

Tolerance to abiotic stress in *Trebouxia* and *Asterochloris* lichen microalgae: physiological standpoints

Francisco GASULLA¹, Pedro CARRASCO², Eva BARRENO¹

¹Universitat de València, Inst. "Cavanilles" de Biodiversidad y Biología Evolutiva, Botánica, Fac. CC. Biológicas, Valencia, 46100 Burjassot, Spain.

²Universitat de València, Dpto. Bioquímica y Biología Vegetal, Fac. CC. Biológicas, Valencia, 46100 Burjassot, Spain.

Lichen microalgae are poikilohydric organisms able to undergo under diverse abiotic stresses like salt, light irradiance, heavy metals, etc., including a continuous series of desiccation-rehydration cycles. Each rehydration cycle causes a reactive oxygen species (ROS) burst, contributed greatly by microalgae' photosynthetic machinery. However little is known about the induction of cellular responses in these phycobionts.

The lichen phycobiont *Asterochloris erici* is able to survive rapid desiccation through several constitutive protection mechanisms, such as a powerful antioxidant system, late embryogenesis abundant proteins and others. However, we have demonstrated that desiccation also triggers physiological responses that contribute to anhydrobiosis adaption, among them the activation of an alternative energy dissipation mechanisms in the photosystem II or the modification of lipid membrane composition. In these induced responses the phospholipase D pathway seems to play a key role through the activation of mitogen activated protein kinases cascades and the subsequent phosphorylation of proteins.

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 the lichen *Ramalina farinacea* and its isolated phycobionts, *Trebouxia* sp. TR9 and *T. jamesii* (TR1), which are coexisting in the thalli. These algae show distinct physiological responses to acute photooxidative stress (through the ROS propagator CuHP), irradiance or incubation with Pb. TR9 exhibits lower decay in photosynthesis and photosynthetic pigments than *jamesii*. Similarly, antioxidant enzymes like glutathione reductase, superoxide dismutase, ascorbate peroxidase and catalase were only induced in TR9, and the stress-related protein HSP70 decreased in TR1 but increased in TR9. On the other hand, TR9 immobilized Pb extracellularly while the intracellular accumulation was three times higher in TR1. The better physiological performance of TR9 under oxidative conditions may reflect its greater capacity to undertake key metabolic adjustments, including increased non-photochemical quenching, higher antioxidant protection and the induction of repair mechanisms. We also find out remarkable differences in composition, structure and physicochemical features of the cell walls and EPS between TR1 and TR9 that could account for the differential responses to stress conditions. Lastly, to investigate the genetic basis of other physiological features of this algal taxon, we generated a survey of the genomic sequences of *Trebouxia* sp. TR9 by NGS, and detected several genes that can support CO₂ concentrating mechanisms. To corroborate this surveillance ¹³C discrimination experiments were performed. The ¹³C discrimination values calculated in TR9 were similar to C₄ plants (-15.84) and in TR1 to those of C₃ plants (-21.7). GVA_PROMETEOII/2013/021; MINECO_CGL2012-40058-C02-01; FEDER)