



Conference Abstract

# Using single cell/filament PCR method for study non-cultured cyanobacteria

Laura García Abad<sup>‡</sup>, Alžběta Vondrášková<sup>§</sup>, Antonia Dolores Asencio Martínez<sup>‡</sup>, Jan Kaštovský<sup>§</sup>, Esther Berrendero Gómez<sup>‡</sup>

<sup>‡</sup> Miguel Hernández University of Elche, Elche, Spain

<sup>§</sup> University of South Bohemia, České Budějovice, Czech Republic

Corresponding author: Laura García Abad ([laura.garciaa@umh.es](mailto:laura.garciaa@umh.es))

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## Abstract

Cyanobacteria are photosynthetic prokaryotic organisms, and they are believed to be the Earth's oldest life form. They were responsible for forming the oxygen on the Earth and from then they have been able to adapt to a multitude of environmental conditions, which allowing to be present in a wide range of habitats. When it comes to perform ecological and/or taxonomic studies one of problems which we must confront is the fact of some cyanobacteria are present in the nature in a few amounts, going unnoticed. Moreover, some of them show large mucilaginous sheaths, which making difficulted its isolation and growth later in culture. All these aspects have hindered the molecular assessment of a large portion of traditional cyanobacterial taxa, as *Rivularia* and *Stigonema*, especially necessary evaluation in the taxonomic studies. *Rivularia* genus grows on submerged stones, rocks, and damp soils near riverbanks, often on calcareous substrates and in clear, unpolluted, and streaming or stagnant waters, but also there are several species known from marine littoral. However, *Stigonema* genus grows aerophytically or subaerophytically on bark of trees or wet rocks, from lowland to the alpine zone of high mountains, but also several species are known from pools, swamps and moors. In this study, several *Rivularia* colonies collected in the Croatian Adriatic coast and several *Stigonema* species from Spanish alpine terrestrial environments are being analysed. The used methodology consists of a number of stages. In the first step, the samples are washed in EDTA disodium

salt solution for dissolving the associated of calcium carbonate deposits (STEP 1). For the isolation of single cells and filaments, we are using a modified single filament isolation technique for planktonic cyanobacteria applying a glass capillary under sterile conditions (Zapomělová et al. 2008) described by Mareš et al. 2015 (STEP 2). Then, an optimized protocol we are utilized for amplification of the 16S rRNA gene, the associated 16S–23S internal transcribed spacer (ITS) region and the partially the 23S rRNA gene (2,000–2,500 bp) (Berrendero et al. 2016) (STEP 3). Finally, sequences analysis will carry out to determine the phylogenetic position of the different genotypes found inside a same *Rivularia* colony to be able to relation them with the morphotypes observed by the microscope. In the case of the *Stigonema* samples, we have successfully gotten the isolation of several filaments and, at the present, we are working on the amplification the studied region. This methodology, based on a glass capillary isolation technique and a PCR protocol for amplification of specific sequences, has been applied with success in the *Rivularia* and *Stigonema* samples without the need to cultivate them. Our future research will focus on the amplification of several taxonomic marker genes at the same time.

## Keywords

16S rRNA, cyanobacteria, single cell PCR, taxonomy, *Rivularia*, *Stigonema*

## Presenting author

Laura García Abad

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