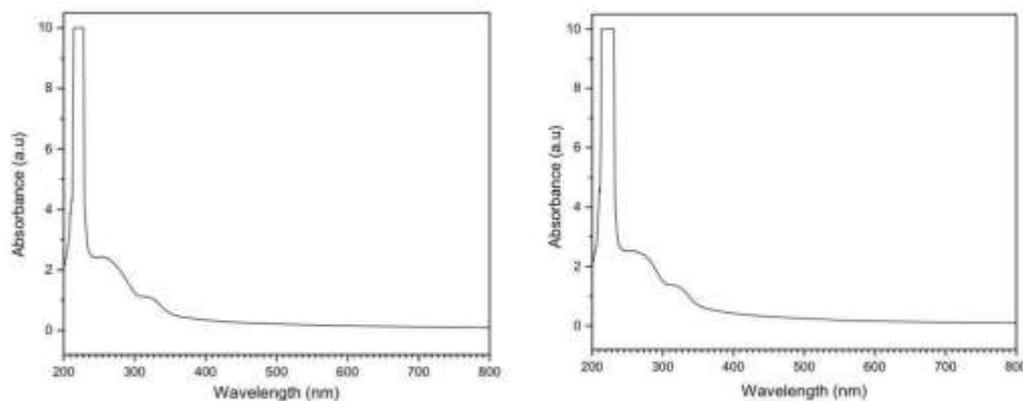


Fig 4.6 Uv- vis spectroscopy for pink pigment

Fig 4.6 Uv- vis spectroscopy for yellow pigment

With an absorbance value of 10 a.u., the pink pigment showed a maximum absorption peak at roughly 200–300 nm, suggesting that the UV area is where it is primarily absorbed. Likewise, the yellow pigment had an absorbance value of 8–10 a.u. and a high absorption peak at about 200–300 nm.

5. DISCUSSION

In order to potentially be employed in fabric dyeing, the study concentrated on the extraction and isolation of bacterial pigments from soil samples. The findings showed that it is feasible to use naturally occurring microbes to produce pigment, which has encouraging ramifications for the textile sector. The significance of the results will be examined, along with how they stack up against previously published research and any potential uses or limitations.

The discovery that coloured bacteria can be isolated from a variety of soil samples, especially from waste-filled areas like landfill sites and kitchen waste dumps, highlights the variety and adaptability of microorganisms in these settings. According to previous research, the majority of coloured colonies in soils connected to garbage indicate that the growth of bacteria that produce pigment is favoured in environments rich in organic matter. **Joshi et al. (2008)**, for example, observed comparable results when they recovered coloured bacteria from soil samples and industrial effluent, demonstrating the potential of such habitats as reservoirs of useful microbial species.

The taxonomy of pigment-producing bacteria is supported by the identification of bacterial species such as *Micrococcus luteus*, *Exiguobacterium aurantiacum*, *Rhodococcus ruber*, and *Domibacillus enclensis*. Because of its antibacterial qualities, *Micrococcus luteus* in particular is well-known for producing yellow pigment, which finds use in both industrial and medical settings. *Exiguobacterium* species, which are renowned for their adaptability in harsh settings, are present, which implies that these bacteria may be enhanced for large-scale pigment synthesis in a variety of settings.

Centrifugation, filtering, and the addition of ethanol were the steps in the pigment extraction process that worked best for isolating the yellow and pink pigments produced by bacteria. This technique is frequently used in microbial pigment investigations; for example, **Venil et al. (2013)** extracted and purified yellow pigment and pink pigment from *Micrococcus luteus* and *Exiguobacterium aurantiacum* using comparable methods. Though efficient, the straightforward extraction method used in this work may have limited the pigments'

purity and yield when compared to more sophisticated techniques like chromatography, which may be taken into account for further research aimed at improving pigment quality.

Given that these values imply that the compounds have higher interactions with the stationary phase and less mobility in the non-polar solvent system, $R_f = 0.23$ and $R_f = 0.25$ most likely indicate more polar molecules within the pigment.

Less polar chemicals, represented by $R_f = 0.92$ and $R_f = 0.65$, moved up the plate more readily, suggesting greater solubility in the non-polar mobile phase. The range of R_f values indicates that the yellow pigment is not a single component, but rather a complicated combination. The pigment may be made up of numerous related pigments or it may have a diversified chemical structure, as suggested by the presence of multiple components.

Both pigments have distinct absorption profiles, according to the spectra, with peaks that fall between 200 and 300 nm. This raises the possibility that they are bioactive substances with UV-absorbing capabilities.

Given the commercial potential of yellow and pink pigments in fabric dyeing, it is interesting that the study focused on these. Because of their durability and brightness, yellow pigments like those produced by *Micrococcus luteus* are highly prized and can be a good substitute for synthetic dyes. Conversely, pink pigments offer visual variation and may appeal to specific textile industry niche customers that value natural and sustainable materials.

