# *A*xenic Ectocarpus *cultures*

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*Additional information:*
**Müller, DG, Gachon, CMM, Küpper FC** *(2008) Axenic clonal cultures of filamentous brown algae: initiation and maintenance. Cah. Biol.Mar.* ***49****: 59-65*

**Axenisation of an *Ectocarpus* strain**

1. Place antibiotic discs on Zobell medium with a flamed forceps (4 per dish 60 mm, 8 per 90 mm dish or 2 x 8 per 140 mm dish).

2. Place small fragments of *Ectocarpus* around and between the discs and seal the plate with parafilm.

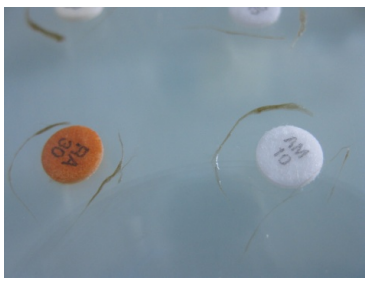
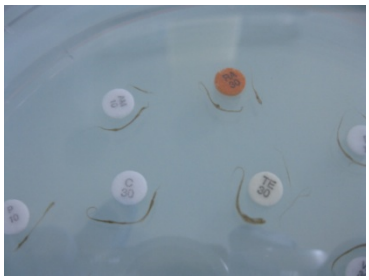
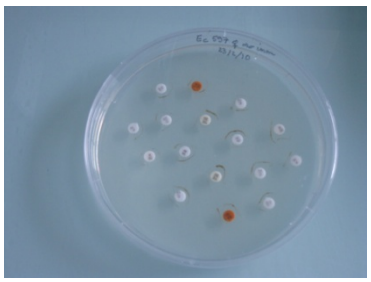
3. Incubate at 18°C and 13°C and monitor the plates daily.

4. Take fragments of *Ectocarpus* from the areas where there is no development of bacteria with flamed forceps and put them in a 55 mm Petri dish containing natural seawater without Provasoli (to limit contamination).

5. Incubate the Petri dishes for 3-4 weeks at 13°C in a plastic box with a top or equivalent to limit contacts with Petri dishes containing non-axenic material.

6. Place a fragment on Zobell medium again, this time without the antibiotic discs. If no bacteria grow, the material can be cultured in natural seawater without Provasoli to produce biomass. If bacteria do appear, repeat the antibiotic treatment.

> *Plates with antibiotic discs can be kept for 1 month before carrying out the test. If large amount of biomass are needed, the material can be grown in a 10 litre bottle in PES for 1 month (without changing the medium, to limit the risk of contamination).*



