JOURNAL OF MICROBIOLOGY AND INFECTION
Jan-Jun 2026, VOL. 4, ISSUE 1, pp.10-13
https://doi.org/10.61577/jmi.2026.100002

ORIGINAL ARTICLE

Characterization of pigment from gram-positive and gram-negative bacteria as alternatives to synthetic dyes

Mahnoor Fatima, Saira Bano and Sayyada Ghufrana Nadeem

Department of Microbiology, Jinnah University for Women, Karachi, Pakistan

ABSTRACT

The use of synthetic dyes is used to revolutionize the activities in food industries as well as the textile industries and pharmaceutical industries. The production of synthetic dyes requires harsh chemicals in addition to energy consuming processes that further increase their adverse effects to the environment. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the bacteria strains that were used in this study because they produce pigments. The isolates were determined by using the biochemical tests and purified their pigments using the appropriate solvents. When using methanol, a pale golden pigment was obtained when using *S. aureus* and a blue-green pigment when using *P. aeruginosa* and a chloroform and hydrochloric acid and sodium hydroxide sequence of treatment followed by Thin Layer Chromatography (TLC) purity analysis was done. This natural pigment has potential, according to the laboratory results, as an alternative to the traditional synthetic dyes. The attractive characteristics of these pigments are that they have bright colors as also they can be found in the natural sources and that they can be degraded naturally making them applicable in many applications. Pigment durability in the face of environmental conditions is dependent on economic reliance on scalability tests and pigment performance of lightfastness tests. This proves that bacterial pigments are potentially viable alternatives to dyes as they can be used successfully in extracting P. aeruginosa in conjunction with S. aureus. Potentially, these promising research findings, further steps are required to translate them into commercial applications.

KEY WORDS

Bacterial pigments; Pseudomonas aeruginosa; Staphylococcus aureus; Thin Layer Chromatography (TLC); Synthetic dyes

ARTICLE HISTORY

Received 15 January 2026;
Revised 05 February 2026;
Accepted 11 February 2026

Introduction

Although synthetic colors are more affordable and consistent compared to natural ones, their possible disadvantages, such as reduced color intensity and stability and reported allergenic and carcinogenic effects and the world is turning to natural colorants as a substitute of synthetic ones because of health issues [1]. Natural pigments traditionally have been used on a wide scale and they tend to make the products more marketable since they are inherently bio-functional such as antibacterial, antioxidant, anticancer and antiviral. Nevertheless, their extensive use is dampened by the toxicity issues [2]. The fact that pigment is produced aids bacteria to sustain life in their habitat in a variety of industrial opportunities. The carotenoids and melanin are the two major pigment groups produced by bacteria although there are two less popular groups: phycobilin and flavonoids [3]. The microorganisms will be able to grow in simple and low-cost growing solutions which will enhance their economic sustainability. According to Numan et al., bacteria synthesize pigment in processes that are simple and involve simple culture and extraction methods in addition to being highly scalable and growing rapidly [4]. Current studies shift their focus to the idea of bacterial carotenoid production as the plant-derived carotenoids keep the manufacturing cost very high [5]. There are a few bacterial pigments that exhibit significant antibacterial effects that offer effective therapy to multidrug-resistant bacteria and chronic infections [6]. Synthesis of the microorganisms generates several pigmentation products. Carotenoids, flavins, prodigiosins and violaceins along with zeaxanthins are some of the known microbial pigments [7]. Production of pigments by the microorganisms not only have advantages in terms of stability but also biodiversity protection and removal of the risks of pollution by synthetic dyes during the production process (Figure 1).

Figure 1. Pigmented microorganisms grown on nutrient agar showing diverse natural pigment production by different microbial colonies.

