Chapter 12
Extremophilic Patagonian Microorganisms Working in Biomining

Patricia Chiacchiarini, Laura Lavalle, María Sofía Urbieta, Ricardo Ulloa, Edgardo Donati, and Alejandra Giaveno

Abstract The microorganisms known as extremophiles have become a powerful tool in the field of biotechnology. Among them, acidophilic and thermophilic microorganisms capable of oxidizing iron(II) or sulfur compounds are very important in ore-processing operations as they are able to enhance the dissolution of sulfide ores. The aim of this chapter is to describe the physiological and phylogenetic characteristics of the main acidophilic species and communities found in geothermal and mining environments in Neuquén Province, Patagonia Argentina, and the advances done by our research group in their application to biomining and bioremediation of heavy metals. Additionally, the chapter includes the description of a novel thermoacidophilic archaeon from the genus *Acidianus* (*Acidianus copahuensis*) autochthonous of the Copahue geothermal area isolated and characterized by our research group.

12.1 Introduction

The extremophiles are the microorganisms able to grow at one or more environmental conditions outside the range considered normal for human life, that is, the thermophiles, psychrophiles, halophiles, acidophiles, piezophiles, and alkaliphiles. Their capacity, not only to survive but also to function under harsh conditions, gives them vital importance in biotechnology, and they have forever changed our perceptions of the limits of living organisms. In this chapter we pay special attention to acidophiles because of their relevance in commercial-scale biomining and their potential applications in bioremediation (Du Plessis et al. 2007; Schippers 2007; Watling 2015).

P. Chiacchiarini • L. Lavalle • R. Ulloa • A. Giaveno (✉)
PROBIEN (CONICET, UNCo), Facultad de Ingeniería, Universidad Nacional del Comahue, Buenos Aires 1400, 8300 Neuquén, Argentina
e-mail: agiaveno@hotmail.com

M.S. Urbieta
CINDEFI (CONICET-UNLP), Fac. Cs. Exactas, UNLP, Calle 47 y 115, La Plata, Argentina

E. Donati
Industrial Fermentation Research and Development Center (CINDEFI), CONICET-UNLP, School of Science, National University of La Plata, La Plata, Argentina

N.L. Olivera et al. (eds.), *Biology and Biotechnology of Patagonian Microorganisms*, DOI 10.1007/978-3-319-42801-7_12

Acidophiles can be classified according to their optimum growth temperature: mesophiles up to ~40 °C, moderate thermophiles between ~40 °C and ~55 °C, and extreme thermophiles between ~55 °C and ~80 °C. The most relevant acidophiles to biomining applications are the iron- and sulfur-oxidizing bacteria and Archaea that grow autotrophically by fixing CO_2 from the atmosphere. The first used and better characterized leaching acidophilic bacteria belong to the genus *Acidithiobacillus* (formerly *Thiobacillus* (Kelly and Wood 2000) in the order *Gammaproteobacteria*. The species of this genus are the first isolates of extremely acidophilic sulfur- or iron(II)-oxidizing bacteria: the mesophilic *Acidithiobacillus (At.) thiooxidans* and *At. ferrooxidans* together with the moderately thermophilic *At. caldus*. Other important bacteria in bioleaching are the members of the genus *Leptospirillum* in the phylum Nitrospirae; they are also mesophilic, extreme acidophiles that derive energy only from the oxidation of iron(II) but not from sulfur compounds (Schippers 2007). All the extreme thermophilic metal sulfide-oxidizing microorganisms belong to the domain Archaea, specifically to the genera *Acidianus*, *Metallosphaera*, *Sulfolobus*, and *Sulfurococcus* in the order *Sulfolobales*; most of the mesophilic and moderately thermophilic Archaea used in biomining belong to the order *Thermoplasmatales* and the genus *Ferroplasma*.

Our research team has been working since 1997 in fields mainly related to isolation and characterization of acidophilic microorganisms from natural environments, especially from Neuquén Province, and their application in biotechnological processes such as metal ore bioleaching/biooxidation and heavy metals bioremediation at the laboratory scale. We have contributed to the study of the diversity of acidophilic microorganisms in Copahue-Caviahue geothermal region (CCG) using culture-dependent and culture-independent approaches (Chiacchiarini et al. 2009, 2010; Giaveno et al. 2013; Urbieta et al. 2012, 2014a). For some years now it has been clear that the high throughput of modern molecular ecology techniques to assess diversity and the need to obtain the microorganisms isolated for physiological and biotechnological studies oblige the use of a variety of techniques for the study of any habitat. The culture-dependent studies demonstrated that typically only 1–10 % of the total population from any biosphere is cultivable; in contrast, culture-independent approaches, mainly sequencing of the 16S rRNA gene, allow a more realistic picture of the ecosystems because almost the entire microbial community is considered. Nevertheless, traditional microbiological procedures remain essential because isolation and biochemical and physiological characterization of novel species in pure culture is fundamental to develop whole-cell applications (Lutton et al. 2013).

The aim of this chapter is to describe the physiological and molecular characterization of the acidophilic microorganisms isolated from different environments from Neuquén Province, Patagonian Argentina, and their applications in biomining and bioremediation of heavy metals. The main topics described are (1) a summary of the native acidophilic microorganisms isolated and characterized from the CCG system, from "La Silvita" and "Andacollo" mining areas in Neuquén, Argentina; (2) a description of a novel thermophilic archaeon isolated from the CCG system, its versatile metabolic characteristics and its phylogenetic characterization; and an outline of several examples of (3) biomining applications carried out at laboratory scale using ores from Argentinean mines and (4) heavy metals bioremediation assays.

12.2 The Copahue-Caviahue Geothermal System

12.2.1 Site Description

The Copahue-Caviahue geothermal (CCG) system is located on the Northwest corner of Neuquén Province, Patagonia Argentina. Its geology, geochemistry, volcanism, and thermalism have been studied during the past 25 years (Accorinti et al. 1991; Delpino and Bermúdez 1995; Mas et al. 1996; Vallés et al. 2004; Gammons et al. 2005; Caselli et al. 2006; Varekamp et al. 2006; Pedrozo et al. 2008). Nowadays, this area is still very attractive for diverse scientific fields (Vélez et al. 2011; Agusto et al. 2013; Temporetti et al. 2013; Monasterio et al. 2015). Interest in the biodiversity of microorganisms that inhabit this extremely low pH natural environment has increased significantly in recent years. Some of the studies focused on the ecology of the bacteria, yeast, plankton, and algae communities (Juárez and Vélez 1993; Pedrozo et al. 2001; Wendt-Potthoff and Koschorreck 2002; Lavalle et al. 2005; Russo et al. 2008; Chiacchiarini et al. 2009; Giaveno et al. 2009b; Chiacchiarini et al. 2010; Urbieta et al. 2012; 2014a; b).The CCG system has been the most important study area in our research projects and it deserves special attention. Table 12.1 shows the location, the physicochemical characteristics of the water, and the metal concentrations obtained by inductively coupled plasma mass spectrometry (ICP-MS) during a sampling campaign in the dry season.