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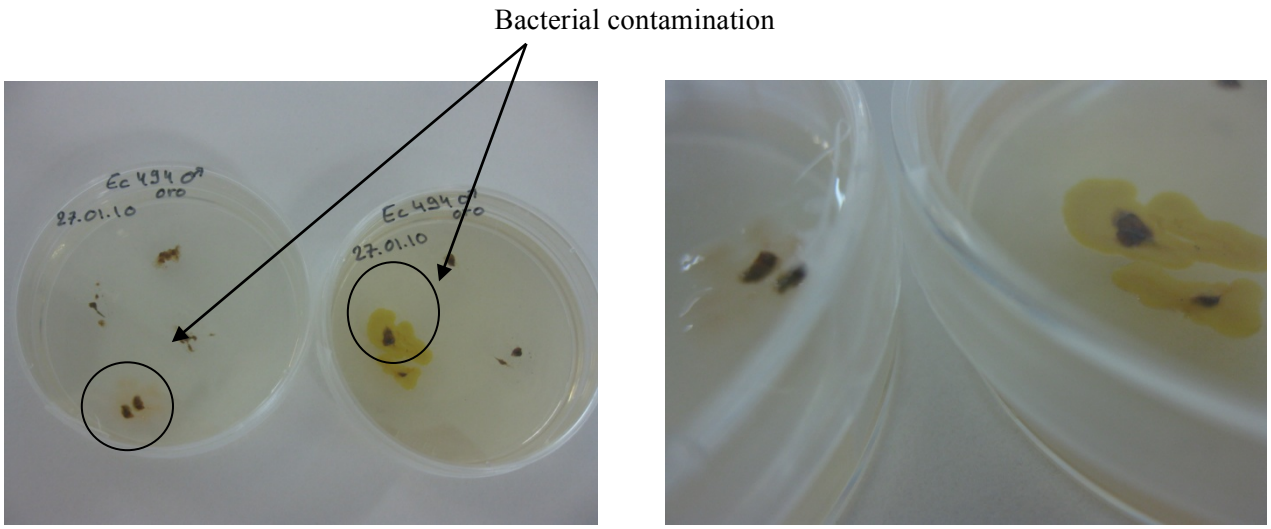
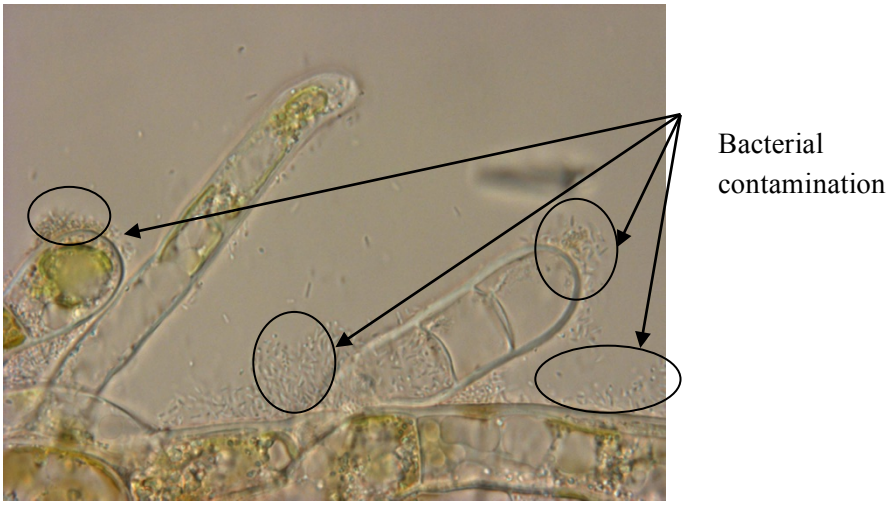
**Verifying that a strain is axenic**

Working under a horizontal laminar flow hood, place some *Ectocarpus* material onto Zobell medium in a 60 mm Petri dish and incubate at 18°c (and, if possible, also at 13°C) for between 2 days and 4 weeks. Score by eye and under the microscope.




The original material can also be observed under a microscope to look for the presence of bacteria.



SOLUTIONS

ZOBELL MEDIUM (bacteriological solid medium)

| Reagent | Quantity (for 500 ml) |
|---|---|
| Bactotryptone (Triptone caséine triptone) | 2.5 g |
| Yeast extract | 0.5 g |
| Filtered natural sea water | 400 ml |
| Distilled water | adjust the volume to 500 ml |
| agar (for solid media) | 5 g |

Pour into a 1 litre glass bottle and autoclave for 20 min at 120°C
Allow to cool slightly for 15-30 mn and then pour the culture medium into Petri dishes under the hood (if you don't pour the medium into the Petri dishes after 30 min, store the bottle in the oven at 60°C until use),
Allow the plates to cool with the lids open.
Seal with parafilm and store upside down at 13°C.

MATERIALS

Antibiotic discs

| **Antibiotic** | **Antibiotic/disc (µg) (Bio-Mérieux discs)** | **Colour (disc with initial)** |
|---|---|---|
| Ampicillin | 10 | white |
| Chloramphenicol | 30 | white |
| Erythromycin | 15 | white |
| Kanamycin | 30 | white |
| Penicillin G | 10 | white |
| Rifampicin | 30 | orange |
| Tetracycline | 30 | yellow |
| Vancomycin | 30 | white |

*Axenisation of Ectocarpus cultures using the method published by*

*Müller et al. (2008).*