Search for perchlorate resistance genes in microorganisms of a hypersaline lake of Atacama

Jorge Díaz-Rullo*†[1], Gustavo Rodríguez-Valdecantos[2], Felipe Torres[3], Luis Cid[2], Ignacio T. Vargas[3], Bernardo González[2], and José Eduardo González Pastor[1]

[1]Laboratory of Molecular Adaptation, Department of Molecular Evolution, Centro de Astrobiología (CSIC/INTA) – Madrid, Spain

[2]Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez - Center of Applied Ecology and Sustainability – Santiago, Chile

[3]Department of Hydraulic and Environmental Engineering, Pontificia Universidad Católica de Chile – Santiago, Chile

Abstract

In 2008, the NASA Phoenix Mars Lander discovered the presence of perchlorate anions in the Martian surface regolith, at a concentration of 0.4–0.6 wt%. This finding questioned the possible presence of life on Mars, since perchlorate is toxic for most of known microorganisms, including humans. In addition, perchlorate ion is very soluble in water leading to very low eutectic temperatures ranging from –74 to –34∘C, which would allow the presence of flowing liquid water containing hydrated salts on Mars. For these reasons, perchlorate has a major astrobiological interest at present. It is known that some bacteria are resistant to perchlorate and are able to degrade it, as *Dechloromonas sp.* and *Azospira sp.* The mechanism consists of two steps: first, a perchlorate reductase transforms perchlorate into chlorite, which quickly dissociates into molecular chlorine and oxygen by a chlorite dismutase. However, recent studies reported the identification of novel perchlorate resistant halophilic microorganisms which do not use this mechanism. It is known that only 0.1 to 1% of microorganisms are culturable on standard laboratory media, therefore culture independent techniques may unveiled information about novel resistance mechanisms of uncultured organisms. Thus, in this project a functional metagenomic approach was used to identify novel genes responsible for perchlorate resistance in microorganisms exposed to perchlorate from sediments of a hypersaline lake in Atacama Desert (Salar de Maricunga, Chile).
Environmental DNA was isolated from samples and a metagenomic library was constructed using *E. coli* DH10B as host. This library was screened for resistance to perchlorate, which allowed the identification of recombinant clones containing fragments of environmental DNA that are responsible for conferring the perchlorate resistance. In total, 18 resistant-clones were identified. When analyzing their resistance profile, clones showed a survival rate from 10 to 100.000 times higher than the control *E. coli* DH10B.

The environmental DNA fragments of these clones were sequenced and genes were annotated. Our preliminary data shows that clones pJR-2 and pJR-12 encode for two enzymes related to the metabolism of the nucleoside queuosine. In particular, pJR-2 contained a gene

*Speaker

†Corresponding author: evolucion17@cab.inta-csic.es

encoding for QueF, an enzyme involved in the biosynthesis of queuine, and pJR-12 was shown to harbour a gene encoding for a tRNA-guanine transglycosylase (Tgt). This enzyme catalyzes the post-transcriptional incorporation of pre-queuine typically into the first position in the anticodon of tRNAs for histidine, aspartic acid, asparagine and tyrosine. Further, the pJR-35 contained a gene encoding for a pseudouridine synthase (RluA). Pseudouridine is the most abundant post-transcriptional nucleobase modification in cellular RNAs. Interestingly, in previous studies of this laboratory, another Tgt and pseudouridine synthase enzyme was reported to conferred resistance to arsenic and UV-radiation. Therefore, RNA modification could be a global stress response.

In addition, pJR-10 contained a gene encoding for a Hfq RNA chaperone. This protein binds small regulatory RNAs (sRNAs), facilitates base pairing between sRNAs and their mRNA targets, and directly binds and regulates translation of certain mRNAs. Thus, Hfq may be essential for adaptation to different environments and growth conditions, as sRNAs regulate many stress response pathways. Also, pJR-10 contained an ORF encoding for a ClpP protease, involved in processes such as degradation of misfolded proteins and housekeeping removal of dysfunctional proteins. Therefore, as perchlorate is an oxidant agent, this protease would help degrading oxidized and dysfunctional proteins.
Other genes that were identified in this work encoded for proteins related to DNA (DNA primase, RNase E) and nucleoside metabolism (e.g., IMP dehydrogenase), membrane transport (e.g., MFS transporter), lipid and amino-acid metabolism (e.g., N-methylhydantoinase A, Acetyl-CoA acetyltransferase), and also for hypothetical proteins. Further characterization of the identified genes will improve the knowledge of the molecular mechanisms and metabolic pathways involved in perchlorate resistance.