The area includes the Copahue Volcano, an andesitic stratovolcano that presents a small acidic crater lake (pH 0.2–1.1) (37°51’S, 71°10.2’W), 2977 m a.s.l. On the east flank of the Volcano just below the crater lake an acid hot spring (NA) emerges and feeds the Rio Agrio, downstream there are two acidic hot springs, the South source (VA1) and the North source (VA2). Both hot springs feed the Upper Rio Agrio (URA, pH 0.5–2.5), the main water inputs of which are Rio Rojo, Rio Blanco, and Rio Jara (pH 6.0). After several waterfalls the URA discharges into the glacial Caviahue Lake (LC, pH 2.1–3.7), with a north and a south basin that overflows in one arm through the Lower Rio Agrio (LRA, pH 2.1–6.0) (Varekamp 2004; Pedrozo et al. 2008; Chiacchiarini et al. 2010). Approximately 10 km away LRA presents a great waterfall named “Salto del Agrio” (SA). Finally, the LRA mixes with the Rio Ñorquin and is further diluted (Varekamp et al. 2009). There is an anthropogenic influence on this area from the presence of two small villages, Caviahue and Copahue, that vary in population throughout the year, attracting many tourists to the therapeutic thermal complex in the summer and to a sky sports center in the winter. The water and sediment samples for isolation were collected during different field campaigns from URA and LRA, the crater lake of the volcano, Caviahue Lake (LC); and the hot springs of Copahue village: Las Maquinitas (LMi) and Las Máquinas (LMa). Pyrite, sulfur deposits, hematite, and jarosite were found on the banks of the streams and pools of the system. Four of the sites sampled, Laguna Verde Este (LVE), Baño 9 (B9), Agua del Limón (AL), and Laguna Sulfurosa (LS) are situated in the Copahue village. Three pools have been constructed to use the naturally acidic hydrothermal water and muds for therapeutic purposes. LVE received its name from the microalgae mats that cover its surface and borders

Table 12.1 Location and water chemistry of some sampling sites in the Caviahue geothermal region (CCG) system

Site	Location	pH	T (°C)	Conc. (mS cm^{-1})	SO$_4^{2-}$	Cl$^-$	Fe	K	Mg	Ca	Na	Mn
NA (Naciente del Agrio)	37°51′24″S, 71°09′14″W	0.8	70	65.1	5542.7	7408.5	816.1	485.0	552.2	13.2	867.4	18.8
VA1 (Vertiente Agrio Sur)	37°51′23″S, 71°09′04″W	0.8	45	63.1	8578.6	11428.6	834.6	504.9	696.2	14.8	911.7	21.2
VA2 (Vertiente Agrio Norte)	37°51′19″S, 71°09′09″W	1.0	42	42.0	8604.5	5053.8	537.8	151.6	341.7	17.1	364.9	10.2
CG (Cascada del Gigante)	37°53′11″S, 71°04′15″W	1.9	10	24.3	978.3	2239.8	510.0	147.5	281.2	13.0	326.0	11.3
CC (Cascada de la Culebra)	37°53′08″S, 71°04′14″W	1.9	10	24.5	932.9	2182.3	447.4	136.0	239.7	14.9	282.6	9.7
CV (Cascada de la Virgen)	37°52′59″S, 71°04′00″W	2.3	13	23.5	881.0	2124.9	450.6	134.5	266.3	15.0	282.6	10.1
LC (Lago Caviahue)	37°53′14″S, 71°02′46″W	3.1	16	1.17	280.9	48.2	21.3	4.4	18.1	3.2	14.0	0.7
PG (Puente Gendarmería)	37°49′42″S, 70°58′06″W	3.3	16	0.72	326.4	49.4	2.7	4.6	11.0	1.6	11.6	0.4
SA (Salto del Agrio)	37°48′43″S, 70°55′31″W	3.7	16	0.35	128.1	28.7	Nd	2.9	7.7	2.3	7.5	0.1
LS (Laguna Sulfurosa)	37°49′5.36″S, 71°5′54.38″W	3.0	55	1.12	611.7	28.7	3.2	12.6	4.8	5.8	25.2	0.1
LVE (Laguna Verde Este)	37°49′6.31″S, 71°5′49.01″W	2.4	28	2.60	410.8	3.4	6.6	8.7	1.8	3.2	17.3	0.1
B9 (Baño 9)	37°48′59.8″S, 71°5′48.52″W	2.0	50	3.38	196.6	13.8	7.7	5.4	2.2	0.8	12.6	0.1
AL (Agua del Limón)	37°49′0.13″S, 71°5′36.18″W	2.0	55	5.10	807.5	3.8	31.4	11.1	1.5	2.3	23.2	0.1
LMi (Las Maquinitas)	37°49′09″S, 71°05′12″W	2.5	85	8.60	2040.0	5.7	43.2	9.7	4.4	3.2	26.4	0.7
LMa (Las Máquinas)	37°50′2.61″S, 71°5′3.20″W	1.8	39	3.81	212.5	17.2	10.6	5.7	8.3	2.8	15	0.2

The metals and anions concentration, are expressed in mg l^{-1}

(Laguna Verde means green lagoon). The water of Laguna Sulfurosa (LS) is generally muddy from the presence of colloidal sulfur and gas that emerges in some places and becomes visible by bubbling at the surface. Las Maquinitas (LMi) site is located near the Copahue village and present the most extreme temperature found in the area. The sample site named Las Máquinas (LMa) is located in the homonymous geothermal manifestation area; there the water samples were taken from a moderate-temperature hydrothermal pool chosen because it is the least affected by human activity.

12.2.2 Isolation and Characterization of Native Acidophilic Microorganisms from the CCG System

In the CCG system, we have detected and isolated chemolitho-autotrophic and chemolitho-heterotrophic bacteria, archaea, yeasts, and filamentous fungi. This biodiversity is described in the following sections.

12.2.2.1 Mesophilic and Moderately Thermophilic Prokaryotes

In a first report, we described the isolation of iron-oxidizing strains of the genus *Acidithiobacillus* (on 9 K-agarose solid medium at pH 1.8), the physiology, and molecular characterization (Lavalle et al. 2005). The analysis of 16S rRNA genes by PCR/ARDRA using E*co*RI and E*co*RV enzymes was done to identify acidophilic mesophilic bacteria.

Figure 12.1 shows the influence of temperature on specific maximum growth rate (μ) at pH 1.8 on Fe(II) for isolated strains from the CCG system and a collection strain (DSM11477). At 30 °C almost all the isolates presented the highest μ

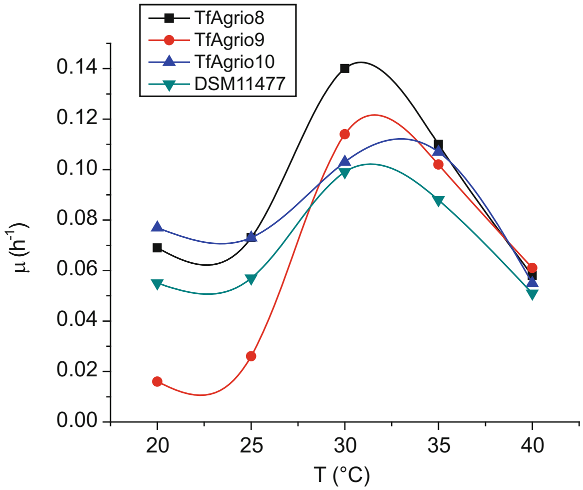

Fig. 12.1 Effect of temperature on specific maximum growth rate (μ) at pH 1.8 for iron(II)-oxidizing acidophilics isolated from the Caviahue geothermal region (CCG) system and a collection strain

values, except for TfAgrio10 that showed maximum μ values at 35 °C. All the native species of *Acidithiobacillus (At.) ferrooxidans* found in the CCG system proved to be less susceptible to 30 °C than the *At. ferrooxidans* collection strain (DSM11477), confirming the major capacity of indigenous strains for Fe(II) oxidation. One of the *At. ferrooxidans* species isolated (TfAgrio8) presented a highest specific growth rate on Fe(II) ($\mu = 0.14\ h^{-1}$) and a high productivity of iron(III) (5.6 mmol $Fe^{3+}\ l^{-1}\ h^{-1}$) at pH 1.8 and 30 °C. On the other hand, TfAgrio8 presented the highest sulfuric acid productivity (17.7 mmol l^{-1} day^{-1}) on sulfur growth. This better performance makes it suitable for possible use for acidification in the process of mineral leaching, solubilization of metals in groundwater, dissolution of phosphates, etc. (Lavalle et al. 2005).

