Bioprospecting Pigmented Soil Bacteria from Varied Ecosystems in the Surat Region of Gujarat

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Abstract:

Soil microorganisms represent a vast and largely untapped reservoir of biologically active metabolites, including natural pigments. These pigments are known to play crucial roles in microbial survival and adaptation. Pigment production is often linked to microbial stress adaptation, making varied ecosystems—particularly those with anthropogenic pressures—valuable sources for bioprospecting. In this study, 30 soil samples were systematically collected from natural, agricultural, and industrial/urban sites across Surat, Gujarat, and screened for pigmented bacteria. A total of 78 distinct pigmented isolates were obtained on Nutrient Agar using the spread plate method. Phenotypic characterization revealed a predominance of Gram-positive rods, followed by Gram-negative rods and cocci. Pigments were extracted in 4% acidified ethanol and analyzed by UV–Vis spectroscopy (300–600 nm). The findings indicate that yellow and orange pigments were most frequent in natural and agricultural soils, whereas industrial soils exhibited a broader diversity, including red and pink hues. UV–Vis profiles of the extracted pigments were indicative of specific chemical classes, with absorption peaks suggesting the presence of carotenoid-like pigments (340–400 nm) and prodigiosin- or flavin-like pigments (405–475 nm), particularly among isolates from polluted sites. This study highlights the ecological potential of pigmented soil bacteria in the Surat region by providing a baseline for their diversity and characteristics. Future work should focus on molecular identification and advanced pigment profiling to further characterize these isolates.

1. Introduction:

Bacterial pigments are secondary metabolites that serve a variety of critical functions in microbial physiology, including protection from UV radiation, defense against reactive oxygen species, and adaptation to environmental stressors (Srinivas et al., 2018). These compounds are of interest as they offer a natural, biodegradable, and often non-toxic alternative to synthetic dyes. The chemical diversity of bacterial pigments, such as carotenoids, prodigiosin, violacein, and melanin, is well-documented (Venil& Lakshmanaperumalsamy, 2009; Dufossé, 2006; El-Naggar et al., 2021). Pigments from microbes are increasingly explored for their beneficial properties, including potential anticancer activity (Bharti et al., 2014 ;Solievet al., 2011; Narsing Rao et al., 2017).Soil ecosystems, in particular, are rich and complex environments teeming with diverse microbial populations. The composition of these microbial communities is heavily influenced by factors such as organic content, nutrient availability, and the presence of pollutants (Khanna & Solanki, 2017). Industrial and urban soils, subjected to high levels of stress and contamination, have been shown to host microorganisms with enhanced metabolic capabilities, including the production of novel pigments for survival (Bhosale, 2004). This makes such environments prime targets for bioprospecting. Despite India's rich biodiversity and its potential for yielding unique microbial resources, the diversity of pigment-producing soil bacteria in the region remains largely underexplored (Patel et al., 2019). This study aims to bridge this gap by systematically investigating the pigmented bacterial population from three distinct soil ecosystems in Surat, Gujarat. The research focuses on the ecological distribution of these isolates, their pigment diversity, UV–Vis spectral characteristics, and preliminary phenotype-based correlations.

2. Materials and Methods

2.1 Sample Collection

Thirty soil samples were collected from diverse sites in Surat, Gujarat. The sampling sites were categorized into three groups:

- Natural: forest edges, gardens, and canal banks, representing relatively undisturbed environments.
- Agricultural: active farmland plots.
- Industrial/Urban: sites near pharmaceutical facilities, sugar mills, petrol pumps, pathology labs, and dump yards, characterized by potential anthropogenic contamination.

Samples were collected in sterile containers, and stored at 4 °C until processed (Aneja et al., 2014).

2.2 Isolation of Pigmented Bacteria

Aliquots of the soil samples were serially tenfold diluted in sterile distilled water. The spread plate method was used to inoculate Nutrient Agar (NA) plates with 100 µL of the diluted suspension. Plates were incubated at room temperature for 24–48 h (Tajima et al., 2001). After incubation, colonies with distinct and visible pigmentation were selected and subcultured to obtain pure isolates. Each isolate was assigned a unique label (e.g., 1.1). Uninoculated NA plates were used as negative controls to ensure media sterility.

2.3 Pigment Extraction and UV–Vis Spectroscopy

To extract pigments, the isolates were cultivated in NA broth at room temperature for 48 h. The bacterial cultures were then centrifuged at 10,000 rpm for 10 minutes to pellet the cells. The resulting cell pellets were resuspended in 4% acidified ethanol to lyse the cells and extract the pigments. The extracts were further clarified by centrifugation to remove cellular debris. The supernatant, containing the extracted pigments, was analyzed using UV–Vis spectrophotometry in the range of 300–600 nm, with 4% acidified ethanol serving as a blank (Parmar & Singh, 2017; Suthar et al., 2022).

