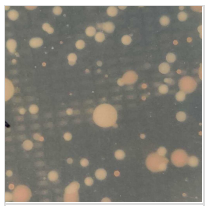

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Isolating bacteria from algal monocultures

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1 *Works for me* Share

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Symbiodiniaceae-bacterial protocols at CEE Lab


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DISCLAIMER

Recommended to work in a biosafety cabin/hood or if that's not available, around fire (Bunsen burner)

ABSTRACT

To isolate bacteria from non-axenic, algal (we did this with *Symbiodinium* but may work with other algae) monocultures. Our aim is to isolate bacterial members of the phycosphere to use in re-inoculation and genome wide association studies to better understand how microalgal-bacterial relationships influence marine ecosystems.

This protocol was conducted using a three-year, laboratory monoculture of *Symbiodinium linucheae*, isolated from the anemone *Aiptasia pallida*, as described previously (https://www.protocols.io/edit/isolating-non-axenic-monoclonal-symbiodinium-cultu-cg9stz6e).

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MATERIALS TEXT

- 0.2 µm filtered seawater (FSW)
- prepared R2A marine agar (made with 0.2 µm FSW instead of DI water) plates
- prepared R2A marine broth (made with 0.2 µm FSW instead of DI water), 2 L
- 30 mm, 0.22 µm PES sterile filters
- 3 mL sterile syringes
- 200/1000 µL sterile filter-capped pipet tips
- Vortex
- 2 mL microcentrifuge tubes
- 2mL cryovial tubes
- 15 mL Eppendorf tubes
- 50 mL Eppendorf tubes
- Parafilm
- Bunsen burner
- Loops
- 100% glycerol, autoclaved 120°C for 15 minutes
- 4.5 mm plating beads, sterilized
- GenElute Bacterial Genomic DNA Kit (NA2110, Sigma-Aldrich, USA)
- 16S rDNA primer pair 1492R/27F
- Incubator 25- 27 ºC, lights PAR ~10-50 µmol with a timer of 14:10 (light:dark)
- - 80ºC freezer

SAFETY WARNINGS

We work around fire, mostly using a Bunsen burner to maintain aseptic technique. You can work in a biosafety cabinet as well.

Working in a lab bench without fire or a biosafety fume hood is not recommended for this protocol.

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Recommended to work in a biosafety cabin/hood or if that's not available, around fire (Bunsen burner)

BEFORE STARTING

NOTE: Assure you have a non-axenic, monoclonal *Symbiodinium* (or other dinoflagellate/algal) culture that has never been subjected to thermal stress or antibiotic treatments!

The algal phycosphere may range according to available exudates and organic substances. Therefore, the bacterial community may differ between varying stages of algal growth (lag, log, stationary and death). Please keep in mind the health of your culture, along with the appropriate date of collection for your individualized experiment.

We collected at ~ stationary phase (1.5 month old culture).

Initial isolation from *Symbiodinium* 5d

1 Transfer 1 mL of a non-axenic *Symbiodinium* culture to a 2mL microcentrifuge tube. Centrifuge the algae and bacteria at 10,000 rpm for 2 minutes.

2 Discard the liquid and rinse the pellet with 1 mL of 0.2 µm FSW. Vortex. Repeat.

3 Add 2 - 3, 4.5 mm sterile glass plating beads to the microcentrifuge tube and vortex at maximum speed for 1.5 minutes. This breaks down the *Symbiodinium* cell but leaves most bacteria intact.

4 Transfer the slurry (omit the beads) onto a 3mL syringe, attached to a sterile 0.45 µm PES, 30mm filter.

5 Pass the slurry through the filter and into a new, sterile microcentrifuge tube.

6 This is your concentrated slurry (10^0) . Dilute this to 1:100, 1:1000 and 1:10000 in three different microcentrifuge tubes.