This work provides a more comprehensive view than previous studies since it isolates different bacterial species and pigments from several soil samples. For instance, **Kumar et al. (2015)** highlighted the potential for antibacterial and anti-cancer applications of the red pigment Prodigiosin from *Serratia marcescens*. Although the bioactive characteristics of the pigments were not investigated in this study, the discovery of several species that produce pigments points to possible multifunctionality, which may be investigated in subsequent research.

The results of the study align with the wider movement towards discovering sustainable substitutes for artificial dyes. Using bacterial pigments has been shown to benefit the environment and human health, especially when it comes to lowering the hazardous waste involved with the creation of synthetic dyes, according to research by **Vasanthabharathi et al. (2011)**.

In our research, only a limited number of pigment-producing strains were identified. Of the six bacterial strains capable of producing pigment, only two successfully produced pigment in nutrient broth. The inability of the remaining strains to produce pigment in this medium could be attributed to several factors, including the potential for higher solubility of the pigment or suboptimal conditions for pigment production. Additionally, the scope of our research was constrained by a small sample size and a focused examination of specific soil properties, which may have limited the broader applicability of the findings.

6. CONCLUSION

Soil is one abiotic component that exists everywhere on Earth. It is composed of elements such as water, minerals, organic and inorganic components that aid in the growth of plants, and microbes that aid in the breakdown of complex substances into simpler forms that plants can absorb for sustenance.

Numerous bacteria found in soil can be quite helpful for bioremediation and sustainability, both of which are critical for the years to come. This investigation aided in the identification of bacteria that produce pigment in soil samples taken from various locations. The generated pigment has various applications, including use as an antioxidant-rich food colouring and as a fabric dye with antibacterial properties. In comparison to pigment that is chemically generated, bacterial pigment can be grown more quickly and at a lower cost.

The increasing need for sustainable and non-toxic textile production is in line with the extraction and separation of bacterial pigments from soil samples for fabric dyeing, which offers a viable, environmentally acceptable substitute for synthetic dyes. Pigmented bacteria were effectively recovered and identified from a variety of soil samples in this investigation, including those from a car park, garbage dump, rhizospheric soil, and kitchen waste disposal. *Micrococcus luteus*, *Exiguobacterium aurantiacum*, *Rhodococcus ruber*, *Domibacillus enclensis*, and *Lactobacillus paralimnarius* were the main bacteria detected; they were found to produce several

pigments including yellow, pink, light pink, medium yellow, orange, and lightorange. Despite the possibility that they could have harmful effects that can be avoided with technology like genetic engineering which may additionally improve pigment production

To isolate and identify the bacteria, conventional plating, serial dilution, and biochemical testing were the methods used. A number of biochemical tests, such as the Indole, Methyl Red, Voges-Proskauer, Citrate, and Nitrate tests, together with Gram staining were particularly useful in revealing the physiological and metabolic traits of these microbes. The study emphasized the possibility of such areas serving as reservoirs for pigment-producing microorganisms by highlighting the presence of coloured bacteria primarily in soil samples from waste-rich locations.

The viability of using bacterial pigments as fabric dyes was proven by the successful production and extraction of pigments, especially the pink and yellow ones, from isolated colonies grown in nutrient broth. Centrifugation, filtration, and the addition of ethanol were used to extract the pigments, producing workable dyes that could be used in subsequent textile dyeing procedures.

When comparing this work to previous research, it can be seen that a number of investigations have also successfully isolated pigmented bacteria from a variety of environmental sources, such as waste sites, water, and soil. For example, **Kumar et al. (2015)** discovered Prodigiosin, a red pigment with important uses in textile dyeing because of its bright colour and antibacterial qualities, from *Serratia marcescens*. Similarly, *Micrococcus luteus* was the subject of a 2013 study by Venil et al. that highlighted the plant's potential as a natural dye with strong colorfastness characteristics and concentrated on producing yellow pigment. Nonetheless, this study offers a wider variety of pigmented bacteria, indicating the diversity of soil microorganisms capable of producing a range of colours, in contrast to other studies that frequently concentrate on a particular type of pigment or bacterial species. This study is made more novel by including a variety of soil types from various environmental situations, which highlights the impact of habitat on pigment production.

Furthermore, the extraction technique used in this work, which included ethanol extraction, filtration, and centrifugation, is consistent with accepted methods for pigment isolation. Similar approaches were employed in other investigations, such the one conducted by **Vasanthabharathi et al. (2011)**, however extra purification procedures, like column chromatography, were frequently added to improve pigment purity. Although efficient, the comparatively simple extraction method used in this study would benefit from such sophisticated purification methods to raise the dye's quality for use in commercial applications.

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