SCIENTIFIC REPORT

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PROJECT TITLE
3D Electron Tomography of extreme environment fossil microbes (Rio Salado, Chile): the problem of biogenicity and its detection

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Duration of field work: 16 days
Starting date: 07 September 2008
Completion date: 22 September 2008

FIELD TRIP PURPOSE
The aim of this research was to examines and sampled sites with different characteristics (e.g. silica content) from the El Tatio geyser area where the precipitation of amorphous silica affords cyanobateria with an effective screen against UV radiation, and allow to their (cellular) ultrastructures in order to better understand their potential in biosignatures and their fossilizations (see the submitted project). This study was expected to expand the recognition of the actual origin of bacteriomorphs and other alleged microbial morphologies through the reconstruction of their shape and composition with a comparative investigation of modern and fossil examples from a range of environmental setting such as El Tatio, Chile.

PREPARING FIELD TRIP
The El Tatio geothermal area is the highest (4300 m) geothermal fields in the world, and the largest known in the southern hemisphere. The geothermal field at El Tatio, know as Los Géiseres del Tatio, is located at the northern edge of the Atacama Desert in the Antofagasta Province (II Region) of Norte Grande, Chile, near the Bolivia border and 95 km east of Calama city and the copper mine of Chuquicamata, less of 80 km north of San Pedro de Atacama (Fig. 1). The El Tatio area is geologically associated with the Altiplano-Puna Volcanic Complex, in the north–south down-faulted block (El Tatio graben), bounded on the east by the Serrania de Tucle-Loma Lucero horst and on the west by El Tatio volcanics (Lahsen & Trujillo, 1976).

Fig. 1. *Location of the El Tatio geothermal area. Modified from Fernandez-Turiel et al., 2005. APVC: Altiplano–Puna Volcanic Complex.*

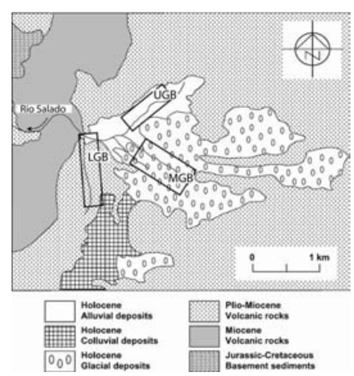

Fig. 2. *Schematic geological map of the El Tatio geothermal area and location of Upper, Middle and Lower Geyser Basin (UGB, MGB, LGB). Modified from Lahsen and Trujillo, 1976, and Fernandez-Turiel et al., 2005.*

The geothermal system is associated with an thick 2000 m, Upper Miocene to Pleistocene volcanic sequence of siliciclastic lavas, breccias and ignimbrites, which is underlain by Upper Cretaceous and Tertiary continental sedimentary formations (Lahsen, 1976). The thermal manifestations scatter over an area of more than 35 km^2. However, the main surface thermal features are concentrated within 10 km^2 and include hosts fountain geysers and cone geysers, intermittent and perpetual boiling and hot springs, rare mud ponds, steaming ground and some fumaroles, broad sinter terraces and aprons in the uppermost levels of the eastern part of the graben that are in three distinct basins, Upper, Middle, and Lower Geyser Basin (Fig. 2). A detailed physiographic description of the El Tatio thermal area and inventory of each related hydrothermal feature identified can be found in Glennon & Pfaff (2003).

DESCRIPTION OF THE FIELD WORK
The studied samples were collected in collaboration with Prof. Roberto Barbieri, Università di Bologna, during September 2008. Based on inventory of Glennon & Pfaff (2003) was relatively easy to recognize the main features of the Upper, Middle and Lover Geyser Basin at El Tatio (Figs 3, 4 and 5). Most of the emission point discharge water near to local boiling temperature (as 86-89°C). The largest Upper Geyser Basin, UGB, is characterized by sinter terraces, opaline deposits, and well-developed geyser cones (Fig. 3). Abundant centimetric siliceous oncoids having their origin related to microbial mediation (Jones and Renaut, 1997)

typified the area around geyser terraces and cones at the UGB (Fig. 3). Most of the silica sinters is in the Upper Basin, siliceous precipitation, however, occurs throughout El Tatio thermal area. In the Middle Geyser Basin a number of boiling hot pools (water temperature as 87°C, the boiling point at El Tatio) with fairly persistent hot water jets typify the area (Fig. 4). Numerous hot streams depart from these relatively deep pools (about 3 meters). Streams are well developed in the Lower Geyser Basin, which is located along the Rio Salado River. A number of intermittent and persistent hot springs are active within and in proximity to the Rio Salado River bank (Fig. 5).

