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Turning Stress Into Color: Enhancing Pyocyanin Yield via Solvent-Induced Responses in *Pseudomonas aeruginosa*

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INTRODUCTION & AIM

Pseudomonas aeruginosa is an aerobic Gram-negative bacterium. Although it can be abundantly found in different environments, such as soil and plants (Tuon et al., 2022), it's often extracted from environments with high human activity (Krell & Matilla, 2024). This opportunistic pathogen is a major cause of nosocomial infections, including ventilator-associated pneumonia, surgical site infections, and urinary tract infections (Tuon et al., 2022; Wieland et al., 2018). A key factor contributing to its virulence and antibiotic resistance is its ability to form biofilm structured microbial communities encased in an extracellular polymeric matrix (Thi et al., 2020). Among the many virulence factors contributing to its persistence, pyocyanin—a phenazine-derived, redox-active pigment—plays a crucial role in biofilm development, oxidative stress modulation, and interspecies competition (Jabłońska et al., 2023). Secondary metabolite production can be triggered in stress situations such as nutrient deficiency and environmental factor alterations like temperature, pH and moisture (Tyc et al., 2017).

This pigment is produced by 90–95% of *Pseudomonas aeruginosa* strains and stands out due to its diverse biological properties and industrial potential (Abdelaziz et al., 2023). Beyond its role in bacterial physiology, pyocyanin demonstrates remarkable antimicrobial properties. It effectively inhibits the growth of pathogens like *Magnaporthe grisea* and *Xanthomonas oryzae* (DeBritto et al., 2020). Industrial applications of pyocyanin and other secondary metabolites are vast, spanning bioremediation, biosensors, agriculture, and medicine (Mudaliar & Bharath Prasad, 2024). Advances in synthetic and systems biology have enabled targeted manipulation of stress-response pathways to optimize metabolite yields (Guan et al., 2017).

Researchers have shown that toluene can be used as a chemical stressor that effectively triggers pyocyanin production, by adding of 0.2% of this organic solvent a production of 33mg/ L of pyocyanin by P. *aeruginosa* was achieved (Ozdal, 2019).

The aim of this research was to study and compare the use of organic solvents as chemical triggers for pigment production in P. *aeruginosa*. Since toluene raises many environmental concerns, other solvents with a lower impact were also studied: ethanol and acetone.

METHOD

Pigment production and extraction
Seven Falcon tubes were prepared, each containing *Pseudomonas aeruginosa* (NCTC 12903) inoculated in 30 mL of Nutrient Broth, that were previously grown in Nutrient Agar. Cultures were incubated at 37 °C under rotary agitation (120 rpm) for 24 hours. After incubation, organic solvents were added to the cultures at the following concentrations:

a) Toluene: 0.1% and 0.2%
b) Acetone: 0.1% and 0.2%
c) Ethanol: 0.1% and 0.2%

One tube was maintained as a control. All samples were further incubated under the same conditions until a blue-green pigment developed in the medium.

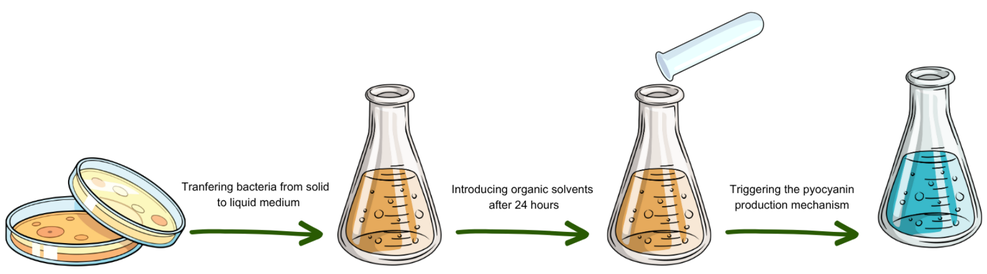

Figure 1: Pigment production scheme

Cultures were centrifuged at 4000 rpm for 10 min at 18 °C. The resulting supernatant was transferred to fresh Falcon tubes, and 2.5 mL of chloroform was added to each. After vortex mixing, a two-phase system formed: pigmented chloroform extract in the lower phase and yellow aqueous medium in the upper phase. The pigmented lower phase was collected and filtered. Chloroform was recovered by rotary evaporation for reuse in subsequent extractions.