7 Aliquot 50 µL of the concentrated slurry onto R2A marine agar plates (triplicates). This will be

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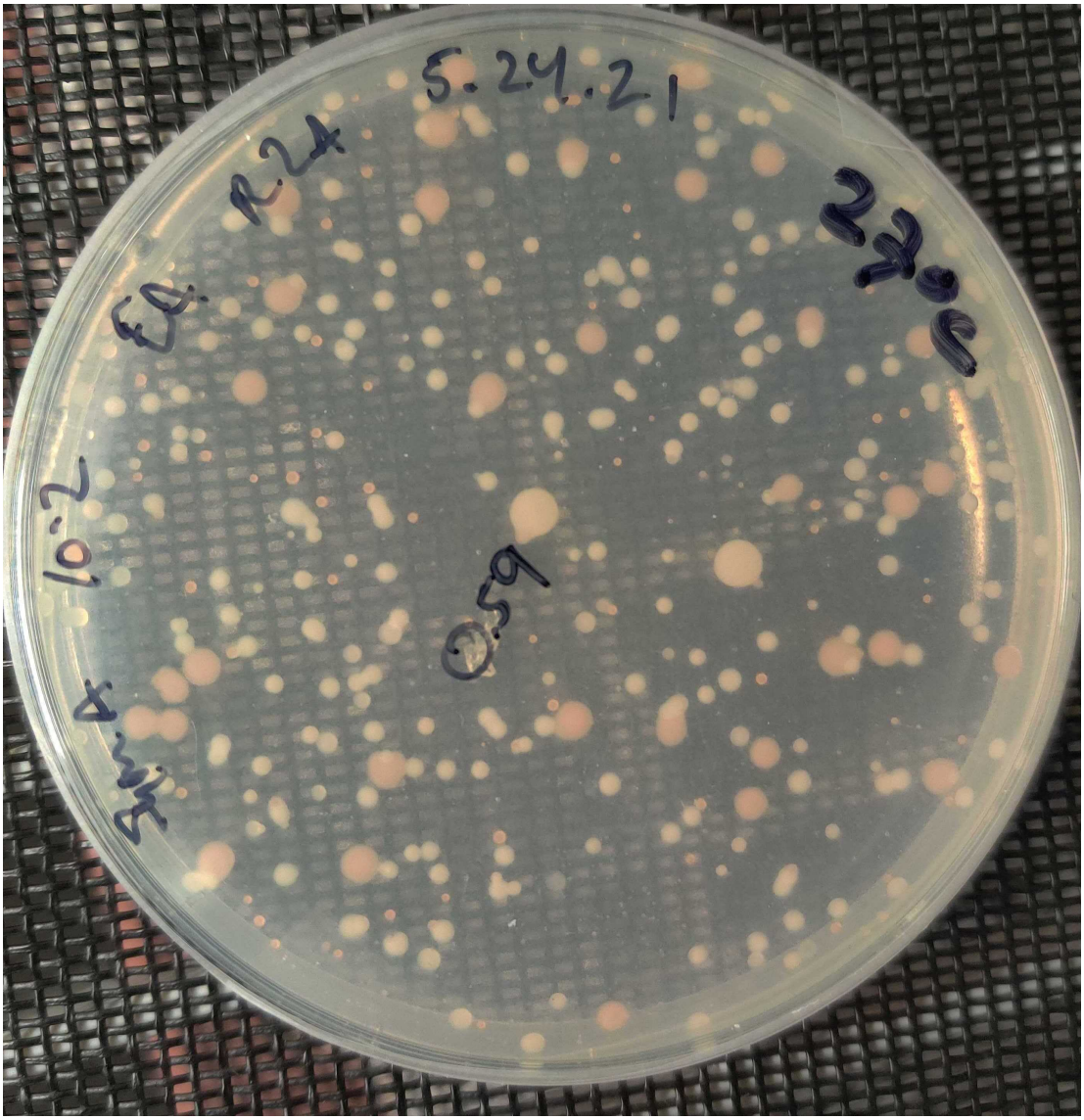
the "positive control" to compare growth, but single colonies will likely not be distinguishable (nor should you use this plate to pick single colonies).