Microorganisms contain pigment compounds that preserve UV radiation damage and regulate oxidative stress besides

***Correspondence:** Dr. Saira Bano, Department of Microbiology, Jinnah University for Women, Karachi, Pakistan, e-mail: sairab2020@gmail.com

enabling survival in the heat and dry environments and genetic control mechanisms and iron absorption and photosynthesis [8]. Bacterial pigments are available in different forms that are of practical uses as discussed below. Prodigiosin is the tripyrrole pigment that is produced by Serratia marcescens, which is red, but is notable in its antibacterial effect especially against Gram-positive bacteria like MRSA and S. *aureus* [9]. Pyocyanin is a blue-green phenazine enzyme synthesized by P. *aeruginosa* which possesses high antibacterial and antifungal effects. It can prevent the development of biofilm on medical devices and thereby decrease nosocomial infections [10]. Also, pyocyanin is antioxidant and was investigated in terms of its application as a green textile dye [11].

Bacterial pigments e.g. carotenoids, melanin, violacein are being increasingly used as food colorants and additives [12]. A large number of bacterial pigments have excellent antioxidant properties, and hence can serve as useful functional food ingredients. Mangrove rhizosphere bacteria pigments have been shown to contain high levels of polyphenols and act as antioxidants. 5-Deinococcus radiodurans pigments such as astaxanthin, myxol, and saproxanthin have been shown to protect against UV damage by marine bacteria [13]. The antimicrobial property and coloration of bacterial pigments are especially useful in textiles. In order to bring further industrial use of bacterial pigments, the studies ought to be directed at discovering economic growth media and streamlining the production processes, in metabolic engineering and synthetic biology provide opportunities to maximize the yield of pigments [14,15].

In a 2024 study concerning Deinococcus bacteria, it was found that their carotenoid pigments that are unique to them and especially, play an important role in the membrane stability of the bacteria under extreme conditions, such as high radiation and low temperatures [16]. Their microbial diversity has attracted clinical isolates, which are adapted to the complex human environment, with the demonstration of the possibility to synthesize pigments. P. *aeruginosa* expresses pyocyanin, a water-soluble blue-green pigment of phenazine color, the characteristic absorption spectrum of which is pH-sensitive. Recent research revealed that pyocyanin has a high antimicrobial effect on clinically relevant microorganisms that include S. *aureus*, *Salmonella typhi* and Candida albicans [6].

Methodology

The isolates were collected from clinical laboratories in Karachi. P. *aeruginosa* and S. *aureus* isolates were cultured on Nutrient agar and Cetrimide agar at 37°C for 24 hrs. Further, microscopic studies were done to determine gram reaction, shape and arrangement of the cells and motility [17]. Biochemical tests were performed including oxidase test identified the existence of the catalase enzyme that is usually found in aerobes and facultative anaerobes for P. *aeruginosa* [18]. S. *aureus* is identified through the coagulase test whereby the coagulase enzyme responsible is detected, causing the coagulation of the plasma. A positive result is formed when a clot is formed and negative is formed when there is no clotting. IMViC was done for the gram negatives [19]. In addition, nitrate reduction test identifies and citrate utilization test [20,21]. Finally, fermentation test of carbohydrates was conducted [22]. These biochemical tests are important in identification of bacteria as species are differentiated according to their metabolic characteristics.

Extraction and purification of pigment

Bacterial pigments were extracted by growing the bacterial strains in nutrient broth at a temperature of 37°C for 96 hrs. The broth was centrifuged at 4000 rpm and made enriched with pigment (20min). The following pellet was disposed of and the supernatant was then filtered with a Millipore filter assembly. Chloroform was added to the filtrate in the same volume in order to extract the pyocyanin. One minute later vortex mixing of the mixture was done. It was followed by another Millipore filing yielding the pyocyanin layer (blue color) [23]. Pigment production was done by incubating the chosen strains of S. *aureus* in Tryptic soy broth 37°C for 48 hours. The broth containing pigment was then centrifuged at 4,000 rpm, 20 minutes after incubation. The cell suspension was resettled in distilled water and put under strong vortex mixing. Centrifugation was repeated at 4,000 rpm in 20minutes. Methanol (5 ml) was added to the cell pellet, after washing. The suspension was vortexed and incubated in a 55°C water bath during 5 minutes and centrifuged a second time at 4,000 rpm during 15 minutes. The pigmented supernatant was taken. Pigment extracts were obtained, put in aluminum foil, and stored at -20°C for subsequent use [16]. Pigment acidification was done by the addition of the same quantity of 0.2 N HCl, and the excess pyocyanin was purified by 30 seconds of vigorous vortexing. This was neutralized by the dropwise addition of 0.2 N NaOH. The molecular solution of the pigment was filtered once again with a Millipore filter assembly that was physically covered with aluminum foil to block light and then stored in -20°C [23].

