**Quantification of the mediator NO (nitrogen monoxide) biosynthetic activities in *Ramalina farinacea* HOLOBIONTS**

Joana R. Expósito1\*; Irene Mejuto1; César D. Bordenave2;Eva Barreno2; Myriam Catalá1

1 Universidad Rey Juan Carlos, Madrid (Spain); 2 Universitat de València, Spain;

\*E-mail: joana.exposito@urjc.es

Nitrogen monoxide (NO) is a necessary mediator in key cellular functions, such as biotic and abiotic stress response. It is also involved in the establishment of symbioses like *Rhizobium*-legume, plant-mycorrhizae and corals. In lichens, NO release increases during rehydration and in presence of heavy metals. NO inhibition increases ROS production during both *Ramalina farinacea* and its isolated phycobionts rehydration. NO biosynthesis is mainly catalysed by nitrate reductase (NR) in plants and nitric oxide synthase (NOS) in animals but is unknown in lichens. Three isoforms of NR are known: a NR using NADH as co-factor, another using only NADPH and a third one that can use both. Our objective is to quantify and characterize NOS and NR activities in *R. farinacea*. A commercial kit for animal cells was used for NOS analysis whereas a specific protocol for lichen NR activity was optimized using *R. farinacea*. Preliminary NOS-like specific activity, 51.03 ± 1.13 µU/mg protein, is in the range of animal cells. NADH-NR specific activity using the optimized protocol is 3.62 ± 0.43 mU/mg protein, one order of magnitude above *Arabidopsis thaliana.* NADPH-NR specific activity is 2.13 ± 0.37 mU/mg protein. The addition of both cofactors renders 2.50 ± 0.11 mU/mg protein. Immunodetection using a polyclonal antibody against plant NR showed the presence of band at 100 kDa in *R. farinacea* identical to that of *Arabidopsis thaliana.* Further analysis of the genome of the most abundant photobiont of *R. farinacea, Trebouxia* sp. Tr9, revealed the presense of a novel gene coding for a NR-like protein highly similar to those of other microalgae. In conclusion, *R. farinacea* exhibits NOS-like and NR enzymatic activity and the presence of plant like NADH-NR is confirmed by immunodetection and sequence analysis. Funding: MINECO (CGL2016–79158-P), FEDER, YEI-CAM (PEJ-2017-AI/AMB-6337) and the Generalitat Valenciana, GVA Excellence in Research (PROMETEO III /2017/039).