8 Aliquot 50 µL of the 1:100, 1:1,000 and 1:10,000 dilutions onto R2A marine agar plates (each dilution in triplicates).

9 Spread the cells in the plates with 4-6, 4.5 mm sterile glass plating beads, each, by shaking gently until the liquid has been adsorbed on the agar. Seal with parafilm.

Incubate all plates at 27ºC on a 14:10, light: dark cycle (since these are the conditions they are used to from co-existing with the algae) for 5-10 days until visible colonies appear.



Various bacterial colony morphologies and colors appear on this 1:100 *Symbiodinium*-slurry diluted R2A plate, resembling the popular and beloved ice cream snack, ***Dippin' Dots***.

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*However, if you would like to attempt culturing more diverse members of the phycosphere, I'd suggest various strategies such as variable media and temperatures as high as 34°C

Isolating single colonies 5d

10 Using a sterile filter-capped pipet tip (200 µm), pick a defined single colony and streak it on a new R2A plate.

Do this for each morphologically distinct colony. Keep a record of the colony morphology, color, texture and smell (if any). Label each distinct colony with a unique sample identifier, like a number.

11 Incubate at 27°C on a 14:10, light: dark cycle until colonies are visible or up to 5 days.

12 Repeat steps 10-11 until it is visibly clear that the plate contains a bacterial monoculture. Collect as many as you'd like!

Growing bacterial monocultures in liquid media for downstream processes 2d

13 Aseptically pick a colony from a plate containing the bacterial monoculture and inoculate it into 100 µL of 0.2 µm FSW. Break up the colony gently by pipetting up and down until the liquid is turbid.

14 Pipet a 25 µL aliquot of the bacterial solution from step 13 and inoculate in 25 mL of R2A marine broth.

15 Incubate at 27°C on a 14:10, light: dark cycle for 2-5 days* or until peak density is approximately 10^7-10^9 cells/mL. Allow oxygen to pass through the cultures, and unless the vial contains a cap with a 0.22 µm filter, loosely cap the vials.

*at this point, most bacteria have acclimated to agar or high nutrient conditions like those in media, so dense, visible growth will be seen in many bacteria monocultures by the second day, although other colonies may take longer.

Glycerol stocks for cryo-preservation 30m

16 Prepare 40 mL of 40 % glycerol solution in 0.2 µm FSW.

17 Transfer 500 µL of the 40 % glycerol solution to a sterile, pre-labeled cryovial tube. For each

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bacterial monoculture, prepare triplicates (or more).

Cap and set aside.

18 Centrifuge 1.5 mL of the bacterial monoculture obtained from steps 13-15 at 13,000 rpm for 2 minutes. Discard the liquid and add 1.5 mL of fresh R2A marine broth. Mix by pipetting up and down until the media is turbid.

19 Transfer 500 µL of the bacterial suspension in a cryovial containing 500 µL of the 40 % glycerol solution. Mix by pipetting up and down, or cap securely and vortex for 3 seconds, max. The final concentration of glycerol in these cryostocks should be 20%.

Repeat in triplicates or more for each bacterial monoculture.

20 Allow the cells to acclimate to the glycerol for 10 minutes prior to storing at -80°C. No need to snap-freeze prior to storage.

Store at -80ºC, indefinitely.

DNA extractions 2h

21 Centrifuge 5 mL of the bacterial monoculture (in a 15 mL Eppendorf) obtained from steps 13-15 at 13,000 rpm for 5 minutes to obtain a pellet. Do this in triplicates.

22 Extract DNA with method of choice.

We used DNeasy Power Biofilm Kit (Qiagen, Germany) or GenElute Bacterial Genomic DNA Kit (Millipore Sigma, USA), both are good choices for this step, but if you're considering doing a genome, we recommend the latter.

16S rDNA PCR 2h

23 To identify the bacteria, we amplified the 16S rDNA region with the DNA obtained (steps 21-22) using the "full-length" primer pair 27F/1492R. PCR products were Sanger sequenced and the resulting sequences were blasted against the NCBI database.

23.1 16s rDNA primers:

- 27F: 5'-AGAGTTTGATCCTGGCTCAG-3'
- 1492R: 5'-GGTTACCTTGTTACGACTT-3'

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