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Isolation Pigment Producing Microorganism and Pigment Characterization

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Abstract: This research focuses on the isolation and characterization of pigment-producing microorganisms from soil samples collected across different regions of Karnataka. As concerns grow over the environmental and health impacts of synthetic dyes, microbial pigments offer a sustainable alternative with broad applications in food, textiles, cosmetics, and pharmaceuticals. Our study aims to isolate diverse pigment-producing microbes using serial dilution techniques and selective media, followed by comprehensive characterization of the extracted pigments. The pigments will be analyzed using multiple analytical techniques including UV-Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Thin Layer Chromatography (TLC), and to determine their structural and functional properties. Additionally, we will evaluate the practical application of these pigments in the textile industry by dyeing silk fabrics and assessing color fastness properties according to international standards. This research not only contributes to the growing field of natural colorants but also promotes sustainable practices by potentially utilizing agricultural and industrial by-products in pigment production. The findings of this study may provide valuable insights into novel pigment-producing microorganisms and their potential industrial applications, advancing efforts toward more environmentally friendly and health-conscious coloring alternatives while supporting local economies through sus- tainable biotechnology.

Keywords: *Pigment, Bacteria, Isolation, Characterization.*

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I. INTRODUCTION

In today's world, where environmental sustainability is a growing concern, the need for natural alternatives to synthetic compounds is more urgent than ever. One such promising area of research is microbial pigments—natural colorants produced by bacteria, fungi, and algae. These pigments not only offer vibrant hues but also represent a sustainable solution to many of the challenges posed by synthetic dyes, especially in industries like textiles, food, and cosmetics. Synthetic dyes, widely used in the textile industry, contribute significantly to environmental pollution. Their production and application involve toxic chemicals and heavy metals, which often end up in water bodies, harming ecosystems and posing risks to human health. It is estimated that 17–20% of global industrial water pollution is caused by textile dyeing and treatment processes. In contrast, microbial pigments are produced through fermentation using low energy inputs and often rely on agricultural or industrial waste as substrates. This makes them an eco-friendly alternative, aligning well with principles of sustainable development. Moreover, microbial pigments often have beneficial bioactive properties, such as antimicrobial, antioxidant, anti-inflammatory, and even anticancer activities. For example, prodigiosin, a red pigment from certain bacteria, shows anticancer potential, while fungal melanins are known for their UV-protective qualities. These additional benefits make microbial pigments valuable not just for their color but also for their functionality. Despite their promise, challenges remain. Pigment yields are often low, and some natural pigments may be unstable under varying conditions. Overcoming these limitations requires detailed research into the isolation and optimization of pigment-producing microorganisms. This dissertation focuses on isolating pigment-producing microbes from various soil samples across Karnataka, characterizing the pigments they produce, and evaluating their suitability for sustainable textile dyeing. Using microbiological and analytical techniques such as serial dilution, UV-Vis, FTIR, and TLC, the study aims to identify microbes capable of producing industrially relevant pigments. By exploring the potential of microbial pigments, this research contributes to environmentally conscious innovation in the textile industry and supports the global transition toward sustainable practices. It highlights the

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possibility of replacing harmful synthetic dyes with natural, multifunctional colorants derived from microorganisms.

Kim et al. isolated and characterized various *Chryseobacterium* species from soil samples collected from agricultural fields in South Korea, establishing their taxonomic diversity within the genus. These bacteria were found to produce vibrant yellow-orange flexirubin-type pigments, confirmed using the KOH test. Their comprehensive taxonomic analysis, including phenotypic, chemotaxonomic, and phylogenetic approaches, provided critical baseline data for subsequent studies and highlighted their potential applications due to pigmentation and environmental adaptability [1].

Bernardet and colleagues developed standardized diagnostic protocols for detecting flexirubin-type pigments in *Chryseobacterium* species, observing their red-brown color change under alkaline conditions. Their KOH test methodology became a foundational tool for rapid pigment identification and supported the classification of *Chryseobacterium* within the *Flavobacteriaceae* family [2].

Herzog et al. demonstrated that flexirubin-type pigments from soil-derived *Chryseobacterium* exhibited significant antimicrobial activity against Gram-positive pathogens and maintained stability across varying pH and temperature ranges, indicating potential pharmaceutical applications [3].

