Original Article

Roseomonas gilardii NCTC 13290 Strain Pigment Extraction and Characterization

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Abstract

Introduction: The study was intended for Roseomonas gilardii NTCC 13290 strain pigment extraction and characterization. Methodology: The pigment-producing bacterial were cultured on Columbia blood agar and nutrient media agar. Then the pigments were extracted by ethanol. The candidate pigment was further characterized by different biotechnological techniques: UV-Vis spectroscopy, FT-IR to analyze the functional group of the targeted pigment, and TLC media. Results: The cultivation of Roseomonas gilardii on media showed pink color and nearly runny texture. The bacterial colonies were microscopically gram stained and examined, the R. gilardii was seen as coccobacillus colonies that mostly form pairs arranged as short chains. The R. gilardii bacteria that produce pink pigment was elected then further propagated for several days. The maximum spectrophotometric absorbance spectrum for the extracted pigment was observed at 500 nm, the functional groups were identified via FT-IR analysis revealed the presence of alcohol, alkenes, alkanes, phenols and carboxylic acid, in addition to iodine. The Rf value was equal to 0.80 in TLC method. Conclusions: Based on the current results, the extracted pigment from Roseomonas gilardii may serve as for food, cosmetic, and textile industries as a natural colorant from bacterial origin.

Keywords: Roseomonas gilardii, NCTC 13290 strain, Pigment, Characterization.

INTRODUCTION

Microorganisms are a source of plentiful bioactive different compounds, these are easily renewable resources, and their production gives rise a potentially greater yield1. The natural pigments of microbial origin have drawn the attention of many industries. These pigments have many beneficial characteristics: safely handled, ease of extraction, degradability, and eco-friendly products with almost no harmful effects2. Bacteria have certain distinguishing advantages over other microorganisms that yield natural pigments like yeast and fungi; for many reasons: shorter life cycle, low sensitivity to climatic changes, massive production capacity, and the diversity of pigments of various colors and shades3.

The broad application of artificial origin pigments that were utilized in the manufacturing of food coloring, fabric dyes, cosmetics, and pills have many various drawbacks and side effects4. These synthetic additives have many consequences like oxidation resulting in cell damage, which lead to immunosuppression and a bad prognosis even into carcinogenesis5. For the afford mentioned reasons, bacterial pigments have a potential raw materials that brings to the sanctuary of novel biotechnological implementation that can be mass-produced time-shortly, and safely-uses focused at enormous industrial sectors, from nourishment and beverages production to the new medications generation as well as biomedical remedies6.

The vast majority of the bacterial pigments are still under research and development stage, the bacterial pigments production needs to be intensified to make them obtainable on the shelves7 [5]; however, bacteria produce a broad range of pigments like but only prodigiosin (red), melanin (Black), carotenoids (orange), violacein (violet), pyocyanin (blue), and zeaxanthin (yellow)8, 9. The study aimed at the extraction and characterization of Roseomonas gilardii strain (NCTC 13290) pigment.

MATERIALS AND METHODS

Bacterial characterization

The Roseomonas gilardii NCTC 13290 strain (National Collection of Type Cultures, UK) were cultured on Nutrient, Columbia Blood, and Brain Heart Infusion media (HiMedia, India). Cultivated plates were incubated overnight at 37°C. The colonies

were identified by colony morphology characteristics, followed by performing the Gram's techniques. The single well-defined colonies were then transferred to Brain Heart Infusion broth (20% glycerol) were added for storage and preservation at -20°C.

Pigment Extraction from Roseomonas gilardii

The pigment extraction from the reference bacteria and the partial purification of bacterial pigment using an organic solvent and Millipore filtration as the previously mentioned method were performed10. R. gilardii cultivated plates were monitoring for pink pigmentation production. Pigment intensity was enhanced by further storing the plates for 10-14 days at 4˚C. Then the bacterial suspension was harvested, and centrifugation (Boeco, Germany) at 6000 rpm for 10 min was applied. The discarded supernatants and suspension in the absolute ethanol of the pellets were following. The suspension re-centrifuged step was repeating until the colorless pellets were collected. Then Millipore 0.22 µm (Sartorius membrane, Germany) filter for the suspension filtration was following. Then alcohol evaporation was at 25 ˚C. Finally, the dried pigment stored for further uses10.

Bacterial Pink Pigment Characterization

1. Ultraviolet Visible Spectroscopy (UV-Vis)Analysis

The Ultraviolet spectroscopy (Bush and Lamb, England) to measure the maximum spectra analysis of the obtained bacterial pigment was performed. The measured wavelength ranging (350 - 750 nm) to reach the maximum absorption spectra versus absolute ethanol as blank was applied11.

2. Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

The FT-IR spectroscopy (Shimadzu, Germany) to analyzes the functional group of the extracted bacterial pigments using the ranges of (4000–400 cm−1)12.