To culture diverse acidophilic bacteria we used the overlay technique proposed by D.B. Johnson with the solid media FeTSBo and FeSo (Johnson 1995). Mesophilic iron-oxidizing bacteria grew at 30 °C as jarosite-encrusted colonies of varying size and morphology on FeTSBo plates from samples collected from different stations in Rio Agrio (CV, CG, LC, PG, SA, PT Puerta del Trolope, and AÑ on the confluence of the Rio Agrio with the nonacidic Rio Ñorquin) and the hot springs in Copahue (LVE, B9, LMi, and LMa). Additionally, moderately thermophilic iron-oxidizing bacteria developed on FeTSBo plates at 45 °C from samples collected from Upper Rio Agrio (NA, VA1, and VA2) and the hot springs in Copahue (LS, LVE, B9, AL, LMi, and LMa). Various species of *Leptospirillum*, also a chemolithotrophic iron(II)-oxidizing bacteria, were isolated and characterized from Rio Agrio (sampling stations CC, LC, PG, and SA) (Chiacchiarini et al. 2010).

On the other hand, several sulfur-oxidizing acidophiles were isolated in basal salt 0 K medium supplemented with 3 g l^{-1} sulfur at pH 3.0 from samples collected all through Upper Rio Agrio where pH was lower than 2.0. One of the isolates characterized as *At. thiooxidans* (TtAgrio6) presented the highest sulfuric acid productivity (26.5 mmol l^{-1} day^{-1}) compared to other native strains and to *At. thiooxidans* DSM11478 (24.3 mmol l^{-1} day^{-1}) (Fig. 12.2). TtAgrio6 was selected to study the feasibility of applying a bioleaching treatment to a contaminated municipal sludge, as we describe on Sect. 4.1.2.

Moderately thermophilic sulfur-oxidizing acidophiles grew at 45 °C on FeSo plates only from the sampling station NA from Upper Rio Agrio. *At. caldus*-like strains were isolated from B9, AL, and LMi.

Mesophilic and moderately thermophilic heterotrophic acidophiles grew on overlay plates inoculated with samples from VA1, VA2, LVE, B9, AL, LMi, and LMa. Heterotrophic colonies were gelatinous cream in color and presented various sizes and morphologies. Sulfate-reducing bacteria (SRB) were cultured at 30 °C and 45 °C from samples collected from the geothermal ponds LMa, LMi, and B9. Fluorescent in situ hybridization (FISH) analysis using specific probes on cultures from LMa samples showed that some of the microbial species were related to the genera *Leptospirillum*, *Sulfobacillus*, *Acidithiobacillus*, *Acidimicrobium*, and *Ferroplasma* (Giaveno et al. 2009a).

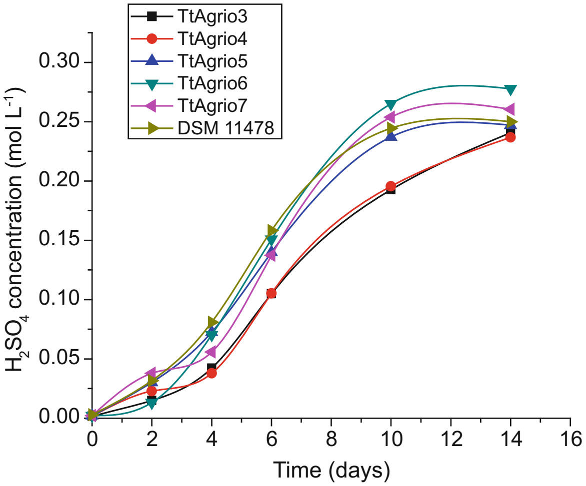

Fig. 12.2 Production of sulfuric acid of different sulfur-oxidizing species isolated from Upper Rio Agrio and the collection strain *Acidithiobacillus thiooxidans* DSM11478

12.2.2.2 Mesophilic Eukaryotes

In the past few years prokaryotic acidophilic microorganisms have been extensively studied in the CCG system as has been described in this chapter. However, the diversity of yeasts and filamentous fungi has received considerable less attention. The first study on yeast isolation from Rio Agrio, that also included the screening of metal-tolerant strains among the isolates, was reported by Lavalle et al. (2007). The concentration of yeast cells observed in the water samples was variable, between 45 and 149 cfu ml^{-1} in CC, CV, and SA samples. The genera *Cryptococcus* and *Rhodotorula* were the most abundant.

Isolated species such as *Rhodotorula mucilaginosa* and *Sporidiobolus salmonicolor* were identified by polymerase chain reaction-restriction fragment length (PCR-RFLP) analysis of the ITS1-5.8S-ITS2 region (Chiacchiarini et al. 2009). Russo et al. presented in 2008 the first complete assessment of yeast biodiversity in Rio Agrio.

The studies done by our research group exclusively focused on copper-, nickel-, cadmium-, and zinc-tolerant yeasts, with the aim of using them in metal uptake assays for bioremediation. A pink pigmented yeast isolate (Agrio-16) presented the highest tolerance to the mentioned metals. It was phenotypically characterized to species level according to the methods and keys proposed by Kurtzman and Fell (1998) and genotypically characterized by PCR-RFLP analysis with enzymes C*fo*, *Hin*fI and *Hae*III. Both results confirmed that the isolate Agrio-16 belonged to the species *Rhodotorula mucilaginosa* (Esteve-Zarzoso et al. 1999; www.yeast-id.com database). Among filamentous fungi, species from the genera *Aspergillus* and *Penicillium* (at least three different species) were identified (Chiacchiarini et al. 2010).

12.2.2.3 Biodiversity in Geothermal Ponds and a Novel Thermoacidophilic Archaea: *Acidianus copahuensis*

The CCG system is the habitat of a wide variety of thermotolerant and thermophilic organisms, which are specially adapted to grow in this environment (Chiacchiarini et al. 2010; Urbieta et al. 2012; Giaveno et al. 2013). Urbieta et al. (2014a) recently reported the prokaryotic biodiversity of five representative ponds using two complementary molecular ecology techniques: phylogenetic analysis of 16S rRNA bacterial and archaeal genes and FISH (or CARD-FISH) for quantitative estimation of biodiversity. The results, supported by multivariate statistical analysis, showed that the biodiversity in Copahue ponds seemed to be determined by temperature. High-temperature ponds were dominated by Archaea, mainly apparently novel representatives from the orders *Sulfolobales* and *Thermoplasmatales* that had no close cultivated relatives. A deeper description of prokaryotic biodiversity on CCG system is given in the work published by Urbieta et al. (2012; 2014a; 2015a, b). These reports describe in detail the species that colonized Río Agrio and the several representative geothermal ponds in the area. The results presented allowed outlining preliminary geomicrobiological models in the CCG system. Detailed reading of these works is recommended to complete an overview of the ecological potential of the system.

Considering that most of the archaeal sequences detected in Copahue were distantly related to cultivated species and formed separate clades in the phylogenetic tree, it is possible to consider the CCG system as an excellent biological reservoir of potential novel species, mainly related to the sulfur cycle. In fact, we have reported a novel indigenous thermoacidophilic archaeon from the CCG system that we named *Acidianus copahuensis* ALE1 strain (DSM 29038).

We have done several studies to characterize this novel archaeon to understand its role in the iron and sulfur biogeochemical cycles in the CCG system and to evaluate its potential applications in biomining processes (Giaveno and Donati 2007; Giaveno 2010; Giaveno et al. 2011; Nodlovu et al. 2014). Some of these applications are described briefly later in this chapter.