2.4 Phenotypic Characterization

The colony morphology of each isolate was characterized on NA plates, observing features such as elevation, opacity, margin, and texture. Gram staining and light microscopy were performed to determine the cell shape, size, and arrangement of each bacterial isolate (Harley & Prescott, 2011).

3. Results

3.1 Isolate Recovery and Source Distribution

A total of 78 distinct pigmented bacterial isolates were successfully obtained from the 30 soil samples. The distribution of isolates across the three types of ecosystems was as follows: 39 isolates from industrial/urban soils, 21 from natural soils, and 18 from agricultural soils. The higher number of isolates from stress-prone industrial sites suggests a correlation between environmental stress and pigment biosynthesis as a survival mechanism.

3.2 Pigment Diversity

The isolated bacteria exhibited a wide range of colors, resulting in eighteen distinct pigment categories. The most prevalent pigments were yellow **(23 isolates)** and orange **(11 isolates)**. A smaller number of isolates produced rare pigment colors, including pink, red, and light green. The highest pigment diversity was observed among isolates from industrial soils.

Table 1. Distribution of Pigment-Producing Isolates

Pigment Type	No. of Isolates	Percentage (%)
Yellow	23	29.5
Orange	11	14.1
Lemon Yellow	8	10.3
Orange Yellow	6	7.7
Light Yellow	5	6.4
Dark Yellow	3	3.8
Pale Yellow	3	3.8
Ochre Yellow	3	3.8
Pink	3	3.8
Red	3	3.8
Light Orange	2	2.6
Orangish Red	2	2.6
Others (rare)	7	9.0

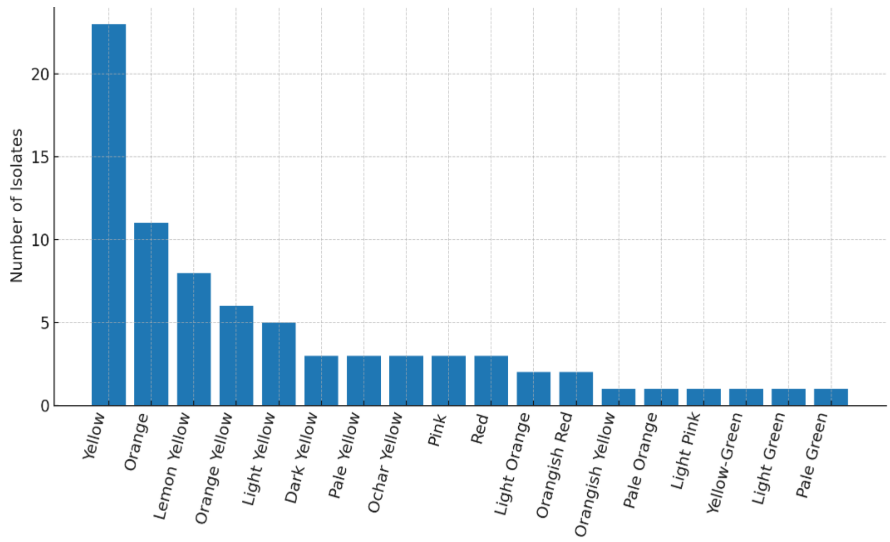

Figure 1. Pigment Diversity Among soil isolates

3.3 Gram Staining and Cell Morphology

Microscopic analysis of the 78 isolates revealed a near-even split in Gram reaction: **41 isolates were Gram-negative (52.6%)** and **37 isolates were Gram-positive (47.4%).** In terms of cell morphology, small rods were the most dominant type **(41 isolates)**, followed by small cocci **(19 isolates)**, big rods **(11 isolates)**, and coccobacilli **(7 isolates).**

Table 2. Gram Reaction Distribution of Pigment-Producing Isolates

Gram Reaction	Number of Isolates	Percentage (%)
Gram-negative	41	52.6
Gram-positive	37	47.4
Total	**78**	**100**

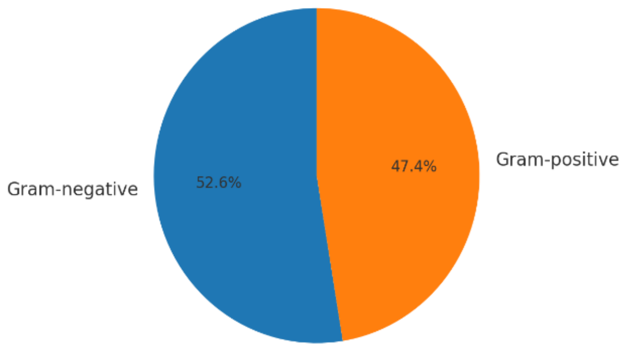

Figure 2. Gram Positive vs Gram Negative isolates

Table 3. Cell morphology Distribution of Pigment-Producing Isolates

Cell Morphology	Number of Isolates	Percentage (%)
Small rods	41	52.6
Small cocci	19	24.4
Big rods	11	14.1
Coccobacilli	7	9.0
Total	78	100

