How to Cultivate *Ectocarpus*

Susana M. Coelho, Delphine Scornet, Sylvie Rousvoal, Nick T. Peters, Laurence Dartevelle, Akira F. Peters and J. Mark Cock

Cold Spring Harb Protoc 2012; doi: 10.1101/pdb.prot067934

Protocol

How to Cultivate *Ectocarpus*

Susana M. Coelho,[1,2,4] Delphine Scornet,[1,2] Sylvie Rousvoal,[1,2] Nick T. Peters,[1,2] Laurence Dartevelle,[1,2] Akira F. Peters,[2,3] and J. Mark Cock[1,2]

[1]*UPMC Université Paris 06, The Marine Plants and Biomolecules Laboratory, UMR 7139, Station Biologique de Roscoff, BP74, 29682 Roscoff Cedex, France*

[2]*CNRS, UMR 7139, Laboratoire International Associé Dispersal and Adaptation in Marine Species, Station Biologique de Roscoff, BP74, 29682 Roscoff Cedex, France*

[3]*Bezhin Rosko, 29250 Santec, France*

This article describes the standard procedure for growing *Ectocarpus* in the laboratory. The culture is started with partheno-sporophyte (or sporophyte) filaments because this is the stage that is usually maintained in strain collections. The standard medium is Provasoli-enriched natural seawater (PES), but *Ectocarpus* can also be grown in artificial seawater, which allows more precise control over the culture conditions. The algae can be cultivated either in plastic Petri dishes or in 10-L bottles with bubbling, if large amounts of biomass are required. Standard growth conditions are 13°C with a 12h/12h d/night cycle and 20 µmol photons m^{-2} s^{-1} irradiance using daylight-type fluorescent tubes. All manipulations of *Ectocarpus* cultures should be performed in a clean environment (if possible, under a laminar flow hood). Forceps should be dipped in ethanol and allowed to dry under the hood.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this article for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Artificial seawater (optional; see Step 1) <R>

Ectocarpus partheno-sporophyte or sporophyte filaments

Ethanol (70%)

Provasoli-enriched seawater (PES) <R>

Provasoli solution <R>

Equipment

Coverslips

Fine forceps

[4]Correspondence: coelho@sb-roscoff.fr

Cite this article as *Cold Spring Harbor Protoc*; 2012; doi:10.1101/pdb.prot067934

Fluorescent tubes (e.g., Philips TL-D)

Pasteur pipettes

Petri dishes (plastic; 60-mm and 140-mm diameter)

Stereomicroscope

METHOD

1. Inoculate autoclaved natural seawater supplemented with Provasoli solution (PES) (or artificial seawater) with small pieces (1 mm) of *Ectocarpus* partheno-sporophyte or sporophyte filaments. Culture at low density (just a few pieces of filament per Petri dish) to ensure that there are sufficient nutrients.

   Cultures grown at high densities will not produce unilocular sporangia. The sporophyte filaments will stick to the bottom of the dish and in a few weeks they start to produce upright filaments. Temperature induction of unilocular sporangia (10–13°C) has been reported for some strains (e.g., E. siliculosus *sensu stricto; Müller 1963). However, this temperature shift may not induce unilocular sporangia in other* Ectocarpus *strains.*

2. Change the medium regularly (once every 2 wk for a 140-mm Petri dish culture).

   About 2 d after the upright filaments appear, plurilocular sporangia are produced. These contain mitospores that allow asexual (clonal) reproduction of the sporophyte. About 1 wk later, the unilocular sporangia that contain the meiospores will appear. The meiospores are the initial cells of the gametophyte generation.