Acidianus copahuensis has been isolated from three geothermal acidic hot springs (B9, LMi, and LMa) in Copahue. It is interesting to notice that although the three ponds presented high temperature and low pH values, the exact environmental conditions (especially temperature) differed were among the three (see Table 12.1), showing that *A. copahuensis* has a natural growth flexibility. The details of the isolation and characterization of *A. copahuensis* ALE1 strain were reported by Giaveno et al. (2013). The M88 culture medium, recommended by DSMZ (http:/dsmz.de/media/media88.htm) for the growth of thermoacidophilic archaea (Vartoukian et al. 2010; Stewart 2012), was supplemented with sulfur, tetrathionate, or yeast extract among other energy sources and cultivated at 65 °C. For solid-plate cultures Gellan Gum was used as the gelling agent because agar melts at temperatures above 50 °C (Lin and Casida 1984; Lindstrom and Sehlin 1989). The polar lipid analysis, carried out by thin layer chromatography, revealed the presence of diphosphatidylglycerol, phosphatidylinositol, phosphoglycolipids, and six different chromatographic mobility types of glycolipids. The cell morphology of *A. copahuensis* ALE1 strain was

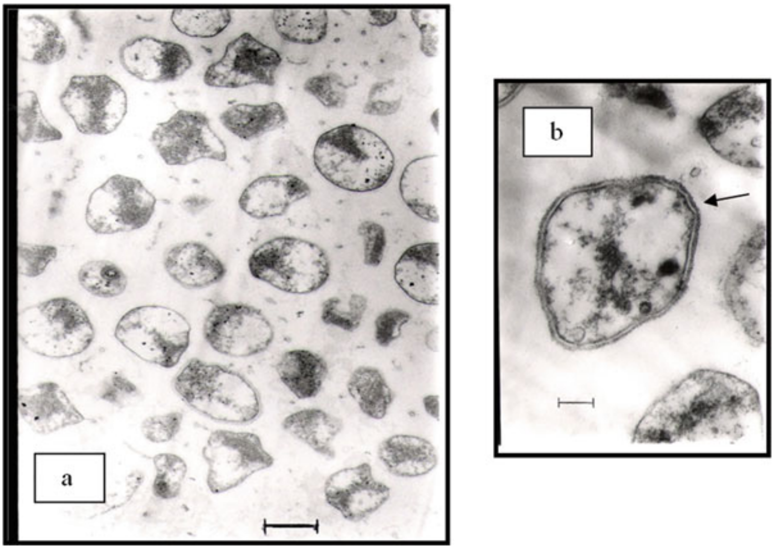

Fig. 12.3 Transmission electron micrographs (TEM) of *Acidianus copahuensis* cells growing in M88 medium. *Arrows* denote typical S-layer present in archaeal cells. *Bars* **a** 850 nm; **b** 210 nm

described by Giaveno et al. (2009b) using different microscopy techniques. Figure 12.3 shows the lobed cells of 0.5 to 1.0 µm with a typical archaeal envelope formed by a single membrane and covered by a paracrystalline glycoprotein layer known as the S layer that serves as protection against a hostile environment. These characteristics are similar to other members of the order *Sulfolobales*.

Phylogenetic analysis based on 16S rRNA gene sequence (Fig. 12.4) shows that strain ALE1 clusters together with members of the family *Sulfolobaceae* in the class *Thermoprotei,* within the phylum Crenarchaeota. However, the ALE1 strain appears in a separate branch from the other members of the genus *Acidianus* reported so far. This isolate has no close relatives according to the NCBI sequence database, not even considering uncultured species, which is even more surprising and reinforces the idea that *A. copahuensis* is autochthonous in the CCG system. Their closest relatives are members of the genus *Acidianus*, but they show low sequence similarity: *A. hospitalis* 91 %, *A. infernus* DSM 3191, *A. ambivalens* DSM 3772, *A. manzaensis* NA-1, and *A. sulfidivorans* DSM 18786 share 90 % of sequence similarity (Segerer et al. 1986; Fuchs et al. 1996; He et al. 2004; Yoshida et al. 2006; Plumb et al. 2007; Kletzin 2008; Liang et al. 2012).

The genome sequence of *Acidianus copahuensis* was obtained using a whole-genome shotgun (WGS) strategy with a 454-FLX Titanium pyrosequencer at INDEAR (Argentina), as recently reported by Urbieta et al. (2014b). The draft genome is 2,454,023 bases in length. The G-C content of the genomic DNA is 35.63 mol%. A total of 2,548 coding sequences (CDSs) and 52 structural RNAs (49 tRNAs and 3 rRNAs) were predicted. Forty-seven percent of the CDSs were classified as hypothetical proteins and 20 % as known enzymes; 34 % of the CDSs were

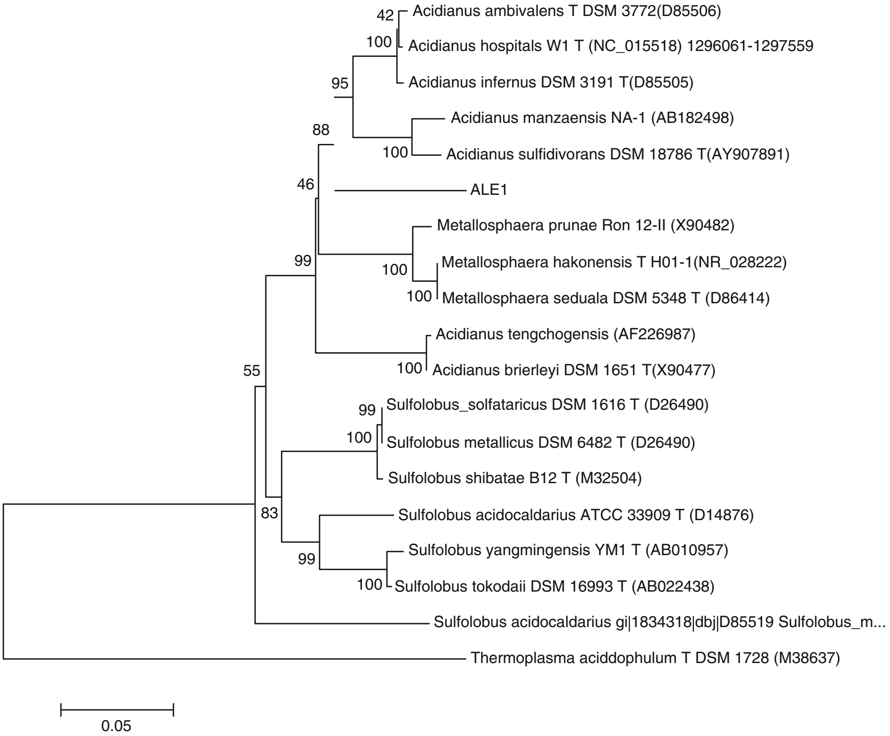

Fig. 12.4 Phylogenetic tree based on 16S rRNA gene sequences showing affiliation of strain ALE1 and selected members of the order *Sulfolobales*. Gene Bank accession numbers are given in *parentheses*. Tree was constructed using neighbour-joining and Kimura two-parameters methods. Bootstraps values are percentages based on 1000 resamplings. *T* type strain. *Bar* 0.05 sequence divergence

assigned to RAST subsystems. The genome of the *A. copahuensis* presents genes that might be related to the relevant metabolic features of the strain. Key enzymes for sulfur compound oxidation, such as sulfur oxygenase-reductase (SOR) and thiosulfatequinone oxidoreductase (TQO), were detected. Homologues of some of the Fox cluster enzymes, associated with iron oxidation, were also found. The genome presents proteins of the five major terminal oxidase complexes of *Sulfolobales*, so far reported in only two species (Auernik and Kelly 2008). Carbon fixation through the 3-hydroxypropionate-4-hydroxybutyrate cycle can be inferred by the presence of key enzymes of this pathway. An interesting discovery is the presence of aioAB genes encoding arsenite oxidase, which might be an indication of a bioenergetics use of arsenite. These genes were reported in *A. hospitalis* and *Sulfolobus tokodaii* genomes but not in other *Sulfolobales*. In an all-versus-all BLASTp comparison (e value, $1e^{-20}$) to *A. hospitalis* (the closest sequenced relative), *A. copahuensis s*howed 789 unique proteins, and in a comparison with *Metallosphaera sedula* (another related archaeon with very similar metabolic features), 766 unique proteins were detected. This whole-genome shotgun (WGS) project has been deposited at DDBJ/EMBL/GenBank under the accession N°JFZT1160895 (http://www.uniprot.org/taxonomy/1160895) (Urbieta et al. 2014b).