Thin-Layer Chromatography (TLC)

TLC served as the technique for testing the purity levels of pyocyanin solution samples. The preparation started by mixing 3.5 g silica gel with 15 ml distilled water until they formed a stable homogenous slurry in a clean beaker (Figure 2). A stroke of pre-cleaned glass slides received the slurry mixture after which it was gently tapped to create a uniform coating. The activated TLC plates underwent heating at 110°C in the oven for a duration of 30 minutes. A combination of 1:1 (v/v) methanol to chloroform mixture was used as the developing solvent system. Commencing from the baselines of the TLC plates a micropipette applied tiny drops of the pyocyanin solution several times. The developed TLC plates needed removal before air-drying took place. The Retention factor (Rf) calculation for the pigment spots happened through use of the following formula:

R_f = distance traveled by the pigment/distance traveled by the solvent

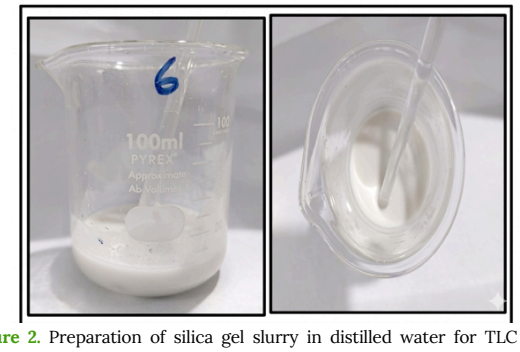

Figure 2. Preparation of silica gel slurry in distilled water for TLC plate preparation.

https://doi.org/10.61577/jmi.2026.100002

Results

On nutrient agar observed smooth, translucent, large low convex colonies with irregular edges. In addition, the isolates produced diffusible blue-green pigment on Nutrient agar and Cetrimide agar at 37°C and on Mannitol salt agar, circular shaped with a smooth, shiny surface and distinct golden-yellow colonies appeared. By performing gram staining observed gram-negative, rod-shaped, scattered cells and gram-positive, cocci in clusters under a 100x oil immersion lens. *P. aeruginosa* isolates demonstrated a positive oxidase reaction. When a single colony was rubbed to an oxidase reagent-impregnated filter paper, a rapid color change to deep purple was observed within 10 seconds. Whereas, *S. aureus* isolates demonstrated a positive oxidase reaction as no color change was observed. A positive reaction is noticed when the *S. aureus* cells are agglutinated by the addition of plasma. The degradation of tryptophan by *P. aeruginosa* was not observed due to the lack of pink or red color on the addition of the reagent of Kovac's after incubation in tryptone broth. The culture of *P. aeruginosa* that was inoculated with MR broth turned yellow, which is a negative result in the Methyl Red test. This finding indicates *P. aeruginosa* negative to glucose fermentation. After the specie confirmation the pigment was extracted following the acid/base extraction was filtered and the measurements were recorded accordingly P1 (1.5 ml), P2 (4.5 ml), P3 (3.5 ml) and P4 (3.5 ml), and placed under the aluminum foil and then stored at -20°C. All pyocyanin samples displayed a single distinct blue-green spot. Retention factors (R_f) were calculated for each spot. The calculated R_f values for all four samples were identical i.e. 0.8. This value matched the standard R_f of pyocyanin, indicating the successful isolation of pure pyocyanin from each isolate. Staphyloxanthin pigment was successfully extracted and purified from all three *S. aureus* samples using methanol and the quantity was measured for each sample respectively: S1 (1.5 ml), S2 (4.5 ml) and S3 (3.5 ml), wrapped in aluminum foil and subsequently stored at -20°C.

