Growing dinoflagellates in the lab

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Here are all the instructions you will need for growing temperate-tropical dinoflagellates in a lab.

Sources

- isolation of single species from a plankton tow
- ordering from various sources:
  - CCMP
  - UTEX
  - Other Algae Collections
  - Univ of Utah (CISE)
  - Sunnyside Seafarms (for more info on this source see "Growing dinoflagellates at home")

Basic Requirements

The following is a basic protocol for growing autotrophic, tropical to temperate marine dinoflagellates. We grow our cultures in Percival incubators on 12 hour light-dark cycles set at **18 ℃** with light levels at **40-140 µmoles/m^2-sec** provided by several cool white fluorescent bulbs. The cultures are kept in various-sized, sterile Erlenmeyer flasks, ranging from 250 ml to 2.8 l, using cotton gauze as stoppers. We make **F/2 media** following the recipe from Guillard, R.R.L. and J.H. Ryther (1962), Can. J. Microbiol. 8:229-239. F/2 refers to enriched seawater that has been shown to be the ideal growth medium for phytoplankton. Our cultures are transferred in a sterile environment into new (sterile) F/2 media every 3 weeks (about 1/4 volume of an old culture is used to inoculate a new culture). Cultures are swirled everyday (except on day of transfer) to prevent clumping of cells and to keep nutrients well mixed.

F/2 Recipe

To 4 l of 0.22 µm filtered open ocean seawater (or artificial seawater) add:

2 ml 15% $NaNO_3$

2 ml 1% NaH_2PO_4

2 ml trace metals stock

2 ml vitamin stock

10 ml soil extract (re-autoclave after each use)

Fill the various sized flasks each to about 1/2 capacity, then plug them with gauze stoppers and autoclave (steam sterilize) for 20 minutes. Remove F/2 promptly from autoclave (any longer than 20 minutes will cause precipitation). Let sit for 24 hours in incubator to cool.

Tips if you have problems with precipitation (some precipitation is normal)

(1) Autoclave for only 20 minutes, with no dry cycle.
(2) Autoclave just the water, then add supplements.
(3) Silicon is not required for dinoflagellate cultures.
(4) Try filter sterilization instead?

Stock Solutions

1. Vitamin Stock Solution

VITAMINS	Concentration	to 500 ml of dist. H_2O add:
Thiamine	1 mg/ml	0.5 g
Biotin	1 µg/ml	0.5 mg
Vitamin B_{12}	1 µg/ml	0.5 mg

2. Trace Metals Stock Solution

METALS	Concentration	to 100 ml of dist. H_2O add:
FeEDTA	2.34 mM	1000.0 mg
$CuSO_4 \cdot 5H_2O$	8 µM	1.86 mg
$ZnSO_4 \cdot 7H_2O$	15.4 mM	4.4 mg
$CoCl_2 \cdot 6H_2O$	8.6 µM	2.0 mg
$MnCl_2 \cdot 4H_2O$	184 µM	36.0 mg
$Na_2MoO_4 \cdot 2H_2O$	5.2 µM	1.26 mg

3. 15% $NaNO_3$: 15 g $NaNO_3$ in 100 ml nanopure dist. H_2O

4. 1% NaH_2PO_4: 1 g NaH_2PO_4 in 100 ml nanopure dist. H_2O

5. Soil Extract:

Collect dirt from oaky area or use "rich looking" top soil. Sieve 3-4 handfuls of dirt through a screen. Add dirt to 1 l nanopure dist. H_2O in a large Erlenmeyer flask. Autoclave 20 minutes. Let sit overnight. Pour off supernatant and centrifuge 10 minutes. Pour supernatant through cheesecloth (Repeat centrifugation and filtering if needed). Divide into subsamples (30-40 ml) and store frozen.

Washing Dino Culturing Dishes

- Day 1: After discarding old cultures, wash the flasks with Liquinox detergent, hot tap water, and a scrub brush. Scrub inside of flask well with brush. Rinse excess soap out with tap water.
- Put one drop of Liquinox in flask and fill to brim with tap water, being careful not to have bubbles around mouth because they will crust. Let flasks soak overnight.
- Day 2: Empty flasks and fill with enough 10% HCl solution to cover typical culture depth in that flask. These flasks should be kept under

the fume hood. Let these flasks sit overnight.

- Day 3: Remove the flasks from the acid bath and scrub out any residue using a designated "acid only" brush. Rinse the flasks 10 times with tap water, then 3 times with DI water. Let air dry. Store in a dry place until flasks are used.

Our Cultures

James Case's lab at UCSB is currently growing:

- *Gonyaulax polyedra* (aka *Lingulodinium polyedrum*)
- *Pyrocystis fusiformis* (picture)
- *Pyrocystis lunula*
- *Glenodinium* sp. (not a bioluminescent species)

All of these species grow well under the conditions described on this page.

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