Chen and Wang optimized flexirubin production in soil-inhabiting *Chryseobacterium* species, identifying glucose and peptone as the most effective carbon and nitrogen sources, respectively. Optimal production occurred at pH 7.2 and 28°C, reflecting conditions in fertile soils, and establishing scalability for industrial use [4].

Lee et al. explored the antioxidant properties of pigments from soil-derived *Chryseobacterium*, reporting strong free radical scavenging comparable to synthetic antioxidants. Their study suggested uses in food preservation and cosmetics as natural alternatives [5].

Park and colleagues conducted structural characterization of flexirubin pigments using HPLC, UV-vis spectroscopy, and mass spectrometry. Their findings revealed unique structural traits linked to pigment stability and bioactivity, offering insights for commercial quality control and structure- function analysis [6].

Zhang et al. discovered significant chitinolytic activity in soil-inhabiting *Chryseobacterium*, in addition to pigment production. This dual capability supported their utility in biocontrol and organic waste management applications, positioning them as multifunctional agents in agriculture [7].

Singh and Kumar performed genomic analyses on soil-derived *Chryseobacterium*, identifying biosynthetic gene clusters for flexirubin and other secondary metabolites. These genetic features were associated with stress resilience and adaptability in soil environments [8].

Rodriguez-Concepcion et al. identified carotenoid components within *Chryseobacterium* pigments that enhanced UV resistance and membrane stability. Their comparative analysis linked pigment composition to ecological success in terrestrial ecosystems [9].

Müller and Schmidt demonstrated the ability of *Chryseobacterium* species to biodegrade aromatic compounds and persistent pollutants in soil, underlining their bioremediation potential in contaminated environments [10].

Wang et al. revealed anti-inflammatory activity of flexirubin pigments, noting suppression of pro- inflammatory cytokines in cell culture models. These findings support exploration of flexirubin as a nutraceutical for inflammatory diseases [11].

Yamamoto and colleagues applied *Chryseobacterium* pigments in textile dyeing, achieving excellent color fastness and UV protection. Their optimization protocols laid groundwork for sustainable use of bacterial pigments in the textile industry [12].

II. METHODOLOGY

➢ *Materials*

Glassware A range of sterile glassware was used to cultivate, isolate, and handle pigment- producing microorganisms:

- Petri plates – For growing microbial colonies on solid media.
- Conical flasks – Used to culture microorganisms in liquid media.
- Test tubes – For serial dilutions and small-volume cultures.
- Beakers – Used in preparation and mixing of reagents and media.
- Measuring cylinders – Ensured accurate measurement of solutions.

Chemicals Chemicals played a vital role in both cultivating bacteria and extracting their pigments:

- Nutrient Broth and Nutrient Agar (Merck) – Essential media for bacterial growth.
- LB-Glycerol solution – For long-term storage of bacterial cultures.
  - Tryptone, Yeast extract, NaCl – Components of LB and peptone media.
  - Peptone water (0.1%) – Used for maintaining
  - Sodium Hydroxide (NaOH) – Used for pH adjustment and pigment solubility testing.
  - Methanol (95%) – Primary solvent for pigment extraction.
  - Acetone (99.5%) – Used in extracting pigments from *Chryseobacterium* species.
  - Ethyl acetate and Chloroform – Used for pigment purification steps.

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- Potassium bromide (KBr) – For preparing FTIR samples.
- Vanillin-sulfuric acid reagent – For visualizing pigments on TLC plates.

Instruments The following instruments facilitated cultivation, analysis, and charac- terization of pigments:

- Autoclave – For sterilizing media and glassware.
- Laminar Air Flow Chamber – Maintained a sterile environment for microbial work.
- Incubator – Maintained ideal temperatures (typically 30°C) for bacterial growth.
- Centrifuge – Separated bacterial cells from media during pigment extraction.
- UV–Vis Spectrophotometer – Measured pigment absorbance between 300–700 nm.
- FTIR Spectrometer (ATR mode) – Analyzed functional groups in pigment extracts.
- Rotary Evaporator – Concentrated pigment extracts after solvent removal.
- Thin Layer Chromatography (TLC) Setup – Used to identify and differentiate pigment types.
- Micropipettes and Spreaders – For accurate sample transfer and inoculation.