It is already known that *Acidianus* species are thermoacidophilic Archaea (Segerer et al. 1986); nevertheless, the novel *A. copahuensis* ALE1 strain shows very interesting metabolic abilities even within the *Acidianus* genus. It was able to grow at all temperatures in a range between 55 °C and 80 °C in M88 medium supplemented with sucrose. Growth rate and cell numbers were influenced by temperature; the fastest growth was achieved at 75 °C with a generation time of 6.7 h. As pH, six different values from 1 to 5 were tested at 75 °C in M88 medium supplemented with sucrose. Growth was detected at all pH values, but was influenced by initial pH conditions. Optimal growth was detected at pH 3.0 with a generation time close to 7.0 h. Additionally, *A. copahuensis* was able to grow aerobically under heterotrophic and also autotrophic conditions at 75 °C when M88 medium was supplemented with sucrose, sulfur, or tetrathionate as energy source, respectively. Finally, *A copahuensis* is also able to grow anaerobically using iron (III) or sulfur as the electron acceptor and sulfur or hydrogen as the electron donor under autotrophic conditions. These results showed that *A. copahuensis* is capable of producing biosulfidogenesis under anaerobic conditions (oxidation of hydrogen and reduction of sulfur) (Giaveno et al. 2013).

12.3 Acidophilic Microorganisms from La Silvita and Andacollo Mining Areas

Besides microbiological studies on the CCG system, our group carried out the assessment of native microorganisms in different mineral areas of the Province of Neuquén. Metal sulfide ores are niches to a wide acidophilic microbiota that offer potential application in biotechnological processes.

La Silvita mine is an epithermal deposit with veins of lead in the andesitic tuffs of Eocene, located at the northeast of the town of Loncopué (38°01′22″S, 70°32′10″W). The average grade for the ore reported is 4.18 % Pb, 3.44 % Zn, 8.2 g t^{-1} Au, and 3.0 g t^{-1} Ag (Leanza et al. 2011). In the La Silvita polymetallic mine the samples were collected from an acid mine drainage with pH 1.5 and from an iron-rich runoff with pH 4.1. The search of microorganisms in these samples was only focused on the species *Leptospirillum ferrooxidans*. The influence of pH and temperature on the specific growth rate and ferrous iron oxidation rate of the isolated species was studied to assess their performance in bioleaching processes. All native *L. ferrooxidans* species showed slightly higher μ than the collection strain *L. ferrooxidans* DSMZ 2705 at pH 1.4 and 25 °C. For example, μ for isolate Lf-LS 04 and DSMZ 2705 at 25 °C and pH 1.4 were 0.050 h^{-1} and 0.020 h^{-1}, and the q_{Fe2+} were 0.19 and 0.17 g l^{-1} h^{-1}, respectively (Lavalle et al. 2008). Additionally, data obtained in growth kinetic assays carried out with native strains of *L. ferrooxidans* in batch culture at different temperatures and pH values were used to develop an artificial neural network (ANN) model. The ANN is a nonlinear estimation technique widely used in data processing that has recently gained much importance because of its range of applicability in assessing biological systems. The final ANN model proved to be an efficient and robust tool in predicting two variables simultaneously, the cell concentration and ferrous iron concentration, in the growth of strains of *L. ferrooxidans*, when the temperature, pH, and time were fed as inputs to the network (Lavalle et al. 2012).

Andacollo mining district, located between the towns of Andacollo and Huinganco, in the northwest of the Neuquén province, is another interesting location for the study of microbial biodiversity with biomining purposes. There gold mines are exploited by gallery excavation with the subsequent metal concentration carried out by froth flotation. Nine samples of water, silt, and rocks were collected from the mines Buena Vista and San Pedro, and their surrounding areas, to search for autochthonous bioleaching microorganisms. The sampling sites included some areas that are currently in operation and others that belong to previous waste stockpiles. Although the results shows that iron and sulfur oxidizers were present in all samples collected, a community obtained from a sample named Relave Viejo (an old stockpile) showed the best performance for reaching the highest iron and copper solubilization and acid production (Ulloa et al. 2012). The biodiversity of the environmental samples was studied by FISH and denaturing gradient gel electrophoresis (DGGE). The results show the presence of microorganisms belonging to the genera *Acidithiobacillus, Leptospirillum*, *Sulfobacillus*, *Desulfovibrio*, and *Acidovorax*. Most of the microorganisms of the genus *Acidithiobacillus* were related to *Acidithiobacillus (At.) ferrooxidans* but high intraspecific variability within this species was found. Archaea species were not detected by the methods used. At present, we are studying biodiversity on Relave Viejo sample by high-throughput sequencing of 16S rRNA genes and bioinformatics analysis tools. This work will allow us to increase knowledge of the biodiversity in the Andacollo mining habitat and to correlate it with other ecological parameters.

12.4 Biomining and Bioremediation Applications

Biomining is the generic term that describes the extraction of metal from ores and concentrates using microbiological technology. This area of biotechnology has seen considerable growth in scale and application since the 1960s following advances in our understanding of microorganisms suitable for the task and improvement in engineering designs for commercial biomining operations (Rawlings and Johnson 2007). Biomining includes two different processes: bioleaching and biooxidation. Bioleaching is an effective technology for metal extraction from low-grade ores and mineral concentrates because of its simplicity, low operating cost, and low environmental pollution. Metal recovery (copper, zinc, cobalt, nickel, etc.) from sulfide minerals is based on the activity of chemolithotrophic bacteria. In contrast to bioleaching wherein the metal of interest is solubilized, in the biooxidation process the valuable metal remains in the solid phase and the surrounding undesired mineral matrix is degraded by selective dissolution mediated by microorganisms. Mineral biooxidation is a viable technology specially used in the pretreatment of refractory sulfide gold ores and concentrates.

In the following sections we discuss the contributions of our research group to the use of native microorganisms from Patagonia in the bioprocessing of ores.

Industrial activities related to metals have increasing impact on the environment and ultimately on human life quality. To mitigate this impact, efforts must be made to enhance metal recovery from industrial waste products, sewage sludge, and soil contaminated with heavy metals. Bioremediation is an eco-friendly biotechnology that has shown successful results. Here, we also provide examples of bioremediation assays carried out with acidophilic bacteria and pigmented yeast isolated from the CCG system.

12.4.1 Mesophilic and Moderately Thermophilic Microorganisms in Bioprocessing

12.4.1.1 Bioleaching and Biooxidation

The characteristics of the most important microorganisms involved in these processes were mentioned in the introduction of this chapter. The main experiments carried out by our research group using indigenous bacteria from Patagonia, described in Sect. 3, are summarized in the following paragraphs. Bioleaching assays performed on La Silvita and Capillitas ores and the biooxidation of a gold concentrate from Andacollo are presented here.

La Silvita is a polymetallic sulfide mine where the main sulfides are sphalerite (ZnS), galena (PbS), pyrite (FeS_2), and chalcopyrite ($CuFeS_2$). The proportions of the valuable metals are 23.87 % Fe, 10.41 % Pb, 8.36 % Zn, and 0.06 % Cu. The capacity of two native *Leptospirillum ferrooxidans* species (Lf-LS02 and Lf-LS04) to catalyze the mineral leaching process was evaluated at laboratory scale (shake flask experiments). Lf-LS04 presented the best performance, achieving 100 % of zinc and copper recoveries in 20 and 12 days, respectively. Iron solubilization was 42 % after 33 days. These recoveries were greater than those obtained using the reference strain *L. ferrooxidans* DSMZ 2705 or in abiotic conditions (Lavalle et al. 2008). Isolate Lf-LS04 was selected to be used in the bioleaching of La Silvita ore in an airlift bioreactor (Giaveno et al. 2007a, b). The experiments were carried out in a 12-l reverse-flow airlift reactor using iron-free 9 K medium at pH 1.8, mineral particle size <74 μm, pulp density of 1 %, and superficial air velocity of 0.01 m s^{-1}. Bioleaching of copper was completed within 44 days; 98.0 % of zinc and 65.0 % of iron were leached after 65 days. The metal recovery from La Silvita sulfide ore with strain Lf-LS04 was higher than recoveries obtained previously with a collection strain of *Acidithiobacillus ferrooxidans*, showing the effectiveness of native species and Lf-LS04 potential in future commercial bioleaching operations.

