Pyocyanin test

Introduction

This protocol is used to extract, isolate, and quantitatively analyze pyocyanin production in *Pseudomonas aeruginosa.* Because pyocyanin expression directly reflects active quorum sensing coordination, this assay is widely used in research as a phenotypic reporter system to evaluate the efficacy.

Reference:
1. King, M. M., Guragain, M., Sarkisova, S. A., & Patrauchan, M. A. (2016). Pyocyanin extraction and quantitative analysis in swarming Pseudomonas aeruginosa. *Bio-protocol*, *6*(23), e2042-e2042.
2. Wu, K., Mo, Y., Duan, M., Zheng, Y., Chang, Y., Ding, M., ... & Duan, N. (2025). A structure-guided bifunctional aptamer mitigates Pseudomonas aeruginosa virulence and biofilms by disrupting las and rhl quorum sensing systems. *Analytical Chemistry*, *97*(37), 20291-20302.

Materials

- Bacterial strain
  - *Pseudomonas aeruginosa* PAO1
- Chloroform 1.8ml
- HCl
- LB broth

Procedure

Pyocyanin test

1. Centrifuge overnight culture at 6000 rpm for 10 min.
2. Resuspend pellet in 5 mL fresh LB and add drug.
3. Incubate at 37 °C for 24 h.
4. Centrifuge at 6000 rpm for 10 min; collect supernatant.
5. Mix 3 mL supernatant with 1.8 mL chloroform
6. Vortex or shake the mixture vigorously to extract pyocyanin
7. Carefully collect this lower blue phase
8. Mix the blue layer with 0.2 M HCl at 2:1

9. Vortex the mixture thoroughly
10. Carefully transfer 200ul of the top red layer into a 96-well plate
11. Use SpectraMax M3 to Read OD_{520}