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Recommendations to grow algal culture - Roscoff Culture Collection

Roscoff Culture Collection[1]

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Roscoff Culture Collection

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ABSTRACT

Recommendations for algal cultures from the Roscoff Culture Collection

SAFETY WARNINGS

- To avoid any risk of contamination of the natural environment, **all culture residues must be sterilized (by autoclaving or by treatment with bleach) before being discarded.**
- Culture transfer should be undertaken using standard sterile microbiological techniques (work under a laminar flow hood or near a bunsen burner flame, clean all surfaces with 70% ethanol, etc.).

Before ordering strains from the RCC

1
- Please make sure that your laboratory has suitable equipment and conditions for the reception and maintenance of the cultures.
- Every culture in the RCC catalogue has a detailed form on our website (http://roscoff-culture-collection.org/), where you will find a range of information concerning culture conditions for the strain in question (temperature, medium, light intensity, etc.).

2 **Prepare media (not supplied by the RCC).**
- Each culture uses a defined medium (see http://roscoff-culture-collection.org/culture-media).
- Sterile (autoclaved and/or 0.22µM filtered) nutrient and trace metal stocks must be added to the sterile seawater under a laminar flow hood.
- You should not autoclave vitamins (sterilization by 0.22µM filtration and storage at -20°C).

Which seawater to use ?
- The media used by the RCC is most often prepared from seawater collected off Roscoff (salinity ca. 33 ‰), stored for at least two months in darkness, then filtered on 0.22µM filters (Millipore filter GSWP09000 plus Millipore prefilter AP1507500) and autoclaved.
- If your laboratory does not have access to natural seawater, you can try using artificial seawater made from mixtures of salts (see the “culture media” section on our website - http://roscoff-culture-collection.org/culture-media). Please note that we cannot guarantee culture growth in artificial seawater based media (except for some cyanobacteria for which it is recommended to use Red Sea Salt artificial seawater).

3 **Prepare room or cabinet with appropriate conditions**

Light
- All RCC strains are exposed to a 12H/12H day/night light cycle.
- We use 'daylight' neon tubes (Tabur-Neons Sylvania Daylight F58W/54/765 ref : 0001440+ starter ref :00007698476).
- Light intensity for culture maintenance rarely exceeds 100 µEinsteins.m^{-2}.s^{-1}. If in doubt, use rather lower than higher irradiances to avoid light stress
- Some of our cultures (notably certain cyanobacteria) prefer blue light and therefore you can wrap your lights or your cultures with a blue filter (« Moonlight Blue 183 » - Minet Eclairages Scéniques).

Temperature
- The optimum temperature condition is indicated for each strain

- Temperature control is absolutely critical for optimum algal culture growth. In particular temperature fluctuations are very detrimental to cultures. At the RCC, we use the following standard conditions:
- Polar strains : 4 °C
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- Temperature strains : 15 °C
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- Tropical strains : 22° C
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4 **Order / prepare culture flasks**

- RCC strains are routinely maintained in single-use sterile polystyrene flasks with a ventilated filter cap (Sarstedt ref 831810.002 or Nunc ref 136196) or single-use sterile polystyrene tubes (CML ref TCU12PS25).
- Our strains generally also grow well in Erlenmeyers or other glass flasks

Shipment of cultures

5

- After receiving your order, RCC staff will contact you in order to arrange a suitable delivery date.
- We usually use DHL courier service.
- Upon pick-up of the package, we will send you the DHL tracking number by e-mail.
- Packages should be delivered within one to three days (depending on location).
- **Upon delivery, cultures should never be stored in a cold room or freezer.**
- Please warn your receiving of of the imminent arrival of the package
- **they should store it at room temperature** and inform you immediately of the arrival of the package.
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After receiving the culture

6

- After opening the package, transfer the flasks into a culture room or culture cabinet with the appropriate temperature and light conditions.
- Cultures are subjected to stress during transport and we therefore recommend that you wait for one to two days before transferring cultures into fresh medium.
- Monitor strain growth daily by one of the following methods (**do not just rely on tube color** because many strains grow at very low concentrations).
- direct microscopy (not appropriate for very small cells sur as *Prochlorococcus*). Small cells can be detected by epifluorescence
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- inverted miscoscopy. This is the best way to check flasks
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- flow cytometry (for *Prochlorococcus* or small eukaryotes
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- Most of our strains must be transferred every 2-3 weeks with 1/10 to 1/50 dilution in new medium.
- If you need any further advice, please contact us at rcc@sb-roscoff.fr

7 **If the cultures are not in good shape upon arrival, please notify us immediately with details (e.g. picture of the flask, microscopy images). Note that none of cultures are axenic, so you will always see some bacteria in the cultures. Under our terms and conditions, if you notify us of any problems within two weeks we will send you a new culture free of charge (except for shipping).**

Specific instructions for viruses

8

- Upon delivery of a virus – host pair of cultures, store the flask containing the virus at 4°C in darkness.
- The host microalgal culture needs to be transferred in your culture incubator upon delivery as described above. The host must be growing in exponential phase to efficiently propagate a virus strain. The host culture can be diluted 1/10 – 1/50 (vol/vol) into fresh culture medium and incubated for 3 to 4 days under appropriate conditions.
- The viral suspension can be added using a virus:host ratio of 1:10 – 1:50 (vol/vol) and incubated under host growing conditions. You can

use an aliquot of non-infected host culture as a control to monitor cell lysis. Cell lysis is detected by complete clearing of the host culture (usually 3 – 7 days after viral inoculation depending on the viral strain, the initial inoculation ratio, and host growing conditions).

- The resulting lysate can either be stored at 4°C or will need to be transferred as soon as possible. Do not keep the lysate for an extended period of time under host growing conditions as it may induce the development of resistant hosts. It is possible to filter the lysate through 0.2 µm filters (use PES, PC or GF filters, but avoid cellulose acetate membranes) to remove remaining host cells and debris.
- Viral suspensions can be stored for several months at 4°C (usually 3 – 4 months). Be aware that viruses are sensitive to intense light irradiance, UV, and heat.