Another sulfide ore from Capillitas (Catamarca, Argentina) containing mainly marcasite (FeS_2), sphalerite (ZnS), and chalcopyrite ($CuFeS_2$) was evaluated in a bioleaching experiment. In this case, a moderately thermophilic acidophilic consortium from the CCG system was used. The consortium included species related to *L. ferriphilum*, *At. caldus*, and an uncharacterized moderately thermophilic acidophilic heterotrophic bacterium. A shake flask experiment was performed at 42 °C, pH 2, pulp density of 2 % w/v, and particle size <74 μm. Zinc and copper solubilization was 80.0 % and 98.3 %, respectively, with daily maximum productivity of 4.8 ppm zinc and 63.8 ppm copper. This moderately thermophilic consortium proved to be 6 and 40 times more efficient than the traditional chemical solubilization for each metal, respectively, and also more efficient than another mesophilic consortium previously used (unpublished results).

Our research group also attempted the biooxidation of a gold concentrate from Andacollo area using an autochthonous consortium obtained from samples from Relave Viejo that showed the highest rates of iron and sulfur oxidation. The inoculated system reached 50.0 % iron solubilization whereas in abiotic systems iron was not released to the media. The presence of soluble iron indicated that the consortium used pyrite and probably arsenopytire as energy source. Gold recovery by cyanidation after biooxidation was 94.8 % in inoculated flasks and 67.0 % in abiotic systems (Ulloa et al. 2012). These results reinforce the advantage of using indigenous microorganisms in biomining processes.

12.4.1.2 Bioremediation

Our research group studies the capability of certain microorganisms to be used in the bioremediation of heavy metal-contaminated environments, mainly through different biotechnologies: bioleaching and biosorption. Here we describe two examples: the bioremediation of sludge by leaching using *At. thiooxidans* and a biosorption assay through yeast biomass.

A strain of *At. thiooxidans* (TtAgrio6) isolated from the CCG system was selected to evaluate the use of bioleaching to remediate a metal-contaminated municipal sludge coming from a secondary sedimentary tank from Parque Industrial of Neuquén wastewater treatment. Bioleaching experiments were carried out in shake flasks containing the sewage sludge and elemental sulfur. TtAgrio6 culture was able to solubilize 70 % of zinc and 25 % of chromium. At the end of the bioleaching process, the metal concentrations in the treated mud were below the legal permitted values (Chiacchiarini et al. 2010).

Biosorption has emerged as an important cost-effective alternative for the removal of toxic metal from industrial effluents because conventional techniques such as ion exchange, membrane filtration, oxidation–reduction, chemical precipitation, adsorption, reverse osmosis, and evaporative recovery are high cost and have negative impact on the environment (Wang and Chen 2009). Microorganisms such as yeasts are potential bioremediators, removing metals via active or passive uptake (Volesky 2003). Consequently, several yeast strains isolated from Río Agrio were tested for copper, nickel, cadmium, and zinc tolerance. The isolate Agrio-16, characterized as *Rhodotorula mucilaginosa*, was selected to study its ability to uptake copper and nickel from diluted solutions. The results showed that Agrio-16 was efficient in the removal of Cu(II) and Ni(II), especially at low pH values (2.5–4.5). The maximum sorption capacity for Ni(II) uptake was 62.4 mg g^{-1} and 68.9 mg g^{-1} at pH 2.5 and 4.5, respectively, and 160.5 mg g^{-1} for Cu(II) (Lavalle et al. 2007).

12.4.2 Thermophiles in Biomining

Although the first microorganisms used in biomining were essentially mesophiles or moderate thermophiles, biodiversity studies (especially sequencing of the whole microbial community by 16S rRNA genes) on samples from industrial applications, particularly tanks, began to reveal the presence of thermophilic bacteria and Archaea. Bioleaching and biooxidation are highly exothermic processes (more than 30 MJ kg^{-1} of oxidized sulfur). The use of mesophiles requires cooling systems that increase the cost of the processes and are energy consuming (Petersen 2010). Some of the thermophiles used in biomining are *At. caldus*, *Acidimicrobium*, *Sulfobacillus*, and *Ferroplasma* with an optimum temperature range between 45 °C and 55 °C and extreme thermophiles with optimum temperatures between 60 °C and 85 °C, as the Archaea from the genera *Sulfolobus, Acidianus*, and *Metallosphaera*. Also, thermophiles present a viable alternative to the bioleaching of refractory mineralogical species such as arsenopyrite (FeAsS), enargite (Cu_3AsS_4), and chalcopyrite ($CuFeS_2$) (Du Plessis et al. 2007).

12.4.2.1 Strategy to Optimize Gold Recovery

To evaluate the behavior of a thermophilic consortium, and more specifically the role of *Acidianus copahuensis*, in a biooxidation process we designed an assay using a polymetallic sulfide refractory gold ore concentrate processed in the Andacollo ore treatment plant and concentrated by flotation (Giaveno 2010). Metals with commercial interest, such as Au (160 ppm), Ag (288 ppm), Fe (24.1 %), Pb (2.8 %), Zn (1.2 %), and Cu (0.3 %) were detected in the sample. It is interesting to remark that arsenic concentration (1523.4 ppm) was high enough to have inhibitory effect. The mineralogical composition exhibited quartz (SiO_2), feldspar ($KAlSi_3O_8$), pyrite (FeS_2), arsenopyrite (AsFeS), sphalerite (ZnS), chalcopyrite ($CuFeS_2$), and traces of galena (PbS). The presence of pyrite and arsenopyrite in the ore indicated that much of the gold might be occluded in the mineral matrix, which is an obstacle to traditional commercial exploitation. The assay, that included an abiotic control, was performed in shake flasks at 150 rpm, 75 °C, with 1 % pulp density in M88 culture medium at pH 2. The abiotic control showed a rapid increase in pH, probably caused by the dissolution of basic species present in the ore. In inoculated systems pH decreased from the activity of sulfur-oxidizing microorganisms (Fig. 12.5). In spite of the high arsenic content in the ore, microbial activity was not completely inhibited.

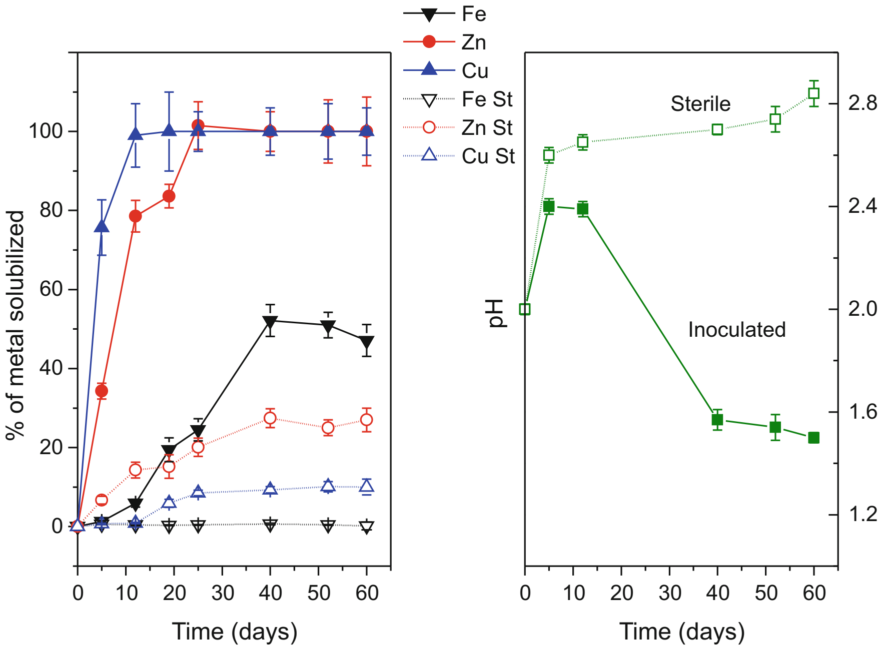

Fig. 12.5 Percentages of metals solubilized and pH evolution along the Andacollo ore biooxidation assay using a thermophilic consortium

The thermophilic consortium efficiently solubilized zinc and copper (Fig. 12.5). Iron solubilization is associated with pyrite dissolution, and it is a parameter that allows following the evolution of the biooxidation process. The abiotic control showed no appreciable iron solubilization because the increased pH favored precipitation of iron compounds and impeded pyrite dissolution. Gold recovery was similar to that obtained using a mesophilic native consortium from Andacollo mines; however, the thermophilic consortium achieved maximum gold recovery faster, increasing the process productivity (Giaveno 2010).

