Phylogenetic diversity of freshwater picocyanobacteria
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time and effort we could not include

METHODS

- A total of 15 lakes (different for origin, thermal regime, maximum depth, trophic conditions, salinity and location (Argentina, Italy, and Mexico) were sampled at different depths (tbs.1). For most samples the lake was filtered through a 40 µm polycarbonate membrane. The remaining water was divided into several 10 ml fractions and mixed with 10% of DMSO.

- Purification was performed by flow cytometry single-cell sorting on an Inius V (Cellix) equipped with a 488 nm excitation wavelength (Argon) and a blue laser (460 nm excitation wavelength, Coherent) at right angles. A defined internal of 750 m·s−1, subcellular DNA (500 m·s−1) was selected and checked for sorting picocyanobacteria. From the events occurring within the selected interval, a single cell-per well was sorted and directly resuspended in 50 µl well plates enriched with F/2F medium (Martiny et al. 2003a) without planktonic cyanobacteria. The plates were kept for two hours at the same conditions for the F/2 medium. After that, the plates were incubated for two weeks and then checked for picocyanobacteria. Any single cell was kept in liquid nitrogen and sent to the laboratories (CNR-Institute of Ecosystem Study, Paliano – Italy) where it was kept at − 70°C.

- To isolate the DNA, the mixture was treated with lysozyme (0.2 mg·ml−1) and proteinase K (10 mg·ml−1). DNA was extracted with phenol-chloroform and then precipitated with isopropanol. DNA quality was checked with 1% agarose gel in 1× TBE buffer, stained with GelRed (Biotium Inc., CA, USA) for 1 h and visualized by UV transillumination. DNA concentration was estimated with spectrophotometry (Shimadzu UV 1800) and verified by gel electrophoresis (1% agarose gel in 1× TBE buffer, stained with GelRed). The DNA was then stored at − 20°C in Tris-EDTA (TE) buffer.

- Purification of PCR products was carried out on a 1% agarose gel in 1× TBE buffer, stained with GelRed.

- Phylogenetic analysis: a total of 41 isolate sequences were used. Quality of sequences was checked with the software Geneious Pro v5.6.1 (Drummond 2011) and imported into the database BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) with the appropriate parameters. The sequences were compared with the sequences available in the database to determine the closest related sequences based on the alignment of the sequences. The sequences were then used to create a neighbor-joining tree, with the Newick format, with the bootstrap option from 100 replications. The resulting phylogenetic tree was evaluated using software Megg’s (http://www.lyceum.edu.co/Departamento-de-Biologia-2014/Software/Megalab) and FastGap (http://www.fasterlab.org). The average gap values in the alignment were used to calculate the alignment of the sequences and the inclusion of sequences, from the first to the last sequences. The average gap values of the alignment were calculated from the first to the last sequences.

RESULTS

The majority of the isolates, both PC and PE, assemblage was found within the Cyanobium gracile cluster group A (Crosbie et al. 2003). Only one L. Magni group was used in group B, whereas many of our isolates clustered in a novel clade (group C) encompassing geographically widespread strains. Other strains displayed distinct habitat differentiation, like those isolated from high altitude tropical athalassochaline lakes in Mexico, which formed a well distinguished group of halotolerants near the marine subclusters 5.3 and 5.2 (Synecococcus WH 5701). Notably, Synecococcus strains from tropical lakes had the highest Fv/Fm, typical of ecotypes with high quantum conversion efficiency of PSI. Synecococcus strains isolated from the ultratrophic and large algal mats of Lake Atitlan, Guatemala, were related to L. Constance subcluster II (Synecococcus B08005 and B00014) and to marine subcluster 5.2, with high Fv/Fm.

CONCLUSIONS

The phylogenetic analysis of our new isolates provides more clear evidence of the non-marine nature of group A as well as of the phylogenetic relationship between marine and freshwater synecococcus Synecococcus strains. The indication of the Fv/Fm as "signature" of putative ecotypes should be taken into consideration and examined something closely.

References