Discussion

Our laboratory work resulted in obtaining and purifying pigments from both *P. aeruginosa* and *S. aureus* bacterial strains by using the isolation, culture, and purification methods [24]. Pyocyanin production through commercial synthesis requires chemical reagents including phenazine methosulfate, nitrogen, chloroform, hexane, methanol and hydrochloric acid Tris-HCL and sodium hydroxide according to Cheluvappa et al. [25]. This manufacturing process through chemicals takes up too much time and money to produce. To overcome this issue, in the present investigation the pyocyanin was naturally produced from the *P. aeruginosa* strains within four days. In our study, seven clinical isolates of *P. aeruginosa* were tested, four isolates were pyocyanin producers while three isolates did not produce pyocyanin. Our findings agreed with El-Fouly et al., reported maximum pyocyanin production from *P. aeruginosa* after the fourth day [26]. Although natural pigment synthesis takes time, pyocyanin can be extracted quickly using a microbiological source and simple basic materials. Our results for pyocyanin pigment extraction correlate with Feghali and Nawas in 2018 for pigment extraction (Figure 3). Similarly, TLC results correlate with the previous studies of Murat Ozdal, analysis of purified pyocyanin showed one single spot with R_f of 0.8. According to Feghali and Nawas, the established R_f of standard pyocyanin pigment is normally between 0.70 and 0.81 [23].

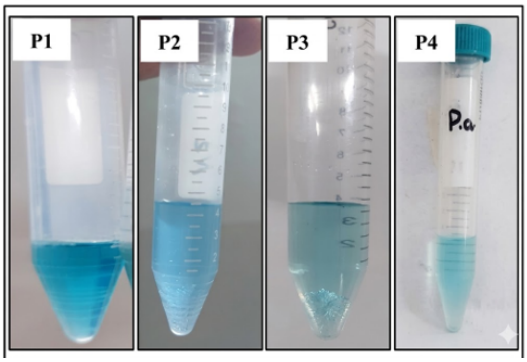

Figure 3. Isolation and purification of pyocyanin pigment from P. aeruginosa using the acid/base extraction method.

Furthermore, our results agreed with Clauditz et al., and Kwieciński, that the optimum incubation time for staphyloxanthin pigment production was 72 hrs., as the pigment is a secondary metabolite that requires time to produce [27,28]. The extracted staphyloxanthin displayed a very light golden color, however, the cell pellet was a dark golden color. Although the reasons for the very light golden color of the extracted pigment remain to be elucidated, our finding demonstrated that staphyloxanthin might be degraded with the addition of methanol or heating in a water bath. Moreover, TLC analysis for staphyloxanthin was not carried out as *S. aureus* only produces a single pigment i.e. staphyloxanthin. However, *P. aeruginosa* produces pigments like pyocyanin (blue), pyoveridin (yellow-green), pyorubin (red) and pyomelanin (brown). Therefore, by comparing the migration distances of the spots with those of known *P. aeruginosa* pigments we can confirm the identify their specific types [29]. The successful extraction and purification of bacterial pigments hold promising implications for various fields. These pigments possess diverse properties with potential applications in food coloring, textile dyeing, cosmetics, and pharmaceuticals. Our study, while showcasing the feasibility of pigment extraction and purification from clinical isolates, has limitations.

