Dinoflagellate Isolation from Cnidarian

Dinoflagellate isolation from cnidarians host tissue procedure

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Preparation of cultures

1. Homogenize cnidarians host tissue in 5-10 ml of Sigma-Guillard's (F/2) medium (Sigma-Aldrich).
2. Filter the homogenate using a 100 µm nylon mesh to a final volume of 10-15 ml.
3. Add EDTA to a final concentration of 0.005M.
4. Recover alga from tissue homogenate by centrifugation for 5 min at 5000 rpm.
5. Wash algal pellets in 5 to 10 ml of fresh f/2.
6. Place in a 35 mm Petri dish for picking.
7. From this stage on all procedures will be treated as sterile and performed under a laminar flow hood.

Equipment : P-2000 micropipette puller (Sutter Instrument), laminar flow hood, water bath, gloves, safety goggles and lab coat, centrifuge (any type with a ss-34 compatible frame), dissecting microscope Carl-Zeiss Ltd.

Solutions : Sigma-Guillard's (F/2) (Sigma-Aldrich)

Plasticware : 96 wells plastic case with cover

Picking and first stage growth technique

1. Isolate single *Symbiodinium* cell from diluted homogenate using a P-2000 micropipette puller (Sutter Instrument) or following the technique suggested by Ki et al. 2005, Mar. Biotechnol. 6, 587–593, 2005.
2. Transfer individual cells into single wells of a 96 well cartridge (0.3 ml PCR plates are recommended) pre-filled with f/2 solution (add Antibiotic Antimycotic Solution (100×) (Sigma-Aldrich)).
3. Place the plate in culture room or incubator in the temperature range that best fits the host environment under a 12:12 h light dark cycle for 3-7 days.
4. Check growth and viability of the culture using a microscope (and a microscope PAM).

Large Subunit rRNA-Encoding DNA (rDNA) Sequencing

1. Isolate a single dinoflagellate cell from the selected well (as described above) and extract to 1 µl of TE buffer and freeze until analysing.
2. Add Proteinase K (1 ll 200 µg/ml), maintain tubes at 55 ^{o}C for 50 minutes with a UNOII Thermoblock (Biometra). Incubate at 95 ^{o}C for 10 minutes to inactivate the proteinase K and facilitate DNA denaturation. Cool to 4^{o}C in preparation for PCR amplification. Standard PCR amplification of nuclear ribosomal DNA was performed by using two sets of primers: (*i*) S-DINO (cgctcctaccgattgagtga) and L-DIN-1

(aacgatttgcacgtcagtaccgc), which are *Symbiodinium*-specific and cover the ITS-1/5.8S/ITS-2/partial large subunit (LSU) rDNA, and (*ii*) D1R (acccgctgaatttaagcatat) and D2C (ccttggtccgtgttt), which are dinoflagellate-specific and target a 5′ fragment of the LSU rDNA. PCR products were purified by using Shrimp Alkaline Phosphatase and Exonuclease (modified from Ki et al. 2005).

Mass culture

1. Transfer the culture from the plate to a 25 ml Erlenmeyer flask prefilled with Sigma-Guillard's (F/2) medium (Sigma-Aldrich) after one week. Incubate under the selected temperature and light conditions.
2. Transfer again the culture in log phase (check using a flow cytometer or measuring chl a) to a 100 ml Erlenmeyer flask with Sigma-Guillard's (F/2) (Sigma-Aldrich). Incubate under the selected temperature and light conditions.
3. Repeat with increasing volume until reaching the desired mass.