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## ANALYSING BACTERIAL COMMUNITIES FROM MICROBIAL MATS AND SEDIMENTS LOCATED IN THE ATACAMA DESERT

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The Atacama Desert has more than 100 basins with interior drainage and most of them contain salt flats. These ecosystems have extreme environmental conditions that allow the development of unique microbial communities. The objective of this was to study the bacterial diversity using independent culture tools of microbial mats and sediments from salt flats in the Atacama Desert. Some physicochemical conditions of the water surrounding these samples were analysed to discover if any physicochemical characteristic could be influencing in its taxonomic composition. Five samples were collected, three of them were microbial mats and two were sediments. The mat samples were taken from Laguna Llamara (samples named LL1 and LL2) and Laguna Cejar (Cej). Sediments were taken from Laguna Jachucoposa (Cop) and Laguna Pujsa (Puj) where microbial mats are not present. Total metagenomic DNA extraction was performed on each sample and the V4 hypervariable region of the bacterial 16S rRNA gene was amplified by pyrosequencing using the Ribosomal Database Project (RDP)-suggested universal primers. Diversity of the microbial community was assessed using the QIIME software package. Lakes that harbor microbial mats have a higher salinity and a lower dissolved oxygen concentration and proportion of organic matter and total phosphorous than lakes where mats are absent. All the samples have important concentrations of arsenic, with an extremely high amount in Puj. *Proteobacteria* and/or *Bacteroidetes* are the major phyla represented in all samples. Also, other phyla as *Spirochaetes*, *Chloroflexi* or *Verrucomicrobia* are found. However, cyanobacterial sequences are only observed in LL2 and Puj. On the other hand, we have found a higher diversity in sediment than in mat samples. The sediments samples contain phyla not observed in mat samples. 16S rRNA gene sequences classified within *Actinobacteria* and *Gracilibacteria* are only found in Puj and related to *Tenericutes*, *Gemmatimonadetes* and *Acidobacteria* are only observed in Cop. Finally, an important fraction of the sequences could not be classified at phylum level. The high diversity found in sediment samples may be explained by the physicochemical conditions in the environment. For example, they have a lower conductivity than mat samples. It is known hypersaline environments have a low diversity, where halophilic microorganisms are able to survive to these extreme conditions because they have specific strategies to balance the osmotic pressure. Besides, we found a low proportion or absence of *Cyanobacteria* in the ecosystems studied, suggesting the possibility that other groups may be playing an essential role as primary producers in these extreme environments. Additionally, the large proportion of 16S rRNA gene sequences that could not be affiliated to any known bacterial phyla suggesting that in these ecosystems there are potential novel representatives of bacterial phyla not yet described.

## EXTREME-HALOPHILES: THEIR ROLE IN THE ARSENIC BIOGEOCHEMICAL CYCLE

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Biofilms, mats and microbialites dwell under extreme environmental conditions (high salinity, extreme aridity, pH and arsenic concentration) in the Argentinean Puna and the Atacama Desert. Microbial communities inhabiting those ecosystems are poorly known. Arsenic metabolism is proposed to be an ancient mechanism in microbial life. Besides, some bacteria and archaea are not only able to use detoxification processes to grow under high arsenic concentration, but also, some of them are able to exploit arsenic as a bioenergetic substrate in either anaerobic arsenate respiration or chemolithotrophic growth on arsenite. Only four aioAB coding for arsenite oxidase and two *arrA* coding for arsenate reductase sequences from haloarchaea were previously deposited in the NCBI Database, but have not been reported in the literature. The *arrA* arsenate reductases are reliable indicators of anaerobic As (V) respiration and catalyze the electron transfer to the As (V) terminal acceptor in dissimilatory arsenatereducing prokaryotes (DARPs). In this work, we are presenting our first steps in the study of the arsenic biogeochemical cycle in these ecosystems. Thus, the aim of this study was to isolate and to study the arsenic metabolism genes of the isolated extreme halophile microorganisms as well as to test the growth in minimal medium using different carbon sources. Mats and microbialites samples were taken from the water's edge of Laguna Tebenquiche, Laguna Brava (Salar de Atacama, Chile)