➢ *Methods*

- *Sample Collection*

Soil and water samples were collected aseptically from various locations including aqua- culture facilities and industrial sites, particularly wastewater treatment ponds and effluent tanks. For this study, samples were specifically gathered from different regions in Karnataka. Soil samples were collected using sterile spatulas and stored in sterile containers, while water samples were collected in 250 mL pre-sterilized Schott bottles. An air gap of approximately 2.5 cm was maintained in the bottles to allow aeration and prevent thermal expansion during transport. All samples were stored in ice boxes and transported to the laboratory within 2–3 hours to preserve microbial activity and chemical stability.

- *Serial Dilution Method*

To isolate individual microorganisms from complex environmental samples, the serial dilution technique was employed. In this process, 1 gram of soil was suspended in sterile distilled water and subjected to ten-fold serial dilutions. From the final dilution tubes, 100 µL aliquots were spread on Nutrient Agar (NA) plates using sterile spreaders. Plates were incubated at 30°C for 24–48 hours. Colonies showing distinct pigmentation were selected and re-streaked several times to obtain pure cultures.

- *Isolation of Microorganisms*

After incubation, pigmented colonies were isolated based on their visual characteristics (color, shape, margin, and elevation). Each unique colony was streaked on fresh NA plates until pure cultures were achieved. Selected colonies, such as those producing yellow, red, or violet pigments, were maintained in glycerol stocks (25% v/v) at -20°C for long-term storage.

- *Ultraviolet–Visible (UV–Vis) Spectroscopy*

UV–Vis spectroscopy was employed to evaluate the absorbance characteristics of ex- tracted pigments. Pigments were dissolved in 95% methanol and absorbance spectra were recorded between 300 and 700 nm using a UV–Vis spectrophotometer. Different pH conditions (2, 7, and 9) were also tested to assess pigment stability and bathochromic changes.

