PCC PROTOCOLS

Several questions about how we do at PCC for:

1. Culture media
2. Purity of the strains
3. Culture conditions
4. Biomass culture
5. Cryopreservation

1. CULTURE MEDIA

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 modification, **medium BG-11o** (nitrate omitted), is employed 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, ASN-III 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.

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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		

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

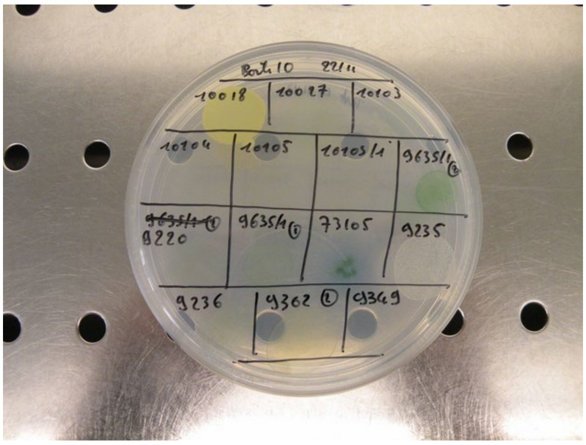
Vitamin B12 (as a filter-sterilized stock solution at 10 µg/mL, giving a final concentration in the medium of about 25 µg/L), stimulates, or is obligatory for, the growth of some cyanobacteria, particularly among marine strains. Il should be noted that for many strains presently maintained in medium ASN-III supplemented with vit. B12 the stimulation by, or requirement for, the added vitamin has not yet been rigorously demonstrated.

Solid media are prepared by mixing, aiter 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.

2. PURITY OF THE STRAINS

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.



On that picture, the contaminations of PCC 10018 and PCC 9235 are visible.

3. CULTURE CONDITIONS

All PCC strains are maintained in the Collection at a temperature in accordance to their origin (18°C, 22°C, 25°C or 37°C). The photon flux density is of approximately 5 µmol m-2 sec-1 and a light regime of 13 h light/11 h dark or continuous light.

It should he noted that the culture conditions employed for the stock cultures in the PCC are optimized for maintenance and not necessarily optimal for heavy growth. 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.

When sufficient growth of the subcultures has occurred, more optimal growth conditions may be investigated.

4. BIOMASS CULTURE

All the strains are not suitable for massive growth, but some are the same way they can form bloom in natural environment. Large-scale cultures should only be inoculated with heavily grown precultures at a dilution of approximately 1/20 (v/v). This implies to grow for example a 50 mL culture, to transfer this one well dense into a 1 L culture.

Direct transfer of the small amount of cell material such as 5 mL provided into culture volumes greater than 50 mL is not recommended.

5. CRYOPRESERVATION

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 PEG/DMSO as cryoprotectant. The PEG/DMSO can be bought from Sigma as a sterile solution.

The volume of 1.5 mL of cell suspensions of a healthy grown culture with 5% PEG/DMSO is placed into Nunc cryovials (2 mL) and plunged directly into liquid nitrogen.

The planktonic strains as Arthrospira, Microcystis, Planktothrix… are more sensitive. 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!

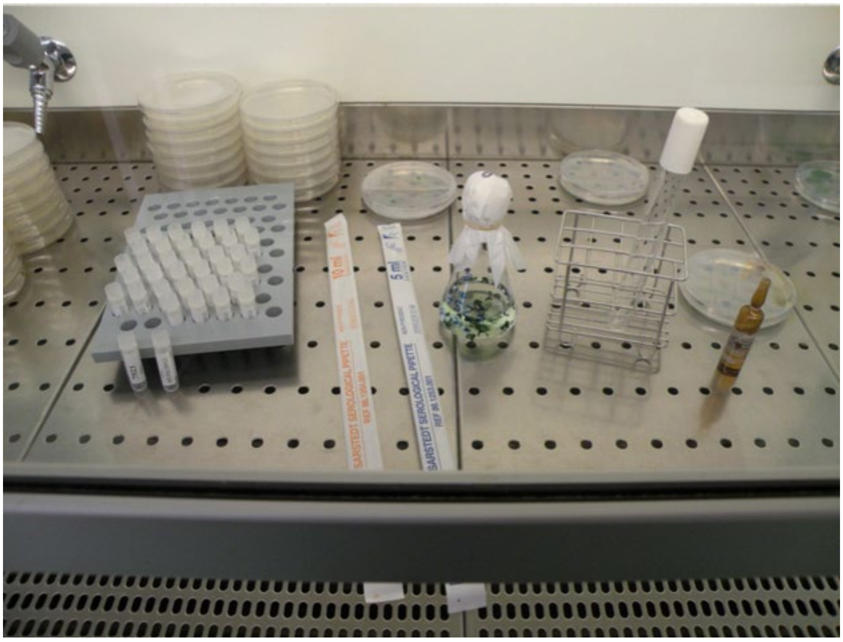
The cryovials are stored in liquid nitrogen tank or in -150°C freezer.

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 yields in a carry-over of DMSO of not more than 0.5 % (v/v). Then, 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 three other tubes per batch for longer storage.

Finally, Cyanobacteria are very sensitive to detergent, which sometimes dry on the Erlenmeyer's wall when cleaning the glass, but afterwards this dry drop of detergent will diffuses efficiently into the medium. Thus, if the cryopreserved culture back to live, that already does not like the DMSO, has to fight also with detergent...it dies very efficiently: within 12 to 24 h the freshly inoculated culture turns from green to yellow (= death).

Some strains just does not matter to be cryopreserved and recover within a month, some are really affected, such as the planktonic ones, and recover **after four months**. Be patient, as we recover at the PCC some strains cryopreserved in the 70's.



References:

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