during December 2012 and from gaylussite crystals (Laguna Diamante) in August 2014. Samples were enriched and plated in WS medium supplemented with arsenic (AsIII 0.5mM and AsV 20mM). Arsenite oxidase (aioB) and Arsenate reductase (arrA) primers specific for haloarchaea were designed using PrimerProspector software. Selected primers were *aioB*-1190F (5'-GCTCMTSACCGGCAGCGTCG-3'), *aioB*-1507R (5'-YGATCTCGTCGATGTCGGCG-3'), *arrA*-417F (5'CCCCGAGTTCGAGCCSATCTC-3') and *arrA*-614R (5'GCRGAGATCGMGCTGTGGGA-3'). In order to identify the isolates we used Archaea-specific primers for 16S rDNA gene amplification: 344F (5'-ACG GGG YGC AGC AGG CGC GA-3') and 915R (5'-GTG CTC CCC CGC CAA TTC CT -3'). Fragments of 577 bp, 317pb and 197pb were obtained from 16S rDNA, *aioB* and *arrA* genes respectively. Universal primers 27F and 1492R were used to amplify 16S rDNA in bacterial isolates. 25 isolates belonging to Archaea and Bacteria Domain were obtained; they are related to the Phylum Euryarchaeota, Firmicutes and Proteobacteria. *AioB* and *arrA* genes were found in most of the isolates and DNA from the samples (mats, microbialites and biofilm). The best carbon source tested was pyruvate and acetate, being pyruvate better in all cases. Promising results were obtained in the search of organisms able to use arsenic in their bioenergetic metabolism. More studies are underway to try to better understand these very interesting systems.

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### IDENTIFICATION OF BACTERIA ASSOCIATED WITH TWO ENTOMOPATHOGENIC NEMATODE SPECIES OF THE GENUS *Steinernema* (RHABDITIDA: STEINERNEMATIDAE)

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Entomopathogenic nematodes of the genus *Steinernema* are obligate parasites of insects and are used as biological control agents of insect pests. Third-stage juveniles (J3) carry symbiotic bacteria of the genus *Xenorhabdus* in their intestine; at the moment of infection, these bacteria are released to the insect hemocoel, where they multiply and become the food source for the nematode. Although this nematode-bacteria relationship is specific, other bacteria related with these parasites have occasionally been identified. The aim of this work was to isolate the bacteria associated with J3 of native isolates of *S. rorum* and *S. diaprepesi*, using two treatments (nematodes subjected or not to disinfection of the cuticle using NaClO). In both treatments, J3 were macerated; the supernatant was used to develop a culture with brain-heart agar medium. The colonies were isolated and total DNA was extracted for further identification based on the 16S rRNA gene. Disinfection of J3 allowed isolation of symbiotic bacteria *X. szentirmaii* and *X. doucetiae* from *S. rorum* and *S. diaprepesi*, respectively. Two strains of *Serratia* sp. were extracted from the nematodes that were not subjected to external disinfection. The results document the unusual foretic association between *Serratia* sp. and the two *Steinernema* species.

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### A POLYPHASIC APPROACH FOR THE TAXONOMIC DESCRIPTION OF TWO NATIVE BLOOM-FORMING CYANOBACTERIA

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The combination of genotypic and phenotypic methods for determining taxonomic positions (known as the "polyphasic approach"), is currently the choice for resolving taxonomic uncertainties. *Raphidiopsis mediterranea* and *Cylindrospermopsis raciborskii*, both planktonic, freshwater bloom-forming cyanobacteria, are of great concern because they can produce cyanotoxins. Although these species are morphologically similar, the presence or absence of heterocysts (in *C. raciborskii* or *R. mediterranea*, respectively) is the only character that has been used to distinguish between them. Importantly, this has led to misidentifications and to question the validity of the genus *Raphidiopsis*. In this work, studies of morphological variation in nature were combined with ecophysiological and molecular analyses to elucidate the taxonomic classification of two strains of *R. mediterranea* native from shallow lakes of the Buenos Aires province. The strains, studied in field for two years, were isolated,