**FAQ- PCC – PROTOCOLS**

### Protocols

All PCC strains are maintained in the Collection at a temperature in accordance with their origin (18°C, 22°C, 25°C, 30°C and 37°C). The photon flux density is approximately 5 μmol photons. m-2. sec-1 and a light regime of 12 h light/12 h dark. Be aware that the culture conditions employed for the stock cultures in the PCC are not necessarily optimal for heavy growth, as they are optimized for maintenance. However, it is suggested to follow these guidelines, preparing both a liquid and plate culture (whose growth is generally superior to slant cultures) upon receipt of a PCC strain, or to keep a backup culture in parallel to the culture of your on-going experiment. When sufficient growth of the subcultures has occurred, more optimal growth conditions may be investigated.

**Q. What culture media for the cyanobacterial strain?**

**A.** A large variety of media have been designed for the cultivation of cyanobacteria (see Rippka. 1988, for review). For convenience, only three standard media are currently employed for the maintenance of strains in the PCC, with minor modifications for certain strains (Rippka et al., 1979).

**Medium BG-11** is used for strains of freshwater, soil or thermal origin, and for those isolated from a marine environment, which do not display the ionic requirements characteristic of true marine strains (Waterbury & Stanier, 1981). A modified BG11 lacking nitrate, **medium BG-11o**, is used for nitrogen-fixing strains after addition of a solution of filter-sterilized NaHC03 (5 mM final concentration).

True marine strains, i.e. those having elevated requirements for Na+, CI-, Mg2+ and Ca2+, are generally maintained in **medium ASN-III** or, more rarely, in **medium MN**, which has a natural seawater base supplemented with medium BG-11 at half strength. All strains that grow in medium ASN-III can also be maintained in MN. However, if medium MN is recommended, it means ASN-III has proved to result in poor growth. **Media ASo-III and MO** are the respective media, lacking nitrate, for the cultivation of marine nitrogen-fixing strains.

**Composition of medium BG-11**

|  |  |  |
| --- | --- | --- |
| Ingredient | Concentration | |
| g. L-1 | mM |
| NaNO3 | 1.5 | 17.67 |
| K2HPO4. 3H2O | 0.04 | 0.18 |
| MgSO4. 7H2O | 0.075 | 0.30 |
| CaCl2. 2H2O | 0.036 | 0.25 |
| Citric acid | 0.006 | 0.029 |
| Ferric ammonium citrate | 0.006 | 0.030 |
| EDTA K2 Mg. 2H2O | 0.001 | 0.0024 |
| Na2CO3 | 0.04 | 0.38 |
| Trace Metal (see A5+Co) | Add 1mL | |
| Deionized water | To 1 L | |
| pH after autoclaving and cooling: 7.4 | | |

**Composition of medium ASN-III**

|  |  |  |
| --- | --- | --- |
| Ingredient | Concentration | |
| g. L-1 | mM |
| NaCl | 25.0 | 428 |
| MgSO4. 7H2O | 3.5 | 14.2 |
| MgCl2. 6H2O | 2.0 | 9.8 |
| KCl | 0.5 | 6.7 |
| CaCl2. 2H2O | 0.5 | 3.4 |
| NaNO3 | 0.75 | 8.8 |
| K2HPO4. 3H2O | 0.02 | 0.09 |
| Citric acid | 0.003 | 0.014 |
| Ferric ammonium citrate | 0.003 | 0.015 |
| EDTA K2 Mg. 2H2O | 0.0005 | 0.0012 |
| Na2CO3 | 0.04 | 0.38 |
| Trace Metal (see A5+Co) | Add 1 mL | |
| Deionized water | To 1 L | |
| pH after autoclaving and cooling: 7.5 | | |

**Vitamin B12** (as filter-sterilized solution, final concentration 10 μg L-1) stimulates, or is obligatory for, the growth of some cyanobacteria, particularly among marine strains. Note that for many strains currently maintained in medium ASN-III supplemented with vitamin B12 the stimulation by, or requirement for, the added vitamin has not yet been rigorously demonstrated.