Figure 3. *The Upper Geyser Basin, UGB, (or Main Terrace) panoramic view. The UGB is a gently sloping valley characterized by well-developed sinter terraces and active and inactive geyser cones. Note the centimetric siliceous oncoids dispersed on terrace surfaces.*

Figure 4. *The Middle Geyser Basin, MGB, (or Middle Valley Group) panoramic view. The MGB is a flat sinter plain characterized by a large and 3–meter deep frothy boiling pools and fountain–type eruptions.*

Figure 5. *The Lower Geyser Basin, LGB, (or River Group) panoramic view. Perpetual and intermittent boiling geysers and hot springs with large splash area, small and large pounds, fumaroles and mudpots are distributed along and around the banks of the Río Salado River.*

Since their discovery in sub-aerial thermal spring environments of Yellowstone National Park, thermophiles have been intensely studied (Brock, 1967). Hot springs provide a temperature range from boiling at the source (100°C at sea level, and lower temperature at the higher elevations) to ~40°C down stream with different microbial populations existing along the gradient. The steep thermal gradient along the thermal spring outflows represent the most important ecological parameter that allow the establishment of different microbial biofacies, mat forming thermophilic and hyperthermophilic communities (Walter, 1976; Casternholtz, 1984). Physiologically diverse Archaea and Bacteria exist at elevated temperatures and extensive microbial mats can develop, supported by chemolithoautotropy. The organisms associated with thermal springs are distributed based on their temperature optimum, and ecotypes of the same species may exist at different temperatures. While photosynthesis seems to be limited to temperatures below 75°C (Madigan, 2003), sulfur, iron, and nitrogen metabolism can occur at higher tempratures (Reysenbach, 2007). The community composition in thermal environments are also impacted by pH. In the spring outcrops, the biofacies generated by termophilic (bacteria and archaea) prokaryotes, appear commonly dominated by distinctive chromatic variations (microbial photosynthetic pigments) that occur associated to specific thermal gradients (Figs 4 and 6). At the El Tatio geyser area the biofacies distribution was dominant and better exposed features in perpetual boiling pool and associated streamers of Middle and Lower Geyser Basins (Figs 4, 6 and 7). Closed to the warm thermal water of the spring source (84-86° C measured), the chromatic variations associated to pigmented microbial communities go from black-dark to dark green biofacies (Fig. 4). An yellow and intense orange biofacies are associated to temperature ranging from less of 65° C to 50° C (Figs 6 and 7). The biofacies transition are characterized by dramatic and transitional change in color. The yellow to orange biofacies are locally characterized, were the fluid flux coming more dynamic, by well developed more centimeter long and mobile streamers generated by filamentous bacteria (Fig. 7). Pigment have an important protective function of cells. For instance, carotenoid pigments provide protection of proteins, DNA, and membranes against chemical damages, such as that produced by the intense solar radiation which occurs at high altitudes. In the Middle Geyser Basin sinter deposits are well developed especially on its eastern sides, and consist of amorphous/colloidal silica precipitated over altered ignimbrites rocks. Rock samples as well as (living) microbial mats were collected from 2 sites in the Middle and Lower Geyser Basin, respectively. Each site was mapped and described, and characterized by thermal gradient and related pH variations (Fig. 8). Each samples was photographed, described and labeled. For each samples temperature and pH values was recorded. Each samples was stored in glutharaldehyde diluted with natural filtered water. For each samples, water (filtered) samples ware collected in order to know their Si-content, and their role in microbe fossilization processes.