12.5 Conclusions

In our research work during the past years, we have detected and/or isolated quite interesting acidophilic species and consortia from regional mine ores and the Copahue Caviahue Geothermal system in Neuquén, Patagonia. Particularly, we have isolated, characterized, and reported a novel species of thermoacidophilic crenarchaeon, *Acidianus copahuensis*.

We have presented several reports concerning technical application in biomining bioremediation of heavy metals at the laboratory scale. Bioleaching using acidophilic iron- and sulfur-oxidizing microorganisms has been successfully applied to the recovery of metals from sulfide minerals and in the pretreatment (biooxidation) of refractory gold minerals.

On the other hand, techniques based on noncultured microorganisms have shown the great biodiversity present in mining environments such as Andacollo and in the fascinating CCG system. Our results show there is an extremophilic microbial richness waiting to be characterized and, most importantly, cultivated. To achieve these goals, it will be necessary to extend our understanding on various aspects of acidic, high-temperature ecosystems as well as the biochemistry and growth requirements of the extreme microbial life that inhabits them.

Acknowledgments Our research work has been supported by Universidad Nacional del Comahue and CONICET grants. The authors are very grateful to Dr. Ana Monasterio from EPROTEN (Ente Provincial de Termas del Neuquén) for getting the permission to work in the CCG area and the management and technical staff of CORMINE (Corporación Minera del Neuquén S.E.P.) for their invaluable help. The authors thank Professors and staff from Asentamiento Universitario Zapala (Fac. Ingeniería, UNComahue) for assistance during sampling and conditioning of the mineral samples.

References

Accorinti J, Squadrone M, Wenzel M, Perez A (1991) Valoración de las propiedades antimicrobianas del agua del volcán Copahue Neuquén Argentina. Arch Argent Dermatol 41:229–237

Agusto M, Tassi F, Caselli AT, Vaselli O, Rouwet D, Capaccioni B, Caliro S, Chiodini G, Darrah T (2013) Gas geochemistry of the magmatic-hydrothermal fluid reservoir in the Copahue–Caviahue Volcanic Complex (Argentina). J Volcanol Geoth Res 257:44–56

Auernik KS, Kelly RM (2008) Identification of components of electron transport chains in the extremely thermoacidophilic crenarchaeon *Metallosphaera sedula* through iron and sulfur compound oxidation transcriptomes. Appl Environ Microbiol 74:7723–7732

Caselli AT, Dapeña C, Agusto M, Delgado Huertas A (2006) Geothermal Copahue volcano system, Argentina. New stable isotope and geochemical data. In: Proceedings of the Vth South American Symposium on Isotope Geology, Uruguay, pp 332–336

Chiacchiarini P, Lavalle L, Giaveno A, Donati E (2009) Acidophilic microorganisms from geothermal Copahue Volcano system. Assessment of biotechnological applications. Adv Mater Res 71-73:87–91

Chiacchiarini P, Lavalle L, Giaveno A, Donati E (2010) First assessment of acidophilic microorganisms from geothermal Copahue-Caviahue system. Hydrometallurgy 104:334–341

Delpino D, Bermúdez A (1995) Eruptions of pyroclastic sulphur at crater lake of Copahue Volcano, Argentina. In: International Union of Geodesy and Geophysics. XXI General Assembly, Abstracts, p 128

Du Plessis CA, Batty JD, Dew DW (2007) Commercial applications of thermophile bioleaching. In: Rawlings DE, Johnson DB (eds) Biomining. Springer, Berlin, pp 57–80

Esteve-Zarzoso B, Belloch C, Uruburu F, Querol A (1999) Identification of yeast by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacer. Int J Syst Bacteriol 49:329–337

Fuchs T, Huber H, Burggraf S, Stetter KO (1996) 16S rDNA-based phylogeny of the archaeal order *Sulfolobales* and reclassification of *Desulfurolobus ambivalens* as *Acidianus ambivalens* comb. nov. Syst Appl Microbiol 1:1956–1960

Gammons CH, Wood SA, Pedrozo F, Varekamp JC, Nelson B, Shope CL, Baffico G (2005) Hydrogeochemistry and rare earth element behavior in a volcanically acidified watershed in Patagonia, Argentina. Chem Geol 222:249–267

Giaveno MA (2010) Biolixiación y biooxidación de minerales utilizando cepas nativas con capacidad termofílica Tesis de doctorado, Doctor en Cs. Exactas, área Química; Cs. Exactas UNLP. Available on line in http://hdl.handle.net/10915/2699

Giaveno MA, Donati E (2007) Bioleaching of a zinc sulfide ore by thermophilic consortia isolated from Copahue volcano. Adv Mater Res 20-21:79–82

Giaveno MA, Lavalle L, Chiacchiarini P, Donati E, Sand W (2007a). Airlift reactors: characterization and applications in biohydrometallurgy. In: Donati ER, Sand W (eds) Microbial processing of metal sulfides. Springer, Dordrecht, pp 169–191

Giaveno A, Lavalle L, Chiacchiarini P, Donati E (2007b) Bioleaching of zinc from low-grade complex sulfide ores in an airlift by isolated *Leptospirillum ferrooxidans*. Hydrometallurgy 89:117–126

Giaveno A, Chiacchiarini P, Cordero C, Lavalle L, Huergo J, Donati E (2009a) Oxidative capacity of native strains from Copahue geothermal system in the pretreatment of a gold sulfide ore. Adv Mater Res 71-73:473–476

Giaveno MA, Huergo J, Lavalle L, Sand W, Donati E (2009b) Molecular and morphological characterization of cultures from the extreme environmental area of Copahue Volcano-Argentina. Adv Mater Res 71-73:93–96

Giaveno MA, Pettinari G, González Toril E, Aguilera A, Urbieta MS, Donati E (2011) The influence of two thermophilic consortia on troilite (FeS) dissolution. Hydrometallurgy 106:19–25

Giaveno MA, Urbieta MS, Ulloa JR, Toril EG, Donati ER (2013) Physiologic versatility and growth flexibility as the main characteristics of a novel thermoacidophilic *Acidianus* strain isolated from Copahue geothermal area in Argentina. Microb Ecol 65:336–346

He Z, Zhong H, Li Y (2004) *Acidianus tengchongensis* sp. nov., a new species of acidothermophilic archaeon isolated from an acidothermal spring. Curr Microbiol 48:159–163

Johnson DB (1995) Selective solid media for isolation and enumerating acidophilic bacteria. J Microbiol Methods 23:205–218

Juárez AB, Vélez CG (1993) Sobre la presencia de *Chlorellakessleri* (Chlorococcales, Chlorophyceae) en aguas del Complejo Termal Copahue (prov. de Neuquen, Argentina). Bol Soc Argent Bot 29(1/2):105–107

Kelly DP, Wood AP (2000) Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov. and *Thermithiobacillus* gen. nov. Int J Syst Evol Microbiol 50:511–516

Kletzin A (2008) Oxidation of sulphur and inorganic sulphur compounds in *Acidianus ambivalens*. In: Dahl C, Fredrich GC (eds) Microbial sulfur metabolism. Springer, Berlin, pp 184–200

Kurtzmann CP, Fell JW (1998) The yeasts: a taxonomic study. Elsevier, Amsterdam

Lavalle L, Chiacchiarini P, Pogliani C, Donati E (2005) Isolation and characterization of acidophilic bacteria from Patagonia Argentina. Process Biochem 40:1095–1099

Lavalle L, Portillo M, Chiacchiarini P, Donati E (2007) Heavy metal tolerance and copper uptake in yeast isolated from Patagonia Argentina. Adv Mater Res 20:639–642

Lavalle L, Giaveno A, Pogliani C, Donati E (2008) Bioleaching of a polymetallic sulfide mineral by native strains of *Leptospirillum ferrooxidans* from Patagonia Argentina. Process Biochem 43:445–450

