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Microbial Pigment Isolation, Culturing, and Extraction to Use as Textile Dyes

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Abstract

The textile industry is one of the largest worldwide polluters of clean water due to the heavy use of synthetic dyes. These chemicals negatively affect the environment, especially aquatic life due to their toxic and mutagenic properties. Synthetic dyes cause harm to human health such as skin allergies and respiratory sensitization. Several advantages such as ease of extraction, availability, high yields and no seasonal variation make microbial pigments the most ideal source of natural pigments. This study was done to isolate colour pigment producing bacteria and fungi from soil collected from organic farms from various locations in Sri Lanka. Out of 7 soil samples, 3 yielded pigment producing bacteria and fungi. In total, 9 pigment producing bacteria and 3 pigment producing fungi were isolated. Gause's synthetic agar yielded the most pigmented isolates. Isolates were inoculated in broths and pigment production was observed. Extracellular pigments produced by 5 of the bacterial isolates were extracted by a water-based method. The antibacterial activity of the pigments in their crude and concentrated forms was tested using the well diffusion method against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538P. Inhibition zone against *S. aureus* was observed for both crude (12.33±0.58mm) and concentrated pigments (9.67±0.58mm) extracted from purple pigment producing bacterial isolate (BPU). This pigment has the potential to be used in antibacterial textile preparation. Extracted pigments were used to dye scoured cotton fabric with the use of 3% alum as mordant. Pigment from BPU isolate resulted in better coloured fabric.

Keywords: Extracellular pigments, Microbial pigments, Natural pigments, Textile industry.

Introduction

Following their discovery in the 19th century, synthetic dyes have been used widely in many industries (Venil et al., 2021). However, the incorrect disposal of synthetic dyes into the environment has been found to cause significant harm to aquatic life and human health. Synthetic dyes belonging to the widely used azo compounds are linked to skin cancers, skin rashes, skin scaling and bleeding and respiratory sensitization (Akilandeswari & Pradeep, 2017). Synthetic dyes contain sulfur, phosphorus, nitrogen and heavy metal ions which raise carcinogenic levels when released into the ecosystem. In an effort to counteract the adverse effects of synthetic dyes, attention is being given to replacing them with natural dyes, as humans have used natural dyes for thousands of years before synthetic dyes were invented (Anahas et al., 2022).

Natural dyes have the benefit of being biodegradable, non-hazardous, sustainable, non-allergenic and they produce little to no toxic waste compared to synthetic dyes (Celestino et al., 2013; Usman, et al., 2018). Natural dyes can be sourced from plants, animals, minerals or microorganisms. Of these, microorganisms are a step ahead due to their numerous benefits. Microorganisms are more readily available, stable, less costly, less labour intensive, have higher yield, are capable of downstream processing and have a higher growth rate than that of plants and animals (Kazi et al., 2022). It is important to study microbial pigments as they are the most suitable source for natural pigments that can meet the growing demand for natural dyes. Economically this is beneficial since the dye industry is expected to increase annually by 7% (Anahas et al., 2022). Some of the major sources of microbial pigments are Actinomycetes, bacteria and fungi.

Previous studies have successfully extracted microbial pigments with antimicrobial properties and used them as textile dyes. A violet pigment was extracted from *Chromobacterium violaceum* isolated from the Great Salt Lake in Chennai, India. The extracted dye showed antimicrobial properties against *S. aureus, Bacillus subtilis, E. coli, Pseudomonas aeruginosa*, and *Candida albicans* and was able to dye cotton and silk fabrics (Anahas et al., 2022). *Micrococcus luteus* yielded a pigment which was found to have antibacterial properties against *S.aureus* and *E.coli* with inhibition zones of 19 mm and 20 mm respectively when extracted with methanol. The pigment was used to dye polyester fabric using alum as a pre-mordant (Priyanka & Jayakumari, 2020). In another study, soil samples yielded yellow and orange pigment producing bacteria. Both pigments were used to dye cotton fabric after mordanting with alum (Ki & Thirumalai, 2021).

The widespread application of microbial pigments on a large scale is hindered by several limitations such as costly investment, the inherent instability of the pigments, limited variation in shades, and difficulties in extracting pure and concentrated forms of the pigments (Goswami & Bhowal, 2014). This study aims to offer more variety of microbial colours extracted from an inexpensive source which can be extracted efficiently.

