NOVEL ULTRAVIOLET RESISTANCE GENES REVEALED BY FUNCTIONAL METAGENOMICS

María Lamprecht-Grandio[1], Marta Cortesão[1], Macarena Benguigui[1], Salvador Mirete[1], and José E. González-Pastor[1*], [1]Laboratory of Molecular Adaptation, Department of Molecular Evolution. Centro de Astrobiología (CSIC-INTA). Carretera de Ajalvir km 4, 28850 Torrejón de Ardoz. Madrid, Spain ([1*]gonzalezpje@cab.inta-csic.es)

To disclose limits of life, it is fundamental to study the molecular strategies and adaptation mechanisms of microorganisms to extreme environments on Earth. Culture independent techniques have recently unveiled information on the resistance mechanisms of uncultured organisms [1], correcting our biased understanding of Earth's biodiversity. Extreme ultraviolet (UV) radiation exposure conditions are believed to have existed on early Earth, and are currently affecting several space environments such as surfaces of spacecrafts and planetary bodies. Although UVB and UVC are harmful to life, causing direct and indirect damage to cells, microorganisms have been found thriving under high doses of UV radiation. This has triggered the curiosity of scientists on how life has evolved to adapt and resist to such conditions. In this project a functional metagenomic approach was used to identify novel genes responsible for UV-resistance in microorganisms highly exposed to UV radiation from hypersaline ponds in the Andean highlands from Argentina (Ojo Seco and Diamante at 4.600 m high), and a saltern from Spain (Es Trenc, Mallorca).

Three metagenomic libraries from microbial communities of these environments were constructed using *E.coli DH10B* as a host. Each library was screened for resistance to UVB and to UVC, allowing the identification of recombinant clones harbouring an environmental DNA fragment that conferred UV-resistance. In total, five resistant-clones were identified: pML-5, pML-6, pML-56, pML-84 from the Andean hypersaline ponds, and pML-105 from the Es Trenc saltern. When analysing their resistance profile, all of the clones showed a survival rate 10% higher than the control *E. coli* DH10B. The environmental DNA fragments in these clones were sequenced and the open reading frames (ORF) were identified and annotated. The clones pML-56 and pML-84 were shown to harbour a single ORF each, encoding for a ribonuclease and an endonuclease, respectively. In turn, the pML-5 contains an ORF encoding for the RecA protein, a recombinase previously identified as involved in UV-resistance through DNA repair, mainly within the SOS response. Interestingly, the clones pML-6 (Ojo Seco) and pML-105 (Es Trenc), from a distant geographical origin, encode each for hypothetical proteins sharing a 36% identity between each other. These hypothetical proteins were shown to present DNA-binding domains, which suggests their involvement in UV-resistance through DNA repair. To elucidate the mechanism of resistance of the clones they were treated with 4-nitroquinoline 1-oxide, a compound that only induces DNA lesions mimicking the effect of UV radiation on DNA. In the presence of this compound, the survival rates of the clones were higher than those of the control, which suggests their direct involvement in DNA repair. Further characterization of the identified UV-resistance genes will improve the knowledge of the molecular mechanisms and metabolic pathways behind them.

References

[1] Mirete S., Morgante V., González-Pastor J. E. (2016) *Curr Opin Biotechnol*. *38*, 143-149.