Lavalle A, Curia L, Lavalle L, Giaveno A, Donati E (2012) Artificial neural network to predict the growth of *Leptospirillum ferrooxidans* in 9K defined medium. Int J Eng Res Appl 2:1406–1416

Leanza H, Arregui C, Carbone O, Danieli J, Vallés J Eds (2011) Relatorio del XVIII Congreso Geológico Argentino. Geología y Recursos Naturales de la Provincia del Neuquén. Buenos Aires. Asociación Geológica Argentina ISBN 978-987-22403-3-2

Liang CL, Xia JL, Nie ZY, Yang Y, Ma CY (2012) Effect of sodium chloride on sulfur speciation of chalcopyrite bioleached by the extreme thermophile *Acidianus manzaensis*. Bioresour Technol 110:462–467

Lin CC, Casida LE Jr (1984) Gerlerite as a gelling agent in media for the growth of thermophilic microorganisms. Appl Environ Microbiol 47:427–429

Lindstrom BE, Sehlin HM (1989) High efficiency of plating of the thermophilic sulfur-dependent archaebacterium *Sulfolobus acidocaldarius*. Appl Environ Microbiol 55:3020–3021

Lutton E, Schellevis R, Shanmuganathan A (2013) Culture-dependent methods increase observed soil bacterial diversity from Marcellus shale temperate forest in Pennsylvania. J Stud Res 2:9–16

Mas GR, Mas LC, Bengochea L (1996) Hydrothermal surface alteration in the Copahue Geothermal field (Argentina). Proceedings of the Twenty First Workshop Geothermal Reservoir Engineering, January 22-24, 1996. SGP-TR-151-34. Stanford University, California, 241–246

Monasterio A, Valles J, Pettinari G, Setti M, López-Galindo A, Baschini M (2015) Maduración de peloides en ambiente natural: experiencia en las Termas de Copahue. In Balnea10 IV CICAP BOI; Maraver F, Vela L, Ankli W, Eds. 255-264. ISBN 978-84-606-9368-0

Nodlovu S, Simate G, Mchibwa K, Giaveno MA (2014) Characterization of nanoprecipitates formed from the forced hydrolysis of bioleach liquors under different pH conditions. J Ind Eng Chem 20:3578–3583

Pedrozo F, Kelly L, Diaz M, Temporetti P, Baffico G, Kringel R, Friese K, Mages M, Geller W, Woelfl S (2001) First results on the water chemistry, algae and trophic status of an Andean acidic lake system of volcanic origin in Patagonia (Lake Caviahue). Hydrobiologia 452:129–137

Pedrozo FL, Temporetti PF, Beamud G, Diaz MM (2008) Volcanic nutrient inputs and trophic state of Lake Caviahue, Patagonia, Argentina. J Volcanol Geoth Res 178:205–212

Petersen J (2010) Modelling of bioleach processes: connection between science and engineering. Hydrometallurgy 104:404–409

Plumb J, Haddad CM, Gibson JA, Franzmann PD (2007) *Acidianus sulfidivorans* sp. nov., an extremely acidophilic, thermophilic archaeon isolated from a solfatara on Lihir Island, Papua New Guinea, and emendation of the genus description. Int J Syst Evol Microbiol 57:1418–1423

Rawlings DE, Johnson BD (eds) (2007) Biomining. Springer, Berlin

Russo G, Libkind D, Sampaio J, van Broock M (2008) Yeast diversity in the acidic Rio Agrio-Lake Caviahue volcanic environment (Patagonia, Argentina). FEMS Microbiol Ecol 65:415–424

Schippers A (2007) Microorganisms involved in bioleaching and nucleic acid-based molecular methods for their identification and quantification. In: Microbial Processing of Metal Sulfides. E. Donati and W. Sand Eds. Springer.The Netherlands. 3–33

Segerer A, Neuner AM, Kristjansson JK, Stetter K (1986) *Acidianus infernus* gen. nov. sp. nov. and *Acidianus brierleyi* comb. nov.: facultatively aerobic, extremely acidophilic thermophilic sulfur-metabolizing archaebacteria. Int J Syst Bacteriol 36:559–564

Stewart EJ (2012) Growing unculturable bacteria. J Bacteriol 194:4151–4160

Temporetti P, Snodgrass K, Pedrozo F (2013) Dynamics of phosphorus in sediments of a naturally acidic lake. Int J Sediment Res Issue 1:90–102

Ulloa R, Giaveno A, Donati E (2012) Adaptation of acidophilic microbial community from Andacollo to be used in biomining processes. XI Jornadas Argentinas de Tratamiento de Minerales, Neuquén, Argentina 301-306. ISBN978-987-604-311-3

Urbieta MS, González Toril E, Aguilera A, Giaveno MA, Donati E (2012) First prokaryotic biodiversity assessment using molecular techniques of an acidic river in Neuquén, Argentina. Microb Ecol 64:91–104

Urbieta MS, Toril EG, Giaveno MA, Bazan AA, Donati ER (2014a) Archaeal and bacterial diversity in five different hydrothermal ponds in the Copahue region in Argentina. Syst Appl Microbiol 37:429–441

Urbieta MS, Rascovan N, Castro C (2014b) Draft genome sequence of the novel thermoacidophilic archaeon *acidianus copahuensis* strain ALE1, isolated from the copahue volcanic area in Neuquén, Argentina. Genome Announc 2(3):e00259–14. doi:10.1128/genomeA.00259-14

Urbieta MS, González Toril E, Bazán ÁA, Giaveno MA, Donati E (2015a) Comparison of the microbial communities of hot springs waters and the microbial biofilms in the acidic geothermal area of Copahue (Neuquén, Argentina). Extremophiles 19:437–450

Urbieta MS, Porati GW, Segretín AB, González Toril E, Giaveno MA, Donati ER (2015b) Copahue geothermal system: a volcanic environment with rich extreme prokaryotic biodiversity. Microorganisms 3:344–363

Vallés JM, Baschini MT, Pettinari GR, García N (2004) Characterization of Muds and Waters of the Copahue Geothermal Field, Neuquen Province, Patagonia Argentina. 8th International Congress on Applied Mineralogy 1, 507-510. ISBN 85-98656-01-1, www.icam2004.org

Varekamp JC (2004) Copahue volcano: a modern terrestrial analog for the opportunity landing site? Eos 85(41):401–407

Varekamp J, Maarten deMoor J, Merrill M, Colvin A, Goss A, Vroon P, Hilton D (2006) Geochemistry and isotopic characteristics of the Caviahue-Copahue volcanic complex, Province of Neuquén, Argentina. Geol Soc Am Spec Pap 407:317–342

Varekamp JC, Ouimette AP, Herman SW, Flynn KS, Bermudez A, Delpino D (2009) Naturally acid waters from Copahue volcano, Argentina. Appl Geochem 24:208–220

Vartoukian SR, Palmer RM, Wade WG (2010) Strategies for culture of ‘unculturable’ bacteria. FEMS Microbiol Lett 309:1–7

Vélez ML, Euillades P, Caselli A, Blanco M, Martínez Díaz J (2011) Deformation of copahue volcano: inversion of InSAR data using a genetic algorithm. J Volcanol Geoth Res 202:117–126

Volesky B (2003) Sorption and biosorption. Sorbex, Montreal

Wang J, Chen C (2009) Biosorbents for heavy metals removal and their future. Biotechnol Adv 27:195–226

Watling HR (2015) Review of biohydrometallurgical metals extraction from polymetallic mineral resources. Minerals 5:1–60

Wendt-Potthoff K, Koschorreck M (2002) Functional groups and activities of bacteria in a highly acidic volcanic mountain stream and lake in Patagonia, Argentina. Microb Ecol 43:92–106

Yoshida N, Nakasato M, Ohmura N, Ando A, Saiki H, Ishii M, Igarashi Y (2006) *Acidianus manzaensis* sp.nov., a novel thermoacidophilic archaeon growing autotrophically by the oxidation of H_2 with the reduction of Fe^{3+}. Curr Microbiol 53:406–411