3. Thin Layer Chromatography (TLC) Method

Partial purification of the pigment using TLC with silica gel G-60 (Merck, India) method was performed13. The pigment solution was loaded with 20µl with the interval of 1 cm, then was allowed to dry at 25 ˚C. The plates positioned in a pre-saturated chromatography chamber containing a mobile phase of (chloroform: methanol) with a ratio of (9:1; v/v). The TLC sheet was carefully removed after 45 min under UV light. The Retention factor (Rf) value was calculated using the following formula13.

"Rf value = Distance travelled by the solute/ Distance travelled by the solvent".

RESULTS

Morphological bacterial Characterization

On blood agar, the well-isolated colony revealed the following characteristics: large size, slightly pink color, mucoid, round but may appear like teardrop-shaped (Figure 1). Gram's staining technique showed gram negative, coccobacilli bacteria, mostly in pairs, and formed a short chains (Figure 2).

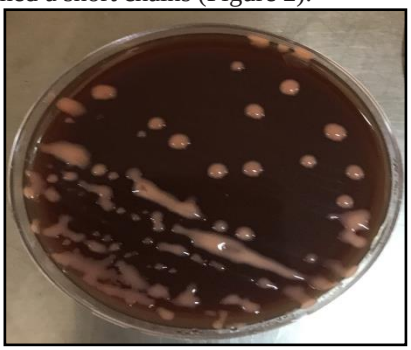

Figure 1: *Roseomonas gilardii* colony morphology on blood media

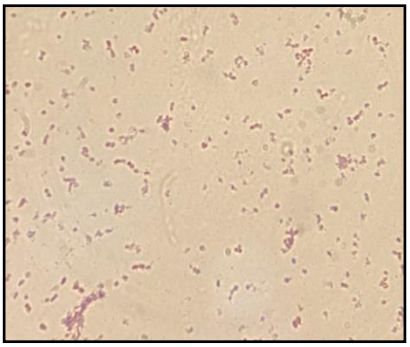

Figure 2: Microscopic conformation of *R. gilardii* (100x).

Bacterial Pigment Extraction

The bacterial pigment was extracted from pigment-producing bacterial isolate after 12 days of culturing and maintaining, the pigment yield was (5.20 g / 32.16 g) of bacterial pallets and the pigment is pink in color. As well as this study improved that, ethanol as a good organic solvent for extraction and partial purification of intracellular pigment (Figure 3).

Figure 3: Partial purified extraction of pink pigment from R. gilardii

Pigment Characterization

UV–Vis Spectroscopy Analysis

Visible wavelength ranges of (350 - 750 nm) were analyzed via spectrophotometric technique. The maximum absorbance (λmax) has observed for partially purified pigment at 500 nm (Figure 4).

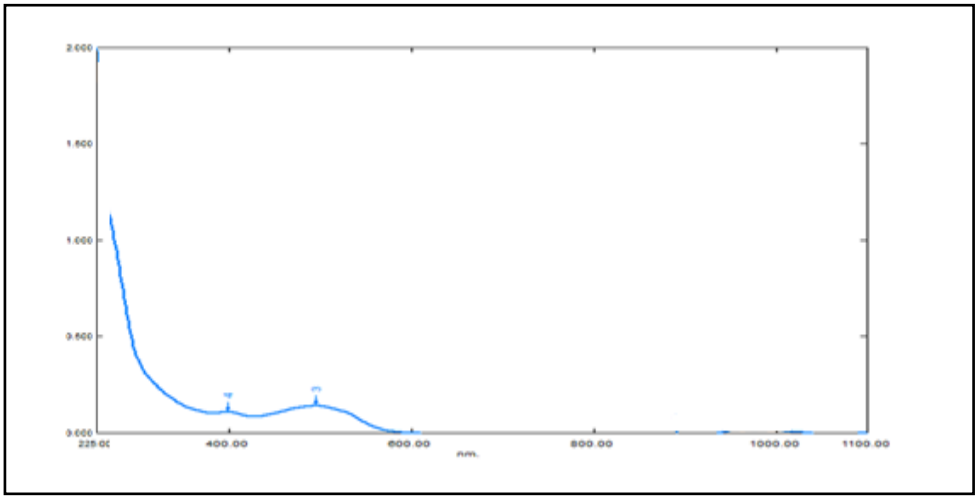

Figure 4: Crude extracted pigment via Ultraviolet spectra absorption

Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

The pigment analysis using FT-IR spectra revealed the presence of the alkynes phenols, alcohol, alkanes, alkenes, and iodine as the functional group that give the pigment its pink color (Figure 5).