**Composition of Trace metal A5+Co**

|  |  |
| --- | --- |
| Ingredient | Concentration |
| g. L-1 |
| H3BO3 | 2.86 |
| MnCl2. 4H2O | 1.81 |
| ZnSO4. 7H2O | 0.222 |
| Na2MoO4. 2H2O | 0.390 |
| CuSO4. 5H2O | 0.079 |
| Co(NO3)2. 6H2O | 0.0494 |
| Deionized water | To 1 L |

**Solid media** are prepared by mixing, after cooling to 50 °C, equal volumes of separately autoclaved double strength solutions of the mineral salts medium and either purified agar (see Waterbury & Willey, 1988) or agarose (more appropriate for planktonic cyanobacteria) to give a final concentration of 0.6 %. A solution of filter-sterilized NaHC03 (5 mM final concentration) is added prior to pouring slants or plates.

**Q. How to test the purity of the strains?**

**A.** The purity of the strains may be confirmed by placing an aliquot of cell material provided onto solid growth medium supplemented with glucose (0.2 %) and casamino acids (0.02 %). We test each transfer at the PCC to control the continuous axenicity of the strain. Test plates should be incubated **in the dark** **for 2-3 days** at the recommended growth temperature prior to microscopic examination using phase contrast objectives and oil immersion.

**Q. How to obtain a large biomass of the cyanobacterial strains?**

**A.** The practical way is to first obtain a nice healthy pre-culture, and to then increase the volume at each transfer. Note that not all strains are suitable for massive growth, but some do the same way they bloom in the natural environment. Large-scale cultures should be inoculated with heavily grown pre-cultures at a dilution of approximately 1/20 (v/v). This implies to grow for example a 50 mL culture, to transfer it, once it has reached high density, into a 1L culture. Direct transfer of the small amount of cell material, such as 5 mL provided by us, into culture volumes greater than 50 mL is not recommended.

**Q. How to cryopreserve the culture I am working with?**

**A.** The most reliable method (though with variable viabilities depending on the strains) of cryoconservation for cyanobacteria we use is storage in liquid nitrogen using 5 % (v/v) of DMSO as cryoprotectant. The DMSO can be bought from Sigma as a sterile solution. The cell suspensions of a healthy grown culture (1.5 mL) are placed into Nunc cryovials (2 mL) and plunged directly into liquid nitrogen. The cryopreserved aliquots are stored in a liquid nitrogen tank or in a -150 °C freezer. Be aware that the planktonic strains, such as *Arthrospira, Microcystis* and *Planktothrix*, are more sensitive to cryoconservation. Before freezing them, it is necessary to collapse the gas vesicles by pressure through a syringe for example and of course to keep all that sterile!

Recovery is done by quick thawing the cryovials at 37 °C, followed by an immediate transfer of the cell suspension into fresh medium respecting a dilution that results in a carry-over of DMSO of not more than 0.5 % (v/v). For several strains, we keep the freshly inoculated culture into darkness for 48 hours (over the weekend), and progressively give access to light from Monday to Wednesday to give them time to adapt. To recover the nice and healthy culture without any DMSO, two to three successive transfers will be needed. It could be useful to test the recovery of the strain from cryopreservation before stopping the live culture. We always have a batch for testing and we prepare 3 tubes per batch for longer storage.

Cyanobacteria are very sensitive to detergents. Sometimes, dry drops of detergent can stay on the Erlenmeyer’s walls after cleaning the glass and can diffuse very efficiently into the medium. Thus, the cryopreserved culture that already does not like the DMSO, once back to life, has to fight also against the detergent...it dies very efficiently: within 12h to 24h the freshly inoculated culture turns from green to yellow (=death).

Some strains show good tolerance to cryopreservation and recover within a month; however, some others are really affected, such as the planktonic strains, which recover **after four months**. Be patient, as we do recover PCC strains, some of which had been cryopreserved in the 70’s.

**References cited**

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