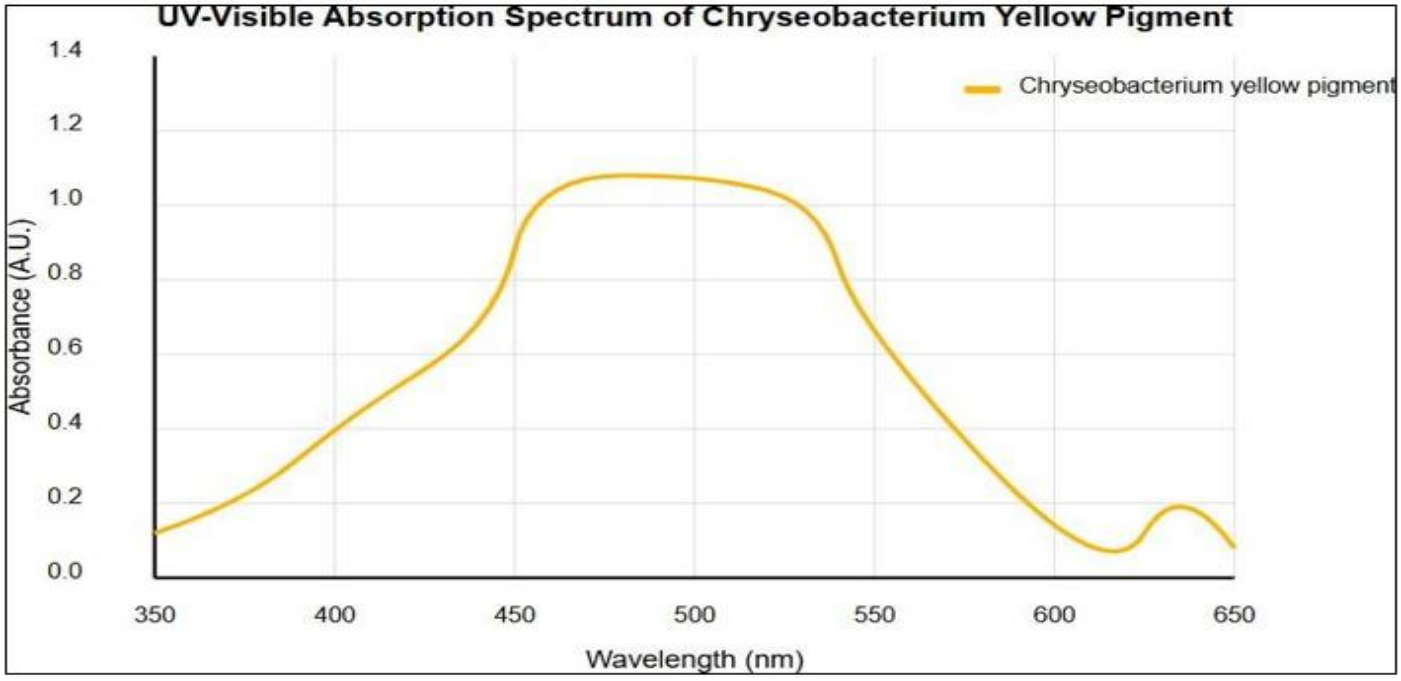

Fig 1 This Image Shows the Ultraviolet–Visible (UV–Vis) Spectroscopy

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- *Fourier Transform Infrared Spectroscopy (FTIR)*

To identify functional groups present in the pigment molecules, FTIR analysis was con- ducted. Samples were ground with potassium bromide (KBr) and pressed into translucent pellets under high pressure. Spectra were recorded in the range of 4000 to 400 cm^{-1}. Characteristic absorption bands corresponding to O–H, C=O, and N–H stretching vi- brations helped elucidate the molecular structure of the pigments, such as violacein and prodigiosin.

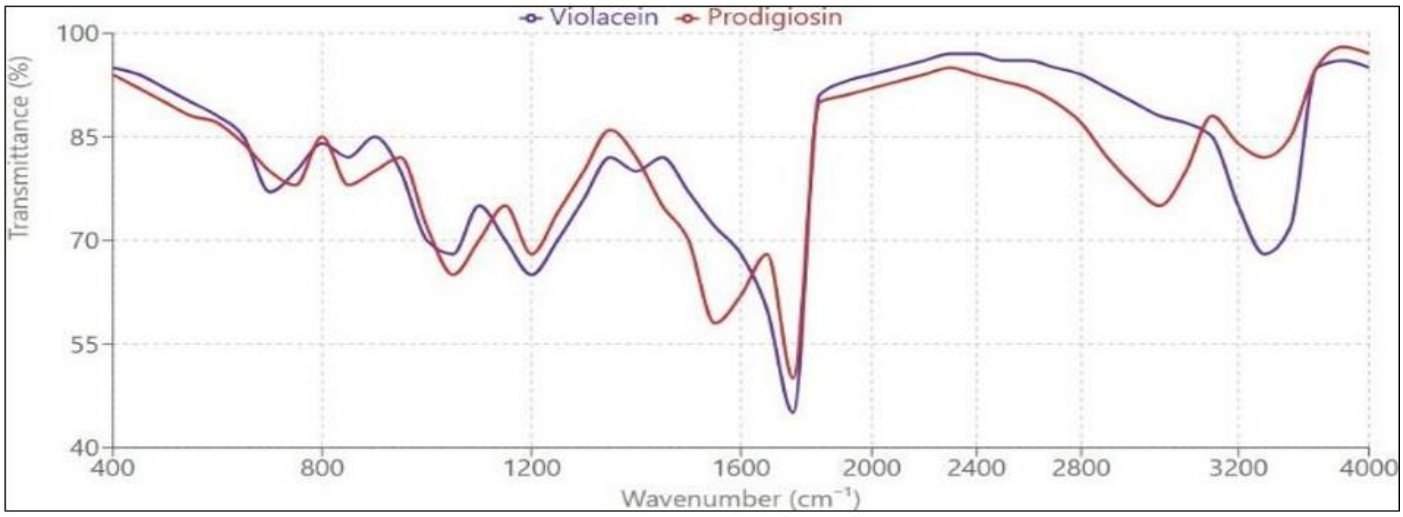

Fig 2 This Image Shows the Fourier Transform Infrared Spectroscopy

➢ *Thin-Layer Chromatography (TLC)*

Pigment extracts were spotted on pre-coated silica gel TLC plates (Merck Kieselgel 60 F254). Solvent systems such as benzene:acetone (2:1) or chloroform:methanol (9:1) were used to develop chromatograms. Pigment bands were visualized under UV light (254 nm and 365 nm). Rf values were calculated using:

$$R_f = \frac{\text{Distance traveled by the pigment}}{\text{Distance traveled by the solvent front}}$$

Pigments like violacein and deoxyviolacein showed distinct Rf values, helping in their preliminary identification.

