

## Microalgal diversity inside a single lichen thallus revealed by HTS approaches (454 amplicon pyrosequencing assay)

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Current literature has revealed that in lichens the intrathalline coexistence of different microalgal lineages or taxa is a more common event than previously thought. In *Ramalina farinacea*, the microalgae *Trebouxia* sp. TR9 and *T. jamesii* (TR1) always coexist even in populations located at great distances far from each other. Most of the studies dealing with molecular phycobiont diversity within entire thalli are based mainly on the Sanger methodology which may underestimate the potential heterogeneity of the microalgae. The aim of this study was to design an accurate protocol (prior to lichen handling) to allow the detection of the vast number of microalgal genotypes that remain concealed when using traditional molecular techniques by using HGS approaches (454 pyrosequencing assay).

In order to analyze the epiphytic algae, a thallus of *R. farinacea* was separated into two sections and washed following different procedures. It was then joined and divided into sections to analyze the intrathalline algal diversity and distribution. To validate the sensitivity of the proposed technique, we included three algal cultures and an equimolar mix. A total of 64955 raw sequences were obtained which allowed the detection of 37 OTUs inside a single thallus including different genera: 26 *Trebouxia*, one *Asterochloris* and other additional algae (3 Chlorophyceae, 4 Trebouxiophyceae and 3 unknown OTUs).

Our study corroborates the results obtained with traditional techniques, but revealed much higher intrathalline microalgal diversity, and a feasible differential phycobiont distribution. Several additional OTUs were detected in middle and low abundances, giving insights into an “algal rare biosphere”. These additional OTUs may also be important components of thalli communities and have to be scrutinized from an ecological perspective. Moreover, this diversity should not be underestimated in phylogenetic and ecophysiological studies. GVA\_PROMETEOII/2013/021; MINECO\_CGL2012-40058-C02-01; FEDER)