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WORKS FOR ME

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Preparing_Duckweed-Bacteria_Cultures

DOI

dx.doi.org/10.17504/protocols.io.36wgqjd7kvk5/v1[Kenneth Acosta](#)¹¹Rutgers University

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COMMENTS 0

ABSTRACT

Protocol to generate duckweed-bacteria cultures to study duckweed-bacteria interactions.

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GUIDELINES

Some bacteria may grow slowly. In this case, more time is needed for bacterial cultures to grow to sufficient concentrations.

Some bacteria do not pellet easily. Longer centrifugation times and higher centrifugation speeds may be needed for these types of bacteria.

MATERIALS TEXT

Note: All reagents/materials should be sterilized.

- H₂O
- media
- 30 mL round-bottom centrifuge tubes
- 50 mL centrifuge tubes
- baby jars

Day 1

- 1 Inoculate 5 mL liquid cultures from bacterial glycerol stocks.
- 2 Grow 5 mL liquid bacterial cultures overnight at 28C at 250 rpm.

Day 2

- 3 Use 500 uL of liquid bacterial culture from Day 1 to inoculate 50 mL liquid cultures.
- 4 Grow 50 mL liquid bacterial cultures overnight at 28C at 250 rpm.

Day 3 (t = 0 days, start of experiment)

- 5 Transfer 25 mL of bacterial cultures to sterile 30 mL centrifuge tubes.
- 6 Spin at 8000 rpm for 5 min at 4C.

- 7 Decant SN.
- 8 Add 25 mL of sterile H₂O to pellets. Resuspend pellets.
- 9 Spin at 8000 rpm for 5 min at 4C.
- 10 Decant SN.
- 11 Add 25 mL of sterile H₂O to pellets. Resuspend pellets.
- 12 Spin at 8000 rpm for 5 min at 4C.
- 13 Decant SN.
- 14 Suspend in 25 mL of media.
- 15 Read OD₆₀₀. In a volume of 50 mL dilute to OD₆₀₀ = 0.2.
- 16 Transfer 50 mL cultures to baby jars.

17 Inoculate cultures with duckweed to cover the surface of the culture.