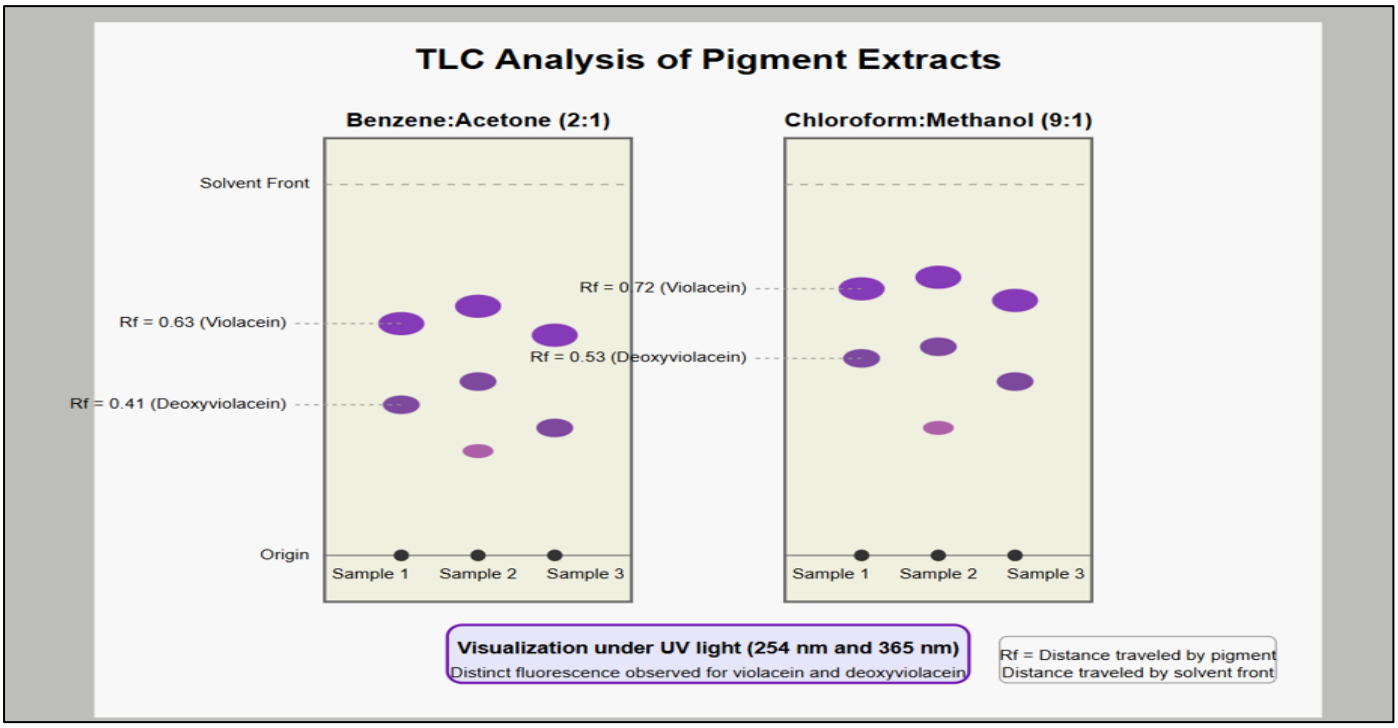

Fig 3 This image shows the TLC analysis for the pigment extracts

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III. RESULTS AND DISCUSSION

➢ *Results*

The following tests and analyses were conducted to isolate pigment-producing microorganisms and characterize the pigments obtained. The results are presented and discussed in detail.

- *Serial Dilution*

Serial dilution was employed to isolate pigment-producing bacteria from soil samples. A known quantity of soil was suspended in sterile saline, and stepwise dilutions were made to reduce microbial concentration. Aliquots from various dilution levels were plated on nutrient agar (NA) and incubated to allow colony formation. This process facilitated the isolation of discrete colonies, enabling identification and further characterization.