Materials and Methods

Isolation of pigment producing bacteria and fungi

Soil from organic farms located in 7 locations, Galaha, Hambantota, Mahiyanganaya, Welimada, Rathnapura, Nuwara Eliya and Yakkala were collected, sieved and weighed. The soil sample (10g) was added to 90 ml sterile distilled water and shaken. The soil solution was diluted according to the ten-fold dilution method (Pepper & Gerba, 2015). Diluted soil samples were cultured on various media to encourage growth of pigment producing bacteria and fungi. Subculturing was done on selected pigmented colonies to obtain pure cultures. A purple fungi (FPU) growing in NBRIP media was inoculated into Gause's synthetic agar to obtain a pure culture.

Growth of extracellular pigment producing bacterial isolates

Each isolate was inoculated into the respective broth Gause's synthetic broth, (50mL) and Czapek Dox broth (50mL) and placed in a shaking incubator at 28 °C at 125 rpm until the desired colour was observed.

Extraction of pigments

Extracellular pigment producing isolates were inoculated into Gause's synthetic broth. The pigment was extracted as described by Dhawane & Zodpe (2017). The broth was transferred into a centrifuge tube and centrifuged at 6,000 rpm for 10 minutes in a benchtop centrifuge (500 - 6000 rpm, max rcf 3800G). The supernatant was collected, and the pellet was discarded. Heating at a temperature of 50°C until the initial volume decreased by half was done to concentrate the pigments.

Antibacterial effect of Selected isolates

The crude (0.1g) and concentrated (0.1g) pigments were subsequently tested for antibacterial properties against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538P using the well diffusion method (Balouiri et al.,2016). The results were observed after incubating at 36°C for 24 hours. Statistical significance was analysed using IBM SPSS Statistics software.

Textile yyeing

Cotton fabric measuring 4 cm by 5 cm were scoured in a solution of 0.5 g/L sodium carbonate and 2 g/L non-ionic detergent for 90 minutes at 90-95°C (Poorniammal et al., 2013). The material-to-liquor ratio (MLR) was 1:50 and the fabrics were air dried. Pre-mordanting and dyeing steps were performed as described by Metwally et al., 2021. Mordanting was performed with a 3% alum solution and with a MLR of 1:20. This process was carried out at 60°C for 20 minutes while stirring. Dyeing was performed using a MLR of 1:40 at 80°C for 60 minutes while stirring.

Results and Discussion

Isolation of pigment producing bacteria and fungi

Bacteria and Fungi capable of producing colour pigments were isolated from soil samples collected from Welimada, Hambantota and Galaha (Table 1).

Table 1.

Isolates of colour pigment producing bacteria and fungi including location of the soil and the soil dilution from which the isolate grew in culture, the type of culture media used for each isolate, the observed colour and the assigned code. Culture media used were Gause's synthetic agar (GSA), Czapek Dox agar (CZA) and National Botanical Research Institute's phosphate growth medium (NBRIP).

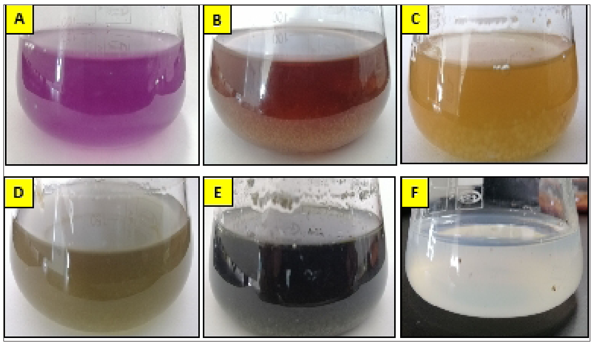
Microorganism	Soil Location	Dilution	Culture Media	Colours Isolated	Isolate Code
Bacteria	Hambantota	10^{-1}	GSA	Purple	BPU
				Brown	BBR
				Yellow	BYL
				Green	BG1
				Blue	BBL
		10^{-3}	GSA	Red	BR1
	Welimada	10^{-2}	GSA	Dark Green	BG2
	Galaha	10^{-2}	GSA	Pink	BPI
Fungi	Welimada	10^{0}	NBRIP	Purple	FPU
	Galaha	10^{-3}	GSA	Yellow	FYL
		10^{-4}	CZA	Red	FRE

Pigment production in liquid medium

Bacterial isolates BPU, BBR, BYL, BG1 and BG2 were found to produce extracellular pigments. Visual observance of extracellular pigment producing bacterial cultures is shown in Figure 1.

Figure 1.

Extracellular Pigment Production in select bacterial isolates A) BPU, (B) BBR, (C) BYL, (D) BG1, (E) BG2 (F) and Control media with no observed pigment production.