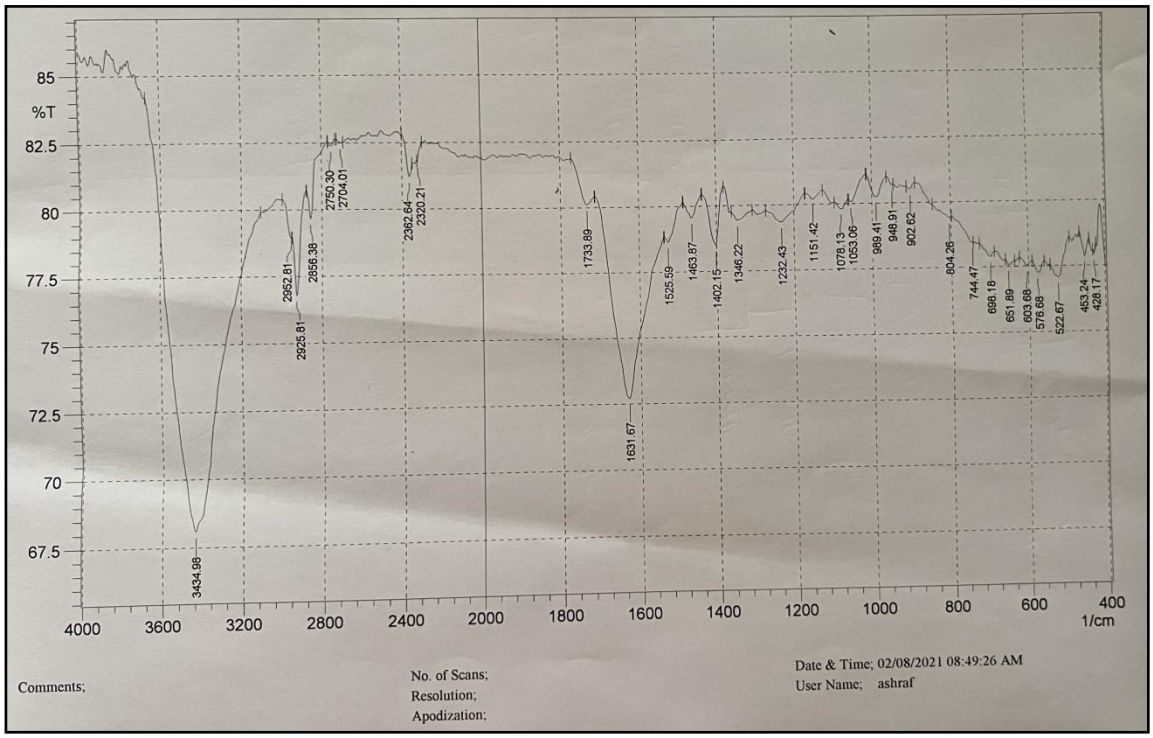

Figure 5: Fourier transforms infrared characterization of pigment.

Thin Layer Chromatography (TLC) Analysis

Through TLC silica-coated plate, the partially purified pigment uses a solvent system of chloroform: methanol (9:1) for the pigment separation. The pigment solution on the TLC plate revealed one component in the mixture which migrate as a brown component in the sheet. According to RF, the value was 0.80 (Figure 6).

Figure 6: Pigment analysis via TLC technique.

DISCUSSION

In 1993 "Gerald L. Gilardi for his many contributions to bacteriology and, specifically, for his contributions in the area of glucose non-fermenting gram-negative rod" first proposed newly Roseomonas species. Roseomonas gilardii is a pink pigment producer, non-fermenter, aerobically coccobacillus gram-negative bacterium, which is causing many infections in humans including bacteremia14. R. gilardii have two subspecies including gilardii and rosea15. The Extraction of novel pigments from candidate bacteria through biotechnological approaches leads to innovations that will advance the industry of pigment production. The Pigments of bacterial origin need improvement in terms of discovering low-cost, eligible nutrient mediums on behalf of the increment of industrial production capacity6. Regarding pink pigment extraction from R. gilardii, this result is compatible with Vora et al. (2014), who found that the intracellular pigment was extracted via different techniques and that ethanol alcohol is good and effective extracted solvent among other organic solvents13.

The pigment characterization which includes the UV-Vis spectrophotometric analysis, this investigation results of λmax for the partially purified pigment is at 500 nm, which is in almost in agreement with a recent study by Siddharthanin et al., (2020) in which the extracted pigment was pink in color from Roseomonas gilardii YP1 strain collected from a soil sample of coffee plantation area in Yercaud, Tamil Nadu and that was the pigment producing was noted at maximum spectrum of 450 nm in UV-visible spectrometry16; Meanwhile, this result was incompatible with Albadri and Alaubydi (2021) findings in that, the pink pigment extracted from Roseomonas mucosa had a maximum spectrum of 595 nm in UV- visible spectrometry17. Indeed, the prodigiosin red pigment from Serratia marcescens showed the strongest absorbance in the UV region12.

The FT-IR analysis results of current investigation is almost similar with Siddharthan et al. (2020) findings, who showed that the extracted pigment from R. gilardii had includes: alcohol, phenols, carboxylic acids, alkenes, alkanes, and primary amines

as the functional groups16. The TLC analysis in current study is RF=0.80, this result is agreed with Siddharthanin et al., (2020)16 findings in which the RF=082 and disagree with a study by Albadri and Alaubydi (2021)17, which showed that extracted pink pigment from Roseomonas mucosa with RF value equal to 0.87.

CONCLUSIONS

Based on the current results, the extracted pigment from Roseomonas gilardii NCTC 13290 strain may serve as for food, cosmetic, and textile industries as a natural colorant from bacterial origin.

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