Co-culture

Introduction

This protocol is used to establish a reproducible anaerobic co-culture system of Escherichia coli and Pseudomonas aeruginosa for studying bacterial interactions, cross-feeding effects, growth dynamics, and downstream metabolomic analysis. This technique allows two bacterial species to grow in the same culture environment while maintaining controlled inoculation ratios and cell densities. Growth is monitored using OD600 measurements and colony-forming unit (CFU) enumeration to ensure comparable starting biomass and accurate interpretation of microbial interactions.

References:
1. Guraka, A., Duff, R., Waldron, J., Tripathi, G., & Kermanizadeh, A. (2024). *Co-Culture of Gut Bacteria and Metabolite Extraction Using Fast Vacuum Filtration and Centrifugation*. Methods and Protocols, 7(5), 74. https://doi.org/10.3390/mps7050074
2. Fazli, M., Almblad, H., Rybtke, M., Givskov, M., Eberl, L., Tolker-Nielsen, T., & Rasmussen, T. B. (2014). Regulation of biofilm formation in *Pseudomonas aeruginosa* and its relevance to chronic infections.
3. Oliveira, N. (2015). Evolutionary competition in microbial communities: from population dynamics to single-cell behavior [PhD thesis]. University of Oxford. https://doi.org/10.1111/1462-2920.12448
4. Elias, S., &Banin, E. (2012). Multi-species biofilms: living with friendly neighbors. FEMS Microbiology Reviews, 36(5), 990–1004. https://doi.org/10.1111/j.1574-6976.2012.00325.x

Materials

- LB broth
- 1.5% LB agar plate
- 1x PBS (Phosphate Buffered Saline)
- Frozen stock or freshly streaked colony of strains
- Antibiotics
- Bacterial strain
  - *Escherichia coli* TOP10/BL21/DH5a
  - *Pseudomonas aeruginosa* PAO1

Procedure

Procedure Seed Culture Preparation and Co-Culture Establishment

1. Culture incubation temperature: **37°C**
2. Use freshly prepared or pre-reduced media.

Seed Culture Preparation and OD600 Evaluation

3. Preparation of Monocultures Pick a single colony of strains and inoculate into LB broth
4. Incubate cultures at 37°C overnight
5. Mix culture thoroughly.

OD600 Measurement

6. Blank the spectrophotometer using sterile LB broth. Measure OD600 of each culture. Record OD600 values.

CFU/mL Determination

7. Serial Dilution Prepare six dilution tubes containing: 900 µL PBS
8. transfer: 100 µL culture into Tube 1. Mix thoroughly.
9. Continue 10-fold serial dilution until 10^-6 dilution is reached.
10. Plate Counting Plate: 100 µL of selected dilutions on LB agar. Spread evenly.
11. Incubate anaerobically: 37°C 24–48 h
12. CFU Calculation Select plates containing: 30–300 colonies. Calculate CFU/mL using: CFU/mL = (Number of Colonies × Dilution Factor) ÷ Volume Plated (mL)

Cell density normalization and co-culture set-up

13. Normalize Culture Density Measure OD600 again before inoculation.
14. Adjust both strains to: OD600 = 0.50 using fresh LB broth. Use: C1V1 = C2V2
15. Incubation Incubate co-culture under 37°C
16. Collect measurements at: 0 h/4 h/8 h/12/h/24 h. Monitor: OD600; CFU/mL; pH (optional)
17. Harvest Criteria Proceed to downstream metabolite extraction when cultures reach: OD600 ≈ 0.9

    corresponding approximately to: 1 × 10^8 CFU/mL which represents mid-log phase growth and maximal metabolic activity.