

**CHARACTERIZATION OF TWO MICROBIAL ISOLATES FROM ANDEAN LAKES IN BOLIVIA.** C. Demergasso<sup>1</sup>, J. Blamey<sup>2</sup>, L. Escudero<sup>1</sup>, G. Chong<sup>1</sup>, E. O. Casamayor<sup>3</sup>, NA. Cabrol<sup>4,5</sup>, E. A. Grin<sup>4,5</sup>, A. Hock<sup>6,4</sup>, A. Kiss<sup>7</sup>, G. Borics<sup>8</sup>, K. Kiss<sup>9</sup>, E. Acs<sup>9</sup>, G. Kovacs<sup>10,4</sup>, R. Sivila<sup>11</sup>, J. Zambrana<sup>12</sup>, M. Liberman<sup>10</sup>, M. Sunagua Coro<sup>12</sup>, C. Tambley<sup>1</sup>, V. Gaete<sup>1</sup>, R. L. Morris<sup>3,4</sup>, B. Grigsby<sup>13</sup>, R. Fitzpatrick<sup>13</sup>, and G. Hovde<sup>3,4</sup>. <sup>1</sup>Univ. Católica del Norte, Chile, <sup>2</sup>Bioscience Foundation, <sup>3</sup>CSIC, Blanes, Spain NASA, <sup>4</sup>Ames, <sup>5</sup>SETI Institute, <sup>6</sup>UCLA, <sup>7</sup>University of Budapest, Hungary, <sup>8</sup>Env. Protec. Inspect. Trans-Tiszanian Reg., Hungary, <sup>9</sup>Hungarian Academy of Sciences, <sup>10</sup>Stanford University, <sup>11</sup>SERNAP, Bolivia, <sup>12</sup>SERGEOMIN, Bolivia, <sup>13</sup>Schreder Planetarium, Project ARISE. Email of first author: cdemerga@ucn.cl

**Introduction:** We are currently investigating the biological population present in the highest and least explored perennial lakes on earth in the Bolivian and Chilean Andes, including several volcanic crater lakes of more than 6000 m elevation, in combination of microbiological and molecular biological methods. Our samples were collected in saline lakes of the Laguna Blanca – Laguna Verde area in the Bolivian Altiplano and in the Licancabur volcano crater (27°47'S/67°47'W) in the ongoing project studying high altitude lakes. The main goal of the project is to look for analogies with Martian paleolakes [1]. These Bolivian lakes can be described as Andean lakes following the classification of Chong [2, 3, 4].

We have attempted to isolate pure cultures and phylogenetically characterize prokaryotes that grew under laboratory conditions.

Sediment samples taken from the Licancabur crater lake (LC), Laguna Verde (LV), and Laguna Blanca (LB) were analyzed and cultured using enriched liquid media under both aerobic and anaerobic conditions. All cultures were incubated at room temperature (15 to 20°C) and under light exposure. For the reported isolates, 36 hours incubation were necessary for reaching optimal optical densities to consider them viable cultures. Ten serial dilutions starting from 1% inoculum were required to obtain a suitable enriched cell culture to transfer into solid media. Cultures on solid medium were necessary to verify the formation of colonies in order to isolate pure cultures. Different solid media were prepared using several combinations of both trace minerals and carbohydrates sources in order to fit their nutrient requirements. The microorganisms formed individual colonies on solid media enriched with tryptone, yeast extract and sodium chloride. Cells morphology was studied by optical and electronic microscopy. Rod-shape morphologies were observed in most cases. Total bacterial genomic DNA was isolated from 50 ml late-exponential phase culture by using the CTAB miniprep protocol. The 16S rRNA genes were amplified by PCR using both *Bacteria*- and *Archaea*-universal primer sets: 27f and 1492r, 21f and 1492r respectively [5, 6]. Sequences of 16S rRNA gene were determined and initially compared with reference sequences contained in the EMBL nucleotide sequence database by using the BLAST

program and were subsequently aligned with 16S rRNA reference sequences in the ARB package (<http://www.mikro.biologie.tu-muenchen.de>).

Aligned sequences were inserted within a stable phylogenetic tree by using the ARB parsimony tool. [7].

In this work we report the morphology and phylogenetic characterization of two isolates belonged to Laguna Blanca sediments.

#### References:

- [1] Cabrol et al., (2003). In: Cambridge University Press. Chapter of Book. (in press).
- [2] Chong Díaz, G. (1984). *Geotektonische Forschungen*, 67: 1-146.
- [3] Chong Díaz, G. (1984). *Terra Cognita*, 4, 1: 91.
- [4] Chong Díaz, G. (1988). *Lecture Notes in Earth Sciences* 17: 137-151.
- [5] Giovannoni SJ (1991). In Stackebrandt E, Goodfellow M (eds) *Nucleic acid techniques in bacterial systematics*. p 177-201. John Wiley & Sons, New York.
- [6] Øvreås L et al. (1997). *Appl. Environ. Microbiol.* 63: 3367–3373.
- [7] Ludwig, W. et al. (1998). *Electrophoresis* 19:554–568.