3. Working under a sterile hood with a stereomicroscope, use a sterile Pasteur pipette to carefully dissect off one or two unilocular sporangia. Put a drop (30 µL) of PES containing the unilocular sporangium on a coverslip that has been placed on a drop of medium in a Petri dish. Add 4–8 drops of PES around the sides of the Petri dish. This creates a moist chamber (Fig. 1) and prevents the drop containing the unilocular sporangium from drying up. A few hours after isolation, the unilocular sporangium should release its meiospores into the medium. When this happens, discard the piece of filament bearing the empty unilocular sporangium using forceps or a Pasteur pipette under the stereomicroscope and fill the Petri dish with PES. Gametophytes should develop in ~2 wk (100–200 per unilocular sporangium).

   Meiospore germlings can be kept under very low light (2 µmol photons m^{-2} s^{-1}) for up to 2 mo. Under these conditions, they will not develop, and can be returned to standard growth conditions when necessary.

4. Isolate the gametophytes under the stereomicroscope. Be careful that they are not contaminated by sporophytes (a proportion of the meiospores will develop as sporophytes, a phenomenon known as heteroblasty). Cultivate ~10 gametophytes in PES in a 140-mm Petri dish.

   The gametophytes should become fertile in ~2 wk, producing gametes in plurilocular gametangia.

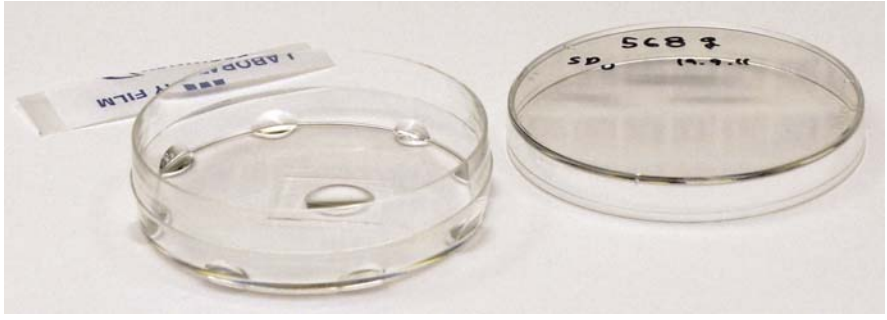

FIGURE 1. "Moist chamber" used for the isolation of unilocular sporangia. After dissection, the unilocular sporangia are transferred to a small drop on a coverslip placed inside a Petri dish. Four to eight drops of PES are placed around the edge of the Petri dish to keep the chamber moist.

5. Monitor the gametophytes under the stereomicroscope until they become mature (i.e., produce plurilocular gametangia). Remove most of the PES so that the gametangia are in a small drop and incubate in the dark overnight at 13°C. In the morning, add 1–2 mL of PES to the drop and place under strong light for 10 min.

   This treatment induces synchronous gamete release. The release of the swimming zoids can be followed under the stereomicroscope.

RELATED INFORMATION

If this protocol for the cultivation of *Ectocarpus* is combined with the procedure described in **Genetic Crosses Between *Ectocarpus* Strains** (Coelho et al. 2012a), the sexual life cycle of the organism can be completed in 3–4 mo, depending on the strain. For further details regarding the biology of *Ectocarpus* and the development of genomic and genetic tools for this organism, see ***Ectocarpus*: A Model Organism for the Brown Algae** (Coelho et al. 2012b).

RECIPES

Artificial Seawater

Reagent	Quantity (for 1 L)	Final concentration
NaCl	26.29 g	450 mM
KCl	0.74 g	10 mM
$CaCl_2$	0.99 g	9 mM
$MgCl_2 \cdot 6H_2O$	6.09 g	30 mM
$MgSO_4 \cdot 7H_2O$	3.94 g	16 mM

Adjust to pH 7.8, autoclave, and store at 4°C.

Provasoli-Enriched Seawater (PES)

Reagent	Quantity (for 1 L)
Natural seawater	1 L
Provasoli solution	20 mL

If possible, seawater should be collected by boat at some distance from the coast. Filter the seawater using a 5-µm mesh. Aliquot into Nalgene bottles (in glass bottles a precipitate can form), autoclave, and store at 13°C. Autoclave the filtered seawater and the Provasoli solution separately to avoid precipitation. This recipe is based on Starr and Zeikus (1993); we use half-strength PES (i.e., 10 mL of Provasoli solution per 1 L of autoclaved seawater).

