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Living Colours: Development of Microbial Culture Collection for Use as Microbial Colour Pigments in 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. Synthetic dyes are harmful to aquatic life and to human health. To overcome this, natural dyes are being explored as a healthier and more eco-friendly alternative. 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. In total, 9 pigment producing bacteria and 3 pigment producing fungi were isolated. Gause's synthetic agar yielded the most pigmented isolates. 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 *E.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.

1. INTRODUCTION

The textile industry is responsible for 20% of all global water pollution making it the second largest polluter of clean water (Jyoti, et al., 2016). This is due to the heavy use of synthetic dyes, found in industry effluent, which are hazardous to the environment and to humans (Lara, et al., 2022, Kramar & Kostic, 2022). Since 10-15% dyes remain unfixed to the fabric during the dyeing stage and is more likely to enter the environment, a less harmful substitute should be studied (Sudarshan et al., 2022). Natural dyes are an eco-friendly alternative to synthetic dyes. They are biodegradable, non-hazardous, sustainable, non-allergenic and produce minimum toxic waste (Celestino, et al., 2013; Usman, et al., 2017). Microorganisms such as actinomycetes, bacteria and fungi, are a rich source of natural pigments (Kazi, et al., 2022). Production of natural dyes using microbial pigments is cost-effective and simple due to their high availability and easy extraction of pigments, resulting in higher yields of the pigment compared to other sources (Kazi et al., 2022).

2. MATERIALS AND METHODS:

2.1. 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. An amount to 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 get pure culture.

2.2. 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 desired colour was observed.

2.3. EXTRACTION OF PIGMENTS

Extracellular pigment producing isolates were inoculated into Gause's synthetic broth. The pigment was extracted as described by Dhawane & Zodpe, 2017. Heating at a temperature of 50°C until the initial volume decreased by half was done to concentrate the pigments

2.4. Antibacterial Effect of Selected Isolates

The crude (0.1g) and concentrated (0.1g) pigments were then 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.

2.5. Textile Dyeing

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.

3. RESULTS AND DISCUSSION

3.1. ISOLATION OF PIGMENT PRODUCING BACTERIA & FUNGI

Colour pigment producing bacteria and pigment producing fungi were isolated from soil samples collected from Welimada, Hambantota and Galaha (Table 1)

Table 1: Isolated colour pigment producing bacteria and fungi

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^{-1}	GSA	Dark Green	BG2
	Galaha	10^{-1}	GSA	Pink	BPI
Fungi	Welimada	10^{-0}	NBRIP	Purple	FPU
	Galaha	10^{-3}	GSA	Yellow	FYL
		10^{-4}	CZ	Red	FRE

3.2. 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 are shown in figure 1.

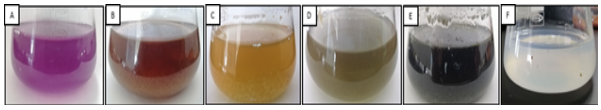

Figure 1: Extracellular pigment production by pigment producing bacteria (A) BPU, (B) BBR, (C) BYL, (D) BG1, (E) BG2 (F) Control

Bacterial strains BPU, BBR, BG1, BG2 took 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.

3.3. 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.

3.4. ANTIBACTERIAL EFFECT OF SELECTED ISOLATES

Crude and concentrated pigment extracted from BPU showed inhibition zones against only *S.aureus* when tested with the well diffusion method (Figure 3). Mean inhibition zone between crude pigment and concentrated pigment shows no statistical significance (P=0.05). There is a statistical significance of crude and concentrated pigments when compared with negative control (P<0.05) (Table 2).

Table 2: Inhibition zone of crude and concentrated purple pigment isolated from BPU isolate tested against *Staphylococcus aureus* ATCC 6538P.

Test sample	Amount of test sample	Mean zone of inhibition±SD (mm)	P
Crude pigment	0.1g	12.33±0.58	0.05 (NS)
Concentrated pigment	0.1g	9.67±0.58	
Crude pigment	0.1g	12.33±0.58	0.00 (S)
Distilled water	0.1g	0.00±0.00	
Concentrated	0.1g	9.67±0.58	0.00 (S)
Distilled water	0.1g	0.00±0.00	

P value – Statistically significant if P<0.05. Statistical analysis: Independent samples t-test. SD: standard deviation. NS – Not significant. S: Significant. Negative control: Distilled water, Positive control: Ampicillin (Mean zone of inhibition = 42.67±0.58)

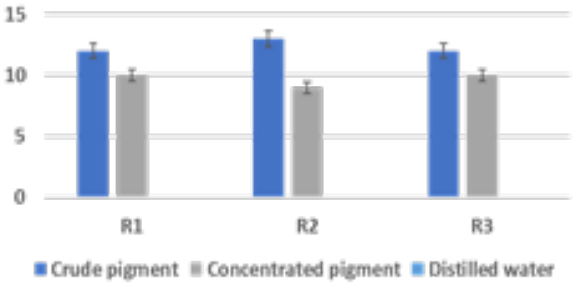

Figure 3: Results of well diffusion method for purple pigment extracted from BPU, against *S.aureus* in both crude and concentrated forms including error bars (y-axis: mean zone of inhibition (mm), x-axis: repeat no.)

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., 2011). This is similar to the results obtained in this experiment with the purple pigment. Therefore, it is likely that the purple pigment is violacein.

3.5. TEXTILE DYEING

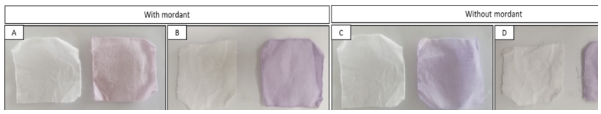

Figure 4: Scoured cotton fabric dyed with purple pigment extracted from BPU. 1st dyeing: (A) & (B) with mordant, 2nd dyeing: (C) & (D) without mordant

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). Purple pigment was successful in imparting a good colour to the cotton fabric (Figure 4). Pigments from isolates BBR, BYL, BG1, BG2 did not impart a satisfactory colour to the cotton fabric.

4. CONCLUSION

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. The extracellular colour pigments extracted using a water-based method were used to dye cotton fabric. The extracellular purple pigment extracted from the bacteria (BPU) exhibited antibacterial property against *S.aureus* ATCC 6538P.

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