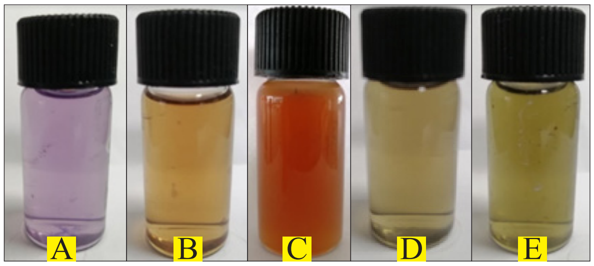
Bacterial strains BPU, BBR, BG1, BG2 have taken more than 5 days to produce pigments. Microbial pigments are mostly secondary metabolites produced during the end of the exponential growth phase in bacteria and are more likely to be produced when substrates are limited (Seyedsayamdost, 2019). Therefore, extracellular pigment production has taken, on average, 7 days.

Extraction of pigments

Different solvent systems are commonly used to extract bacterial pigments. However, the use of these solvents can cause harm to humans and the environment. Therefore, a water-based extraction system was employed over the use of a solvent system and yielded purple, yellow, red and green colours (Figure 2).

Figure 2.

Extracted pigments from bacterial isolates (a) BPU (b) BBR (c) BYL (d) BG1 and (e) BG2.



Antibacterial effect of selected isolates

Crude and concentrated pigment extracted from BPU showed inhibition zones against *S.aureus* when tested with the well diffusion method (Figure 3). Results in Table 2 indicate that the mean inhibition zone between crude pigment and concentrated pigment shows statistical significance ($p < 0.05$). Also, there is a statistical significance of crude and concentrated pigments when compared with negative control ($p < 0.05$). It should be noted that the negative control (distilled water) gave no inhibition zone (inhibition zone = 0.00 mm) in all trials of the antibacterial assay.

Figure 3.

Results of well diffusion method of diluted purple pigment against S.aureus: A - Plate 1, B - Plate 2 and concentrated purple pigment against S.aureus: C - Plate 1, D - Plate 2 (S – pigment sample, PC – positive control, NC – negative control).

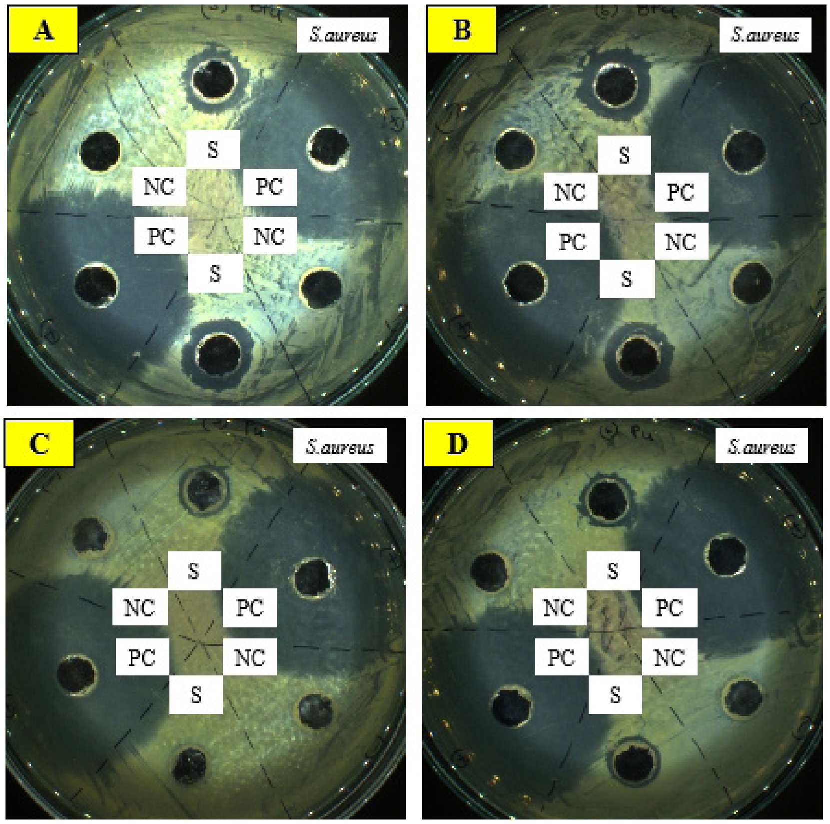


Table 2.

Statistical significance of the antibacterial property of crude purple pigment and concentrated purple pigment extracted from BPU isolate tested against S.aureus ATCC 6538P.

