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HALOARCHAEA FROM THE ANDEAN PUNA: BIOLOGICAL ROLE IN THE ENERGY METABOLISM OF ARSENIC

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Arsenic metabolism is proposed to be an ancient mechanism in microbial life. In fact, some Bacteria and Archaea are able to exploit arsenic as a bioenergetic substrate in either anaerobic arsenate respiration or chemolithotrophic growth on arsenite as an electron donor. The high-altitude Andean lakes (HAAL) consist of several shallow lakes located in a high-altitude desert known as Puna, and these placed are distributed through Argentina, Chile, Bolivia, and Peru along the Central Andes region in South America. This environment host unexplored ecosystems of shallow lakes and salt flats at altitudes of 3700 meters above sea level (masl). In these places high concentrations of arsenic were found in the water and these was attributed to the high Andean volcanism phenomenon which provides the geoenvironmental conditions and determine the availability of arsenic. Recently, our group has reported a metagenomics analysis of a microbialite from Diamante Lake, Catamarca, which was widely dominated by Archaea (96%), assigned to the class Halobacteria (commonly called as haloarchaea). Moreover, these authors strongly suggest that the prevalent haloarchaeal part of the biofilm have all the genes necessary for anaerobic arsenate respiration and arsenite oxidation, suggesting that these haloarchaea use arsenic compounds as bioenergetics substrates to sustain growth. The objective of our study was to investigate the presence and expression of genes (*aioA* and *arrA*) involved in obtaining energy from arsenic compounds in environmental samples and isolated strains. The effect of As [V] and As [III] during isolates growth and the possible role of As as bioenergetic substrate in two selected strains was also evaluated. The presence of *aioA* and *arrA* genes was confirmed in total community DNA from Diamante and Tebenquiche lakes, and the expression of these genes was confirmed by metatranscriptomic RNA samples, suggesting an active expression of both genes in the studied samples. Using selective isolation techniques, eighteen microorganisms belonging to the *Halorubrum* genera (phylum Euryarchaeota) were isolated. The genes encoding for *aioA* and *arrA* were detected in most of the isolates and their expression was verified in two selected strains. The physiological assays using a Chemically Defined Medium (CDM) showed a positive effect of As[III] and As[V] on cell growth. Moreover *Halorubrum* sp. DM2 was able to oxidize and reduce As. The confirmation of oxidation/reduction of arsenic and the transcriptional expression of these genes by RT-PCR in the strain DM2, support the previously raised hypothesis that the arsenic could be used in bioenergetics processes by the microorganisms inhabiting these environments.

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CHARACTERIZATION OF AN ANTIMICROBIAL PEPTIDE PRODUCED BY A CLINICAL ISOLATE AC172 OF *Shigella flexneri* 2

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The objective of this work was to characterize the antimicrobial peptide produced by AC172 strain. This strain was isolated in the summer period 2016-2017 from a pediatric patient with enterocolitis, recovered in the Centro Provincial de Salud Infantil Eva Perón (CePSI-Santiago del Estero province). The biochemical and serology tests allowed us to classify this strain as a member of the *Shigella flexneri* 2 serotype. In this study, we analyzed the antibiotic resistance profile of this strain using the antibiotics-disc technique. We also analyzed the AC172 plasmid profile, detecting the presences of a large number of these extrachromosomal elements. In addition, we determined that AC 172 is able to produce a growth inhibitory substance, using the plate diffusion method and the *E. coli* AB1133 strain as sensitive. This inhibitory compound was characterized using a cell free supernatant obtained after the overnight culture of AC172 in LB. The supernatant's treatment with proteases showed that this inhibitory substance is of protein nature, since it maintained its ability to inhibit bacterial growth after 2 hours of incubation at 37°C. Moreover, it compound was presented as resistant to high temperatures degradation, even after being incubated at 100°C for 20 min. We estimated the size of such substance by electrophoresis in a polyacrylamide gel (12%), where was compared with a molecular weight marker and developed by its growth inhibition activity. The net charge was determined by electrophoresis, running a sample into a 1% agarose gel, at pH 8. The results of this study allow us to characterized this compound as a like-bacteriocin peptide, of low molecular weight (3 kDa, approximately), negatively charged, stable at high temperatures and of protein nature.