Conclusion

We concluded that bacterial pigments represent important natural compounds with significant potential for industrial and biomedical development because of their diverse biological and technological properties. In the present study, pyocyanin and staphyloxanthin pigments were successfully isolated, extracted, and purified from *P. aeruginosa* and *S. aureus* strains using simple microbiological and purification techniques. The findings demonstrated that natural pigment production can be achieved within a shorter period and with lower cost compared to chemical synthesis methods. The study also confirmed the effectiveness of TLC in identifying purified pyocyanin pigment. These bacterial pigments may have promising applications in food coloring, textile dyeing, cosmetics, pharmaceuticals, and biotechnology. Further exploration of bacterial secondary metabolites and pigment-producing strains may contribute to sustainable pigment production, eco-friendly industrial processes, innovative biotechnological applications, and the discovery of novel therapeutic agents for future biomedical research.

https://doi.org/10.61577/jmi.2026.100002

Disclosure Statement

No potential conflict of interest was reported by the author.

References

1. Heer K, Sharma S. Microbial pigments as a natural color: a review. Int J Pharm Sci Res. 2017;8(5):1913-1922.
2. Aman Mohammadi M, Ahangari H, Mousazadeh S, Hosseini SM, Dufossé L. Microbial pigments as an alternative to synthetic dyes and food additives: a brief review of recent studies. Bioprocess Biosyst Eng. 2022;45(1):1-12. https://doi.org/10.1007/s00449-021-02621-8
3. Huang X, Gan L, He Z, Jiang G, He T. Bacterial pigments as a promising alternative to synthetic colorants: from fundamentals to applications. J Microbiol Biotechnol. 2024;34(11):2153. https://doi.org/10.4014/jmb.2404.04018
4. Numan M, Bashir S, Mumtaz R, Tayyab S, Rehman NU, Khan AL, et al. Therapeutic applications of bacterial pigments: a review of current status and future opportunities. 3 Biotech. 2018;8(4):207. https://doi.org/10.1007/s13205-018-1227-x
5. Gong M, Bassi A. Carotenoids from microalgae: A review of recent developments. Biotechnol Adv. 2016;34(8):1396-1412. https://doi.org/10.1016/j.biotechadv.2016.10.005
6. Rai D, Sulthana F, Divyashree M. Production and Antimicrobial Activities of Pyocyanin Extracts from Pseudomonas aeruginosa. J Pure Appl Microbiol. 2024;18(4):2371-2379. https://doi.org/10.22207/JPAM.18.4.10
7. Sutthiwong N, Fouillaud M, Valla A, Caro Y, Dufossé L. Bacteria belonging to the extremely versatile genus Arthrobacter as novel source of natural pigments with extended hue range. Food Res Int. 2014;65:156-162. https://doi.org/10.1016/j.foodres.2014.06.024
8. Sajjad W, Din G, Rafiq M, Iqbal A, Khan S, Zada S, et al. Pigment production by cold-adapted bacteria and fungi: colorful tale of cryosphere with wide range applications. Extremophiles. 2020;24(4):447-473. https://doi.org/10.1007/s00792-020-01180-2
9. Yip CH, Mahalingam S, Wan KL, Nathan S. Prodigiosin inhibits bacterial growth and virulence factors as a potential physiological response to interspecies competition. PLoS One. 2021;16(6):e0253445. https://doi.org/10.1371/journal.pone.0253445
10. Shouman H, Said HS, Kenawy HI, Hassan R. Molecular and biological characterization of pyocyanin from clinical and environmental Pseudomonas aeruginosa. Microb Cell Fact. 2023;22(1):166. https://doi.org/10.1186/s12934-023-02169-0