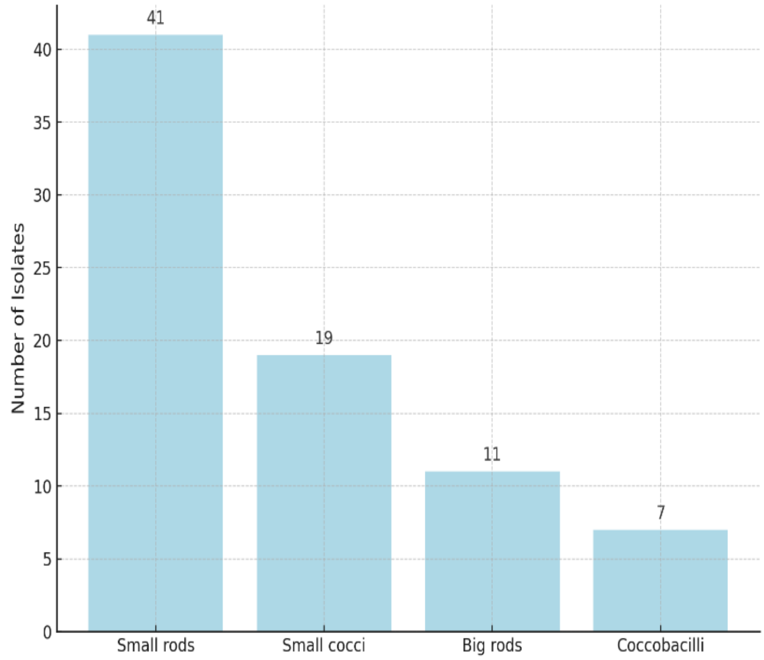

Figure 3. Morphological Distribution of Pigment Producing isolates

3.4 UV–Vis Absorbance

The UV–Vis spectrophotometric analysis of the pigment extracts revealed distinct absorption profiles. The isolates could be broadly categorized into three groups based on their peak absorbance wavelengths:

- **22 isolates** showed characteristic peaks in the 340–400 nm range, which are typically indicative of **carotenoids**.
- **26 isolates** showed peaks in the 405–475 nm range, suggesting the presence of **prodigiosin/flavin-like** pigments.
- **30 isolates** had no significant absorption peak in the tested range, suggesting that they either produced non-aromatic pigments or had a very low pigment yield.

Table 4. UV–Vis Absorbance Peaks of Pigments

Absorbance Range (nm)	No. of Isolates
340–400	22
405–475	26
None	30

3.5 Colony Morphology

Observation of colony features on Nutrient Agar plates showed consistent patterns. The majority of colonies were convex and opaque with smooth margins. Some of the isolates displayed characteristics such as halos or diffusion zones.

Table 5. Colony Morphological Features

Feature	Dominant Characteristic	Frequency (%)
Elevation	Convex/slightly convex	~85
Opacity	Opaque	~90
Margin	Entire/smooth	~80
Texture	Smooth, creamy	~75
Halo or diffusion zones	Present	10–15

4. Discussion

This study provides a comprehensive overview of the diversity of pigment-producing bacteria in soils from the Surat region of Gujarat. The higher recovery rate of pigmented isolates from industrial soils compared to natural and agricultural soils suggests a potential adaptive advantage of pigment production in stress-prone environments. This observation is consistent with a growing body of literature that links pigment production to microbial survival in the presence of pollutants and other stressors (Bhosale, 2004).

The predominance of yellow and orange pigments aligns with previous reports of carotenoid-producing soil bacteria (Fatahi-Bafghi, 2019; Choudhary et al., 2020). The detection of rare red and pink pigments, particularly among isolates from industrial sites, suggests the presence of prodigiosin-like compounds, which are often produced by bacteria in response to nutrient limitation or other environmental stresses (Williamson et al., 2006; Venilet al., 2014). The observed morphological features and Gram staining results suggest that the isolated strains may belong to common pigment-producing genera such as Bacillus, Serratia, and Micrococcus (Patil et al., 2018; Deshmukh et al., 2018).

While this study provides a valuable initial characterization, it has certain limitations. The identification of bacterial strains relied solely on phenotypic and spectral screening. Furthermore, quantitative pigment yields were not measured, which would be crucial for industrial applications.

Future research should therefore focus on:

- 16S rRNA sequencing for accurate taxonomic identification of the isolates.
- Biochemical profiling and antimicrobial assays to determine the functional properties of the pigments.
- Chemical characterization using advanced techniques such as HPLC, MS, and NMR to confirm the identity and purity of the extracted pigments.

5. Conclusion

This study demonstrates that pigment-producing bacteria are abundant in Surat soils, especially under industrial stress. The dominant pigments correspond to carotenoids and prodigiosin-like compounds, highlighting their potential as a natural resource. This work provides the first systematic survey from Surat for pigment producing microbes, forming a baseline for future molecular and applied investigations into these valuable microbial resources.

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