- *Organism Pigment*

The isolates exhibited distinct pigmentation, which served as a key selection criterion. Colonies displaying vivid coloration included:

✓ Violet colonies (e.g., *Chromobacterium violaceum*) producing violacein.
✓ Yellow-orange colonies (e.g., *Chryseobacterium sp.*) producing flexirubin-type pig- ments.
✓ Red colonies (e.g.,) producing prodigiosin.
✓ Color production was stable upon incubation on NA and NB media, confirming the pres- ence of pigment-producing strains.

- *Gram Staining Test*

Gram staining revealed structural characteristics:

✓ *Chryseobacterium sp.* also stained Gram-negative, appearing as short rods. These results were in agreement with known literature on these bacteria.

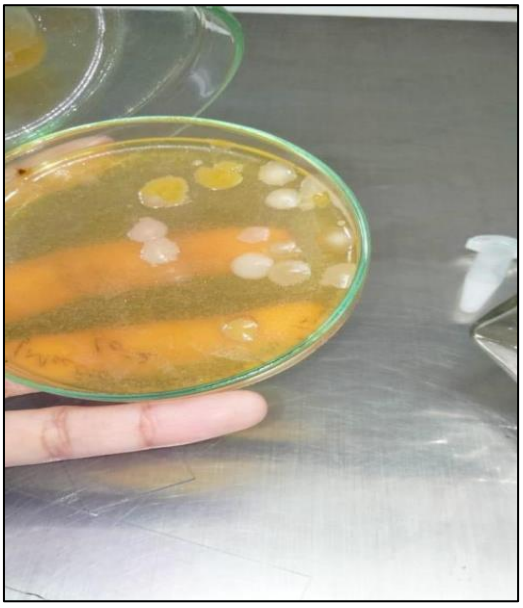

Fig 4 This Image Shows Organism Pigment

➢ *Discussion*

- *Serial Dilution*

Serial dilution was a fundamental technique used to isolate pigment-producing microor- ganisms from environmental samples. This method systematically reduced the microbial load in soil suspensions, allowing the growth of discrete colonies on nutrient agar. By plating aliquots from diluted samples, individual bacterial colonies became distinguish- able and were later subcultured to obtain pure cultures. This careful step was essential for identifying pigmented strains and avoiding cross-contamination, ensuring the reliability of downstream analyses.

- *Organism Pigment Production*

pigment-producing bacterial strains were successfully isolated and characterized:

✓ Chryseobacterium sp. – generated a bright yellow-orange pigment, identified as flexirubin-type based on the reversible color change upon KOH treatment.

- *Gram Staining Test*

Gram staining served as an initial diagnostic step to categorize the isolated organisms based on their cell wall structure. The following results were observed:

✓ Chryseobacterium sp. – Gram-negative rods, producing non-diffusible yellow pigment.
✓ Gram-negative rods, known for producing prodigiosin.

These observations aligned with known taxonomical characteristics, reinforcing the identity of the isolates and guiding selective media use.

