

Interdisciplinary approaches to assess diversity and coexistence in Trebouxiphyceae: ultrastructural, genomic, molecular and functional analyses.

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Lichen thalli are complex biosystems resulting from cyclical symbiotic associations between different organisms (mycobiont, photobionts, bacteria, yeasts) integrated both morphologically and metabolically. The understanding of such complexity requires the implementation of diverse methodologies. A common objective of our research team is to study the identity, genetic diversity and functional features of lichen phycobionts and its role in the complexity of the thalli to gain insights on the knowledge of lichen symbiosis. Interdisciplinary approaches integrating ultrastructural, genomic, molecular and functional analyses have been used to investigate the huge diversity of phycobionts, especially those of the trebouxiphyceae, in both lichenized and cultured states.

The combination of several molecular markers as well as ultrastructural techniques (TEM and CM), both in culture and in symbiotic states, should be utilized to add precision to the phylogenetic analyses and the circumscription of trebouxiphyceae taxa, as exemplified in the case of those microalgae found in *Ramalina fraxinea* or the new taxon *Asterochloris mediterranea*. We reported, for the first time, the coexistence lichens, through the design of new molecular markers (chloroplast, nuclear, mitochondrial). Now, this phenomenon has been detected in several lichens -even with the co-occurrence of different genera. Further, the use of 454 amplicon pyrosequencing assays allowed us the detection of 28 OTUs inside a single thallus of *Ramalina farinacea*, opening new perspectives on the adaptive mechanisms that provide the biological success of lichen symbioses.

Lichen microalgae are poikilohydric organisms able to cope with diverse abiotic stresses. To understand the mechanisms involved in desiccation tolerance and other inducible responses against different stresses (light, ROS, Pb, oxidative pollutants, extreme temperatures, etc.) we have used as models *Asterochloris erici*, *Trebouxia* sp. TR9 and *T. jamesii*. The results of these investigations are summarized in several communications of Theme 3.

The scarcity of genomic information about *Trebouxia* algae prompted us to generate a survey of the genomic sequences of *Trebouxia* sp. TR9 by NGS (454 and Solexa) using DNA and RNA. The assemblage of the DNA obtained sequences from the chloroplast, mitochondrial and nuclear genomes resulted in sizes of more than 300 Kb, 70 Kb and 59 Mb, respectively. *Ab initio* gene predictions calculated 9,500 nuclear genes. Gene annotations based on RNAseq data produced 13,887 genes and 21,076 isoforms. Results showed the absence of genome reductions and suggested the existence of overlapping UTRs. This study provides, for the first time, the organelle genomes and the draft nuclear genome of a *Trebouxia* species and may shed light on evolutionary trends in Trebouxiphyceae. (GVA_PROMETEOII/2013/021; MINECO_CGL2012-40058-C02-01; FEDER)