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SUPPLEMENTARY MATERIAL

Atlas of picocyanobacteria monoclonal strains from the collection of CNR-IRSA, Italy

Cristiana Callieri,[1*] Edoardo Mandolini,[1,2] Roberto Bertoni,[1] Rosaria Lauceri,[1] Antonio Picazo,[3] Antonio Camacho,[3] Pedro J. Cabello-Yeves[4]

[1]National Research Council (CNR), Institute of Water Research (IRSA), Verbania, Italy [2]Department of Microbiology, University of Innsbruck, Innsbruck, Austria [3]Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain [4]Evolutionary Genomics Group, Departamento de Produccion Vegetal y Microbiologia, Universidad Miguel Hernandez, San Juan de Alicante, Alicante, Spain

Picocyanobacteria isolation

Fresh samples from the sampled lakes were kept at the same temperature as measured at the sampling depth, then processed in laboratory (or at the field station) on the very same day of sampling to avoid excessive deterioration by bacteriophages.

In the case of eutrophic lakes, we pre-filtered the sample by gravity (without vacuum) through a 2 µm polycarbonate membrane to eliminate large colonial and most of the large eukaryotes. In the case of oligotrophic lakes, we skipped this passage and directly passed to the next step to concentrate at least 50 ml of sample using a 0.2 µm pore-size polycarbonate membrane mounted on Millipore apparatus (25mm diameter) and a syringe with a luer-lock attack.

We passed the membrane in a 2 ml Eppendorf and shaked up to disperse the cells in the liquid and then, after two days, removed the membrane from the Eppendorf.

The Eppendorf was left at low light (10-15 µmol photons m^{-2} s^{-1}) at 18-20°C. After one week we removed the supernatant and kept approximately 0.5 ml and added new BG11 medium to fill the Eppendorf. When a colour appears in the bottom of the Eppendorf we

sucked 200 µl and passed in a glass flask of maximum 6 ml with new BG11. In these first steps of isolation, it is important to use small flasks or tubes.

When picoeukaryotes were suspected to appear in the sample, cycloheximide was added into the glass vial solution (final concentration between 6 µmol L^{-1} - 0.3 mmol L^{-1}) and incubated for 3 days. We used 5 ml BG11 + 1 ml inoculum + 300 µl cycloheximide (final concentration 0.177 mmol l^{-1}). After 3-4 days, the cultures were transferred to fresh BG-11 medium without cycloheximide.

The phycoerythrin-rich (PE) cultures required at least 2 months to begin their growth. All the work was done using sterile glassware and media to avoid excess bacterial growth, but the cultures were not axenic.

The purification to obtain a monoclonal culture was performed on actively growing cultures with a well-defined colour of the prevalent pigment type, and using a flow cytometric single-cell sorting (InFlux V-GS flow cytometer, Becton Dickinson Inc.) equipped with a UV-laser (355 nm excitation wavelength, 60 mW) and a blue laser (488 nm excitation wavelength, 200 mW). A defined interval of SSC vs autofluorescence (530 nm) was selected and checked for sorting picocyanobacteria. From the events occurring within the selected interval, a single-cell per well was sorted and directly inoculated in 96-well plates enriched with 100 µl of BG-11 substrate per well. Twelve replicates for each pre-culture were done. The plates were kept for two months at the same conditions as the pre-cultures. The colour appearance in a well indicates the successful growth of a monoclonal culture.

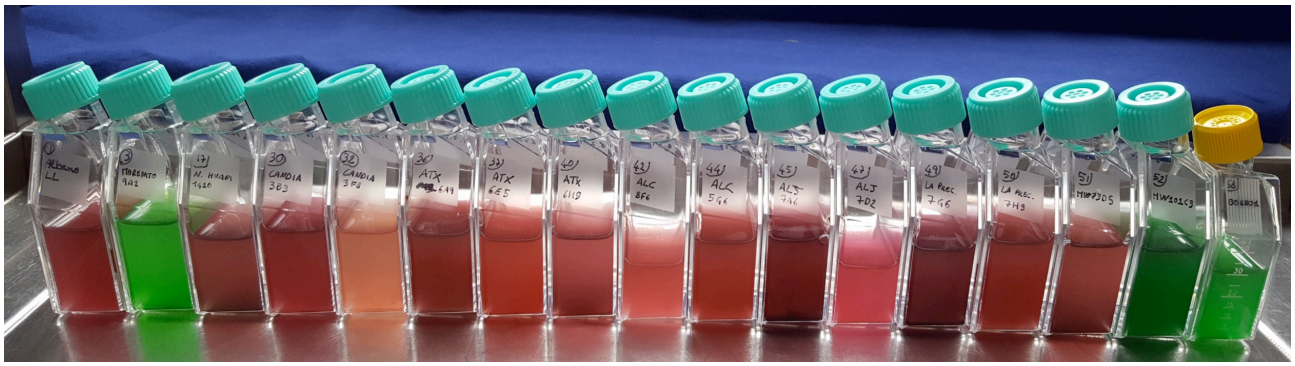

Fig. S1. Picocyanobacteria culture flasks showing the typical colour given by the prevalent pigment type: phycocyanin (green) or phycoerythrin (red).

BG11 MEDIUM (Allen 1968, modified) (65.85 mg N L^{-1} and 2.66 mg P L^{-1})

Stock Solutions		**MW***	**ml of Stock Sol. to add to 985 ml of MilliQ**	**Final concentration in the medium**
Product	**Concentration**			
$NaNO_3$	300 g/l	84.99	1ml	3.53 mM
$MgSO_4 * 7H_2O$	125 g/l	246.48	1ml	1.04 mM
$Ca(NO_3)_2 * 4H_2O$	75 g/l	236.15	1ml	0.46 mM
KNO_3	60 g/l	101.11	1ml	0.59 mM
Na_2CO_3	50 g/l	105.99	1ml	0.47 mM
K_2HPO_4	15 g/l	174.18	1ml	0.09 mM
Na_2EDTA (TitriplexIII)	1 g/l	372.24	3ml	0.008 mM
$CaCl_2 * 2H_2O$	50 g/l	147.02	1ml	0.450 mM
Citric acid	5 g/l	210.14	1ml	0.02 mM
Citrate of $FeNH_3$	1 g/l		3ml	
Micronutrients:			1ml	
H_3BO_3	200 mg/l	61.78	Put in one flask	0.565 mM B
$CuSO_4$	6.4 mg/l	111.59		0.101 mM Cu
$Co(NO_3)_2 * 6H_2O$	5.0 mg/l	291.04		0.102 mM Co
$MnCl_2 * 4H_2O$	14.0 mg/l	197.91		0.253 mM Mn
$NaMoO_4 * 2H_2O$	20.0 mg/l	241.95		0.208 mM Mo
$ZnSO_4 * 7H_2O$	50.0 mg/l	287.54		0.765 mM Zn

*Molecular weight.

Prepare all the stock solution, sterilize in autoclave (20min, 7/10 atm) and keep at 4°C. They can stay in refrigerator for months.
The medium BG11 must be prepared under hood in sterilized conditions. The above recipe is for the preparation of 1 L of medium.
Final pH must be in the range 7.5 – 8.
The pH of the media after the addition of all stock solutions is basic and must be corrected. For 3L of media the addition of 1ml of HCl 1M drops the pH to 7.6. But after sterilization pH is again high (8.95) so it is necessary to regulate with HCl again. In case of lower pH after sterilization, add NaOH.

Allen MM. 1968. Simple conditions for growth of unicellular blue-green algae. J. Gen. Microbiol. 51:199-202.