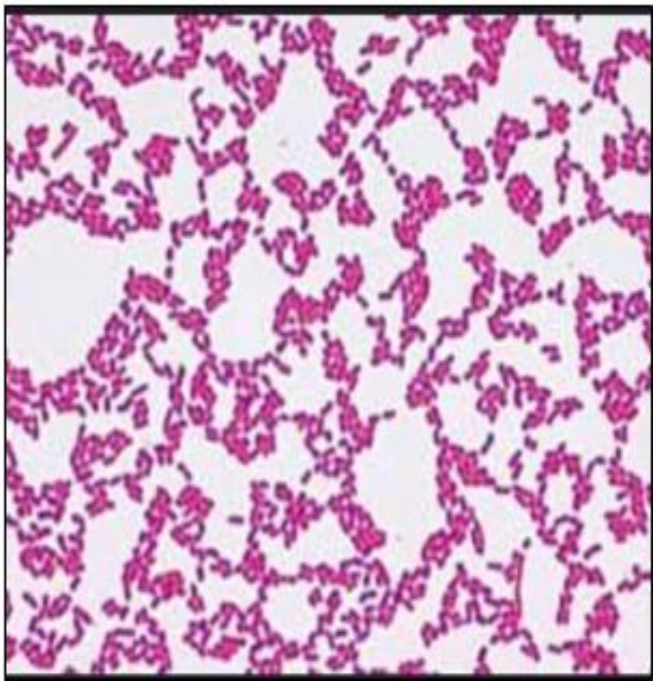

Fig 5 This Image Shows Gram staining Revealed Structure

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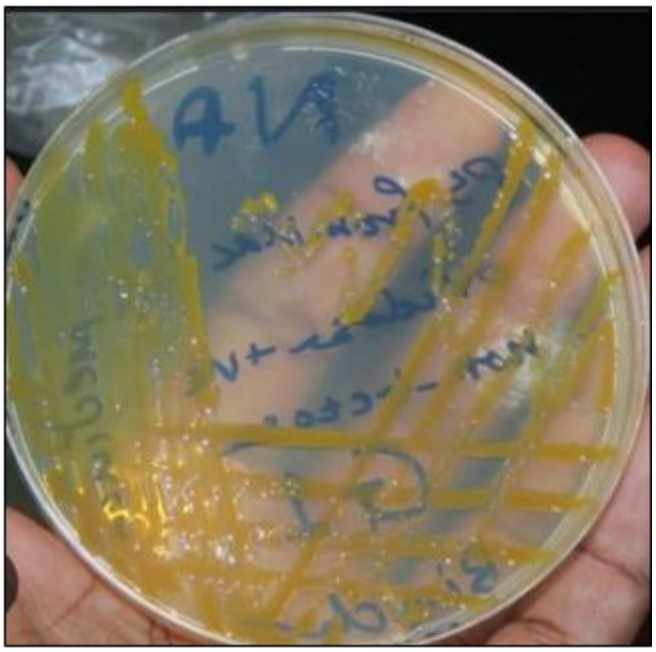

Fig 6 This image shows Organism

IV. CONCLUSION

This study illuminated the hidden world of pigment-producing microorganisms—tiny life- forms that not only thrive in diverse environments like soil and aquatic systems but also contribute significantly to sustainable biotechnological advancements. By isolating microbial strains from distinct environmental samples, including those from brackish water and oil refinery zones, the project showcased nature's remarkable palette—red, yellow, and violet pigments.

The identified bacterial strains—, *Chryseobacterium* sp., and *Chromobacterium violaceum*—produced prodigiosin, flexirubin-type pigment, and violacein respectively. These pigments are known for their vivid colors and valuable biological properties such as an- tibacterial, antiviral, antioxidant, and potential antitumoral activities.

Through meticulous techniques including serial dilution, solvent extraction, and use of low-cost nutrient media, the project emphasized eco-friendly methodologies that avoid synthetic chemicals. The pigments were successfully characterized using advanced ana- lytical techniques like UV–Vis spectroscopy, FTIR, TLC Each method provided crucial insights into their molecular structures and functionality.

Additionally, antibacterial assays confirmed the efficacy of these natural pigments against both Gram-positive and Gram-negative bacteria, underlining their potential in pharmaceutical and textile applications. Their colorfastness on silk fabric was evaluated, revealing practical uses in dyeing processes that align with sustainability.

In essence, this project demonstrated that microbial pigments are not only vibrant and bioactive but also sustainable, biodegradable alternatives to synthetic dyes. Their production from waste materials and applicability across multiple industries make them ideal candidates for future biotechnological innovation.

SUMMARY

➢ *Objective::*

To isolate and characterize pigment-producing bacteria and evaluate their potential use in industrial applications, particularly in textiles.

➢ *Sample Collection:*

Soil and water samples were collected from different environ- mental zones in Karnataka and Malaysia, including oil refineries and aquaculture research centers.

➢ *Isolation Method:*

Serial dilution and selective culturing on nutrient agar/broth were used to obtain pure bacterial colonies producing visible pigments.

➢ *Identified Microorganisms:*

- Yellow-orange pigment: Chryseobacterium sp. (flexirubin-type)
- Pigment Extraction: Solvent extraction was used with methanol or acetone followed by concentration using rotary evaporation.
- Analytical Characterization:

✓ UV–Vis Spectroscopy: Determined absorption peaks and stability un- der various pH conditions.
✓ FTIR: Identified functional groups, confirming pigment class.
✓ TLC: Verified pigment purity and type via Rf values.

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