Figure 2: Pigment extraction scheme

Textile Dyeing
The pigment obtained from the most effective process was dried and dissolved in 5 mL of ethanol, followed by dilution in 50 mL of water. Multifiber fabric samples were pre-treated with 5% (w/w) aluminum potassium sulfate for 30 minutes at 60 °C to facilitate mordanting. This process was expected to improve pigment fixation by promoting coordination between the pigment molecules and available binding sites on the fiber surface.
For the dyeing phase, two different processes were followed, one at 60°C and one at 100 °C.

RESULTS & DISCUSSION

Results
Table 1 expresses the amount of pigment that was extracted from each sample and Figure 3 shows the results obtained from each dyeing process.

Table 1: Amount of pigment obtained from each sample

Organic solvent	Concentration (%)	Pigment weight (µg)
Toluene	0.1	0
Toluene	0.2	0
Acetone	0.1	5.3
Acetone	0.2	5.9
Ethanol	0.1	7.8
Ethanol	0.2	7.4
Control	NA	5.0

Figure 3: Results obtained from dyeing a multifiber fabric composed by: a) acetate; b) cotton; c) nylon; d) polyester; e) acrylic; f) wool, at both temperatures

Discussion
Contrary to previous studies (Ozdal, 2019), the addition of toluene did not stimulate pigment production in *Pseudomonas aeruginosa* (NCTC 12903). No detectable pigment formation was observed in either the 0.1% or 0.2% toluene treatments, suggesting that this strain may respond differently to solvent-induced stress compared to others previously reported. In contrast, ethanol proved to be the most effective organic solvent for enhancing pigment synthesis. At 0.1%, ethanol induced the highest pigment yield (7.8 µg), while 0.2% ethanol produced a slightly lower concentration (7.4 µg). These results indicate that moderate ethanol exposure may provide optimal conditions for pigment production in this strain. Acetone also supported pigment formation, though to a lesser extent, with pigment yields of 5.3 µg and 5.9 µg at 0.1% and 0.2%, respectively.
The control sample, without solvent exposure, produced 5.0 µg of pigment. However, it was observed that pigment production was much slower. In this last case, the production could have been triggered by a lack of nutrients. What this means is that the present colonies consumed the available nutrients until depletion.

Distinct color variations were observed based on fiber type and dyeing temperature. Acrylic dyed at 100 °C turned pink, while all other fibers (acetate, nylon, polyester, wool) showed green hues ranging from very light to dark. Cotton displayed minimal coloration. The pink shift in acrylic likely results from thermal pigment modification or enhanced pigment–fiber interaction at high temperature, as acrylic requires ~100 °C for effective dye uptake. In contrast, the stable green tones in acetate, nylon, polyester, and wool reflect efficient dyeing between 60–100 °C. The poor dyeing of cotton suggests low pigment affinity for cellulose, even after mordanting, likely due to limited binding sites or weak aluminum complex interaction. Figure 4 sums up the fiber-pigment interactions.

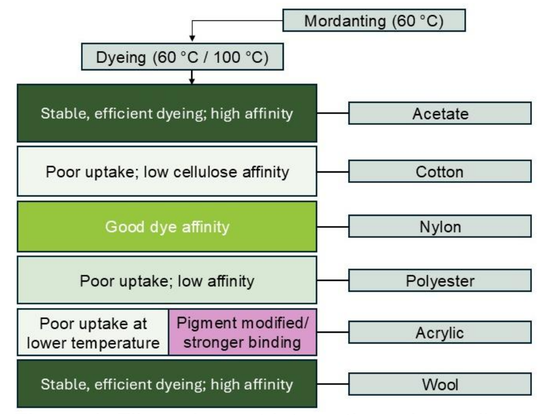

Figure 4: Fiber-pigment interactions depending on dyeing temperature

CONCLUSION

Pigment production in *Pseudomonas aeruginosa* (NCTC 12903) varied with the organic solvent used. Ethanol, especially at 0.1%, induced the highest pigment yield (7.8 µg), while acetone produced moderate amounts and toluene completely inhibited pigment formation. The extraction with chloroform was effective and solvent recovery can be a solution to turn the process more sustainable. After mordanting with aluminum potassium sulfate at 60 °C, most fibers showed green hues, with color intensity depending on temperature and fiber type. Acrylic dyed at 100 °C turned pink, suggesting temperature-induced pigment modification or enhanced binding, whereas cotton exhibited poor dye uptake. Overall, ethanol-induced pigments show promise as bio-based colorants, particularly for synthetic fibers under optimized dyeing conditions.

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