Test sample	Amount of test sample	Mean zone of inhibition ± SD (mm)	*P value of the t statistic*
Crude pigment	0.1g	12.33 ± 0.58	*0.04*
Concentrated pigment	0.1g	9.67 ± 0.58	
Crude pigment	0.1g	12.33 ± 0.58	*0.00*
Distilled water (negative control)	0.1g	*0.00 ± 0.00*	
Concentrated pigment	0.1g	9.67 ± 0.58	*0.00*
Distilled water	0.1g	*0.00 ± 0.00*	
Ampicillin (positive control)	0.1g	*42.67 ± 0.58*	

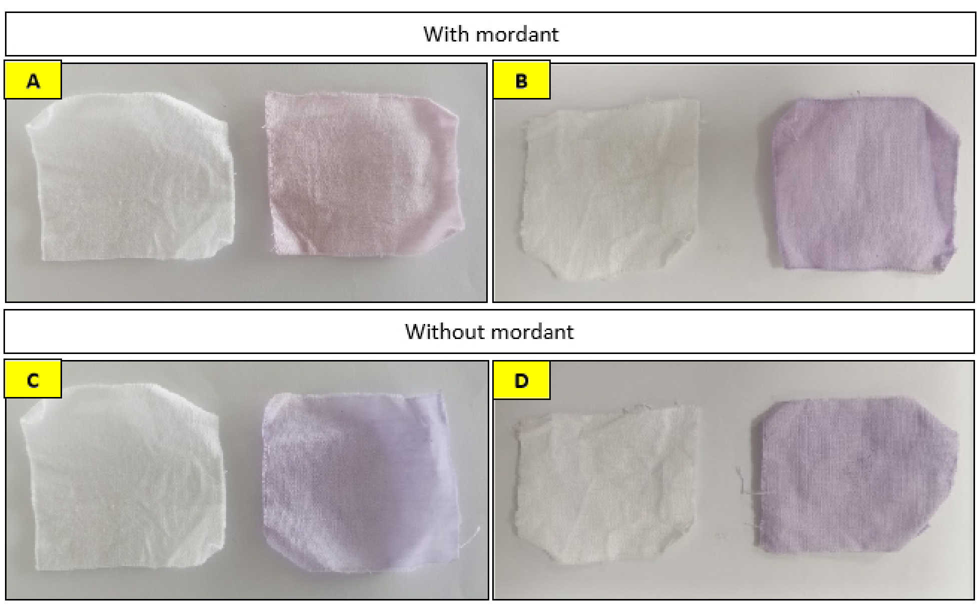
S.aureus is a gram-positive bacteria and is one of the most frequent bacterial infections in humans (Taylor & Unakal, 2023). From the results it is possible to identify the bacterial pigment as a promising source for antibacterial fabrics if the antibacterial property could be transferred to the fabric.

Violacein is a purple-coloured bacterial pigment. It is known to inhibit growth of gram-positive bacteria such as *S.aureus* but does not inhibit growth of gram-negative bacteria such as *E.coli* (Durán et al., 2012). This is similar to the results obtained in this experiment with the purple pigment.

Textile dyeing

Figure 5.

Dyeing of scoured cotton fabric with purple pigment extracted from BPU. First Dyeing: & (B) with Mordant, Second Dyeing: (C) & (D) without Mordant.



Scouring is a process in which the surface of a fiber is treated to make it more hydrophilic, this is advantageous when dyeing of the fiber is necessary (Kiron, 2021). In this experiment alum was utilized as a mordant. Alum, known as aluminium potassium sulphate ($AlK(SO_4)_2$) is an example of a widely used mordant (Haar et al., 2013). During the dyeing process, the dye forms bonds with the mordant, facilitating its attachment to the fabric. The aluminium metal ions in alum form a coordinate complex with natural dyes (Mozaffari, 2018). Purple pigment was successful in imparting a good colour to the cotton fabric (Figure 5). The first dyeing with mordant yielded a paler colour compared to the first dyeing without mordant. Following the second dyeing, the fabric which underwent mordanting yielded a slightly stronger purple colour compared to the fabric without mordant. Pigments from isolates BBR, BYL, BG1, BG2 did not impart a satisfactory colour to the cotton fabric.

Conclusions

A total of 9 pigment producing bacteria and 3 pigment producing fungi were isolated from soil collected from organic farms located in Hambantota, Welimada and Galaha. Of these, 5 of the bacteria produced extracellular pigments. Soil from organic farms in Sri Lanka represents a promising and cost-effective source for isolating pigment-producing microorganism. The extracellular colour pigments extracted using a water-based method to minimize the usage of harmful solvents and were used to dye cotton fabric. The extracellular purple pigment extracted from the bacteria (BPU) exhibited antibacterial property against *S. aureus* ATCC 6538P. This property of the dye can be explored in further studies to produce fabrics with antibacterial properties.

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