

Isolation of algal endosymbionts from protists

PREAMBLE. The method outlined below is based on Achilles Day & Day (2013).

A significant number of freshwater and marine protistan taxa contain endosymbionts. This method was developed for freshwater ciliates, but is directly applicable to other algal endosymbiont containing freshwater and marine organisms. It involves micromanipulation of individual protists, rupturing to release endosymbionts followed by enrichment on complex media and a series of plating steps, under low light (PAR ~10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$).

1. Prepare in advance and label appropriately all medium and plasticware. Multi-well plates (12 well) should be pre-filled with 3.5 ml of 3 different media i.e. four wells with each medium (see Fig. 1).

Choice of medium will be dictated by the origin of protist with freshwater organisms being incubated and/or manipulated in freshwater based medium and marine taxa in marine medium. Complex media containing soil extract, vitamins or cereal infusions will be more likely to enhance growth of endosymbiotic algae than defined mineral-based media.

2. Place the culture, or environmental sample, in a small (4 cm diameter) Petri dish. Observing under an inverted microscope, transfer individual trophs using a capillary pipette to a sterile Petri dish containing non-nutrient agar.

Note : for marine taxa it is optimal to prepare the non-nutrient agar using seawater rather than deionised water.

3. Using a glass Pasteur pipette, (heated by a Bunsen-burner to make it blunt – creating a “glass-drop” at its end) destroy the protist, by pressing gently on the drop previously inoculated on to the surface of the agar.

The manipulation makes a small dint/depression on the agar when the protistan cell was destroyed, ensuring that the endosymbiont cells are not dispersed.

4. Aseptically transfer the green cells released, using a pre-sterilised micropipette to a multi-well plate. Inoculate three wells with the algae derived from each individual ciliate troph, with each well containing 3.5 ml of a different pre-sterilised medium.
5. Transfer the inoculated plates to a 20°C incubator and culture under a 12:12 h light:dark cycle at 10 -20 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ PAR. Incubate for four weeks.

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Protists: Protozoan culture, or environmental sample containing target protists.

Apparatus: a class I biological safety cabinet; photoperiod & temperature controlled illuminated incubator; inverted microscope with 250-400 x magnification equipped with phase- contrast.

Cell culture medium: Modified Provasoli, f/2, or other appropriate medium.

Plasticware: Petri dishes (4cm diameter); Multi-well plates (12 well); sterile disposable pipette tips; sterile loops.

Chemicals used routinely are of Analar grade purchased from Sigma-Aldrich, unless otherwise stated.

Additional information:

Achilles-Day UEM, & Day JG (2013)
Isolation of clonal cultures of endosymbiotic green algae from their ciliate hosts. J. Microbiol Meth. (in press)

It may be necessary to adjust incubation regimes for samples originating from more environmentally extreme niches.

- Where algae have grown (e.g. Fig. 1), aliquots (100µl) should be aseptically transferred and streaked out using a sterile loop onto agar plates using standard microbiological practice (employing the same medium solidified by the addition of 15g l⁻¹ non-nutrient Bacto-Agar). Seal plates with Parafilm and incubate as above for 7 to 28 days.

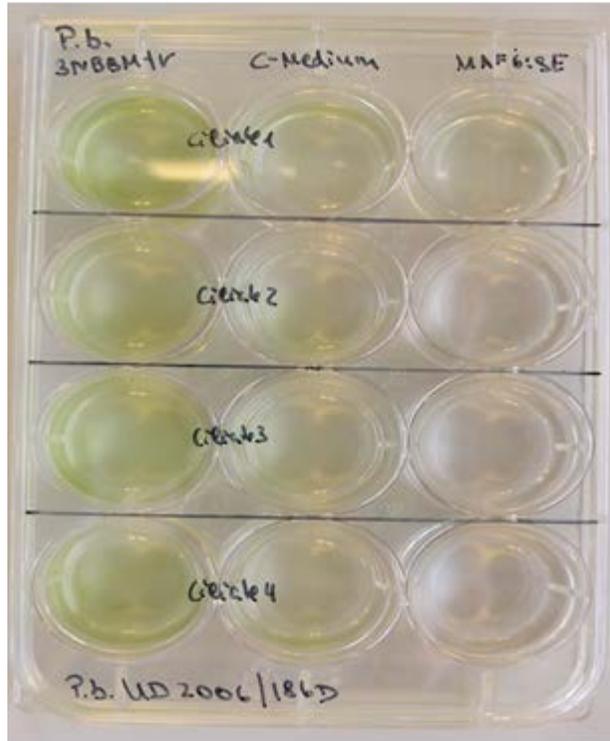


Fig. 1 Example multi-well plate for endosymbionts isolated from *Paramecium bursaria* from Lily Loch, Inverawe, Scotland (UD2006/186D)

- Aseptically remove discrete colonies and transfer, using sterile cocktail-sticks, to multi-well plates, or Petri dishes containing the appropriate medium and incubate as above.

It may be necessary to repeat steps 6 and 7 to ensure clonal cultures are obtained.

- Stock cultures of all successfully established algal endosymbiont cultures can be maintained as liquid cultures, or on agar slants under the above standard environmental conditions and should be routinely transferred by standard serial transfer every 2-3 months.