Provasoli Solution

Solution 1 (10×)

Reagent	Quantity (for 1 L)	Final concentration
H_3BO_3 (MW = 61.83)	1.9 g	30.7 mM
$FeCl_3$ (MW = 162.21)	0.05 g	0.3 mM
$MnSO_4 \cdot H_2O$ (MW = 169.02)	0.273 g	1.6 mM
$ZnSO_4 \cdot 7H_2O$ (MW = 287.54)	0.0367 g	0.127 mM
$CoSO_4 \cdot 7H_2O$ (MW = 281.1)	0.008 g	28 µM
EDTA (0.5 M, pH 8)	11.4 mL	5.7 mM

Cite this article as *Cold Spring Harbor Protoc*; 2012; doi:10.1101/pdb.prot067934

Solution 2 (10×)

Reagent	Quantity (for 500 mL)
Vitamin B12 (cyanocobalamine)	3.35 mg
Thiamine hydrochloride (vitamin B1) (MW = 337.27)	165 mg
Biotin $C_{10}H_{16}N_2O_3S$ (MW = 244.31)	1.65 mg
TRIS (Trisma base) $C_4H_{11}NO_3$ (MW = 121.14)	166.5 g

Solution 3 (10×)

Reagent	Quantity (for 1 L)	Final concentration
$(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$ (MW = 392.14)	1.17 g	3 mM
EDTA (0.5 M, pH 8)	6.8 mL	3.4 mM

Solution 4 (10×)

Reagent	Quantity (for 1 L)	Final concentration
$NaNO_3$ (MW = 84.99)	23 g	270 mM

Solution 5 (10×)

Reagent	Quantity (for 1 L)	Final concentration
$C_3H_7Na_2O_6P{\cdot}5H_2O$ "glycerophosphate" (MW = 216.04)	3.33 g	15.4 mM

Prepare each stock solution separately, autoclave, and store in glass bottles at 4°C. Use a dark bottle for Solution 2.

For 1 L of Provasoli solution, add 100 mL of each of Solutions 1, 3, 4, and 5 plus 10 mL of Solution 2 to MilliQ water (the starting pH should be between 9.6 and 9.8). Adjust to pH 7.8 with concentrated HCl (37%) and adjust the volume to 1 L with MilliQ water. Aliquot into small glass bottles (20, 50, 100, or 200 mL), autoclave, and store at 4°C.

ACKNOWLEDGMENTS

Work was supported by Centre National de la Recherche Scientifique, Université Pierre et Marie Curie, Groupement d'Intérêt Scientifique Génomique Marine, the Interreg program France (Channel)-England (project Marinexus), and Agence Nationale de la Recherche (Project Bi-cycle). N.P. was supported by IRES NSF Grant Number OISE-0652093.

REFERENCES

Coelho SM, Scornet D, Rousvoal S, Peters N, Dartevelle L, Peters AF, Cock JM. 2012a. Genetic crosses between *Ectocarpus* strains. *Cold Spring Harbor Protoc* doi: 10.1101/pdb.prot067942.

Coelho SM, Scornet D, Rousvoal S, Peters N, Dartevelle L, Peters AF, Cock JM. 2012b. *Ectocarpus*: A model organism for the brown algae. *Cold Spring Harbor Protoc* doi: 10.1101/pdb.emo065821.

Müller DG. 1963. Die Temperaturabhängigkeit der Sporangienbildung bei *Ectocarpus siliculosus* von verschiedenen Standorten. *Pubbl staz zool Napoli* **33:** 310–314.

Starr RC, Zeikus JA. 1993. UTEX—The culture collection of algae at the University of Texas at Austin. *J Phycol* **29:** 1–106.