Fig. 6. *Small perpetual boiling source, 81°C, and related outflow channel running parallel to the Rio Salado River in LGB. Note: the biofacies distributions, yellow-orange to green pigments, reflect a cooling thermal gradient.*

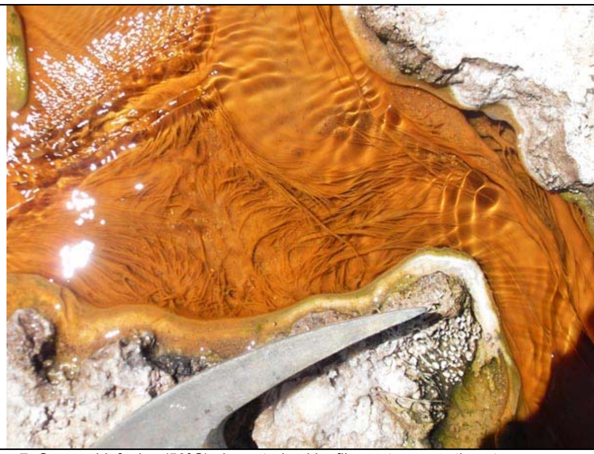

Fig. 7. *Orange biofacies (50°C) characterized by filaments generating streamers.*

Collected rock samples as well as (living) microbial mats have been analyzed and stored in refrigerator of Geomicrobiology and Electron Microscopy Laboratory *(*Prof. S.L. Cady), Department of Geology, Portland State University, Portland, OR, US. Water samples was analized in the Dipartimento di Scienze della Terra e Geologico-Ambientali, Università di Bologna, Italy.

Fig. 8. *Barbara making some notes during field work.*

DESCRIPTION OF THE LABORATORY WORK

Materials sampled for this study were processed and investigated by a multi-analytical approaches. Samples were selected from all different biofacies from a site in the Middle Geyser Basin (22°20.550S, 68°00.774W, 4278m; temperature of boiling water: 87°C) and one in the Lower Geyser Basin (22°20.430S, 68°01.567W, 4244m; temperature of boiling water: 88°C). In laboratory, more of 50 samples were photographed, sub-sampled and sectioned. The sectioned portions were fixed with osmium tetroxide for examination by electron microscopy. For SEM observation, samples were mounted on stubs and Au-Pt or C-coated. The observations were made with a Scanning Electron Microscope (JEOL 35C) equipped with light element EDX System (Kevex X-ray detector) using 10 and 12 kV electron voltage. Unfixed samples, were also observed with a optical fluorescent microscope. Subsequently, all samples coming from 50°C “orange” biofacies (as orange microbial mats and yellow-orange streamers) were sub-sectioned and observed in details using SEM in order to select structures/features to prepare for TEM and 3D-ET experiment. Selected structures, ring-shaped structures typically generated by gliding *Oscillatoria sp*, were prepared for TEM (transmission electron microscope) observations and 3D-Electron Tomography. Unfortunately, at present no significant results (see submitted project) were obtained with this technique. However, in the future, I have plan to apply same technique (3D-ET) on different sub-fossilized microbial structures coming from these samples, in order to verify if the obtained un-results are due to the technique or the “wrong” selected structures. I have already perform on the same structures the FIB-tomography in order to verify the potential of thomografy techniques applied to biogenicity problem.

PROJECTED PUBLICATIONS/ARTICLES RESULTING OR TO RESULT FROM THIS GRANT

Although, some extra analytical work need for better verified the proposed method (3D Electron Tompgraphy), this field experience, and related preliminary results are already included in the publications, and will be presented in national and international congresses:

- Barbieri, R., Cavalazzi, B., Stivaletta, N., Capaccioni, B., SUBMITTED. Life at the extremes: physical environments and microorganisms in the Atacama Region (Chile). GEOACTA.
- Cavalazzi, B., Cady, S.L., Barbieri, R., IN PREPARATION. Attività idrotermale subaerea, distribuzione delle biofacies e dei fossili microbici. PaleoItalia Newsletter.
- FIST-GeoItalia 2009, Rimini (Italy), 9-11 September 2009.

- 9th European Workshop on Astrobiology, 12-14 October, 2009, Brussels, Belgium.
- 2009 GSA Annual Meeting, 18-21 October, Portland, Oregon USA.

In particular, the supplementary analyses are scheduled to be performed at the Portland State University, Oregon, to the Dr S.L. Cady laboratory during the next October. Then, manuscripts could be submitted for publication, as appropriate results.

AKNOWLEDGEMENTS

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