11. DeBritto S, Gajbar TD, Satapute P, Sundaram L, Lakshmikantha RY, Jogaiah S, et al. Isolation and characterization of nutrient dependent pyocyanin from Pseudomonas aeruginosa and its dye and agrochemical properties. Sci Rep. 2020;10(1):1542. https://doi.org/10.1038/s41598-020-58335-6
12. Dufossé L. Microbial pigments from bacteria, yeasts, fungi, and microalgae for the food and feed industries. In: Grumezescu AM, Holban AM, editors. Natural and artificial flavoring agents and food dyes. London: Academic Press; 2018. p. 113-132. https://doi.org/10.1016/B978-0-12-811518-3.00004-1
13. Kamble GR, Sv HI, Mb HI. Evaluation of cytotoxic activity of ethyl acetate extract of pigment from Pseudomonas aeruginosa. Asian J Pharm Clin Res. 2020;13:136-140. http://dx.doi.org/10.22159/ajpcr.2020.v13i6.37217
14. Ahmad WA, Ahmad WY, Zakaria ZA, Yusof NZ. Application of bacterial pigments as colorant. In: Rai M, editor. Application of bacterial pigments as colorant: The Malaysian perspective. Berlin, Heidelberg: Springer Berlin Heidelberg; 2011. p. 57-74. https://doi.org/10.1007/978-3-642-24520-6_4
15. Venil CK, Dufossé L, Renuka Devi P. Bacterial pigments: sustainable compounds with market potential for pharma and food industry. Front Sustain Food Syst. 2020;4:100. https://doi.org/10.3389/fsufs.2020.00100
16. Wang Y, Liu J, Yi Y, Zhu L, Liu M, Zhang Z, et al. Insights into the synthesis, engineering, and functions of microbial pigments in Deinococcus bacteria. Front Microbiol. 2024;15:1447785. https://doi.org/10.3389/fmicb.2024.1447785
17. Smith AC, Hussey MA. Gram stain protocols. American society for microbiology. 2005;1(14):113-144.
18. Shields P, Cathcart L. Oxidase test protocol. American Society for Microbiology. 2010:1-9.
19. MacWilliams MP. Indole test protocol. American Society for Microbiology. 2012:1-3.
20. Buxton R. Nitrate and nitrite reduction test protocols. American Society for Microbiology. 2011:1-20.
21. Jawetz E, Melnick JL, Adelberg EA, Brooks GF, Butel JS, Ornston LN. Med Microbiol. 18th ed. San Mateo (CA): Appleton & Lange; 1989.
22. Reiner K. Carbohydrate fermentation protocol. Energy. 2012;11:12.
23. Abou Raji El Feghali P. NawasT. Extraction and purification of pyocyanin: a simpler and more reliable method. MOJ Toxicol. 2018;4(6):417-422.
24. Narsing Rao MP, Xiao M, Li WJ. Fungal and bacterial pigments: secondary metabolites with wide applications. Front Microbiol. 2017;8:1113. https://doi.org/10.3389/fmicb.2017.01113
25. Cheluvappa R, Shimmon R, Dawson M, Hilmer S, Le Couteur D. Reactions of Pseudomonas aeruginosa pyocyanin with reduced glutathione. Acta Biochim Pol. 2008;55(3):571-580.
26. El-Fouly MZ, Sharaf AM, Shahin AA, El-Bialy HA, Omara AM. Biosynthesis of pyocyanin pigment by Pseudomonas aeruginosa. J Radiat Res Appl Sci. 2015;8(1):36-48. https://doi.org/10.1016/j.jrras.2014.10.007
27. Clauditz A, Resch A, Wieland KP, Peschel A, Götz F. Staphyloxanthin plays a role in the fitness of Staphylococcus aureus and its ability to cope with oxidative stress. Infect Immun. 2006;74(8):4950-4953. https://doi.org/10.1128/iai.00204-06
28. Kwieciński J, Eick S, Wójcik K. Effects of tea tree (Melaleuca alternifolia) oil on Staphylococcus aureus in biofilms and stationary growth phase. Int J Antimicrob Agents. 2009;33(4):343-347. https://doi.org/10.1016/j.ijantimicag.2008.08.028
29. Brenner DJ, Krieg NR, Staley JT, Garrity GM, Boone DR, Vos P, et al. Bergey's manual of systematic bacteriology. 2nd ed. New York (USA): Springer-Verlag; 2005. https://doi.org/10.1007/0-387-28022-7

https://doi.org/10.61577/jmi.2026.100002