# Microbial communities in high altitude altiplanic wetlands in northern Chile: phylogeny, diversity and function

#### **DISSERTATION**

Zur Erlangung des Doktorgrades der Mathematisch-Naturwissenchaflichen Fakultät der Christian-Albrechts-Universität zu Kiel

vorgelegt von

Cristina Inés Dorador Ortiz

aus Antofagasta, Chile

Max Planck Institut für Evolutionsbiologie, Plön IFM-GEOMAR, Kiel Mai 2007

Referent: Prof. Dr. Johannes F. Imhoff

Korreferent: Prof. Dr. Irma Vila

Tag der mündlichen Prüfung: 06. Juli 2007

Zum Druck genehmigt: 06. Juli 2007

Der Dekan

The work of this thesis was conducted between April 2004 and Mai 2007 in the MPIL (now Max Planck Institute for Evolutionary Biology) in Plön under the supervision of Dr. Karl-Paul Witzel and at Leibniz Institute for Marine Sciences (IFM-GEOMAR) in Kiel under the supervision of Prof. Dr. Johannes F. Imhoff.

I received funds from the Deutscher Akademischer Austausch Dienst (DAAD) for the realization of this thesis.



# TABLE OF CONTENTS

Summary
Zusammenfassung
Resumen
Thesis outline
1. Introduction6
2. Materials and Methods
3. Comparative analysis of bacterial and archaeal communities in different high altitude
wetlands in Northern Chile
4. Diversity of Archaea in environmental samples from Salar de Huasco
5. Diversity and composition of photosynthetic bacterial communities in Salar de
Huasco
6. Salt tolerance of enrichment cultures of ammonia oxidizing bacteria from Salar de
Huasco
7. Molecular analysis of halophilic bacteria isolates from Salar de Huasco
8. Discussion
Conclusion
Individual scientific contributions to multiple-author publications
References
Acknowledgements
Curriculum Vitae
Erklärung

#### **SUMMARY**

The phylogeny, diversity and function of microbial communities from several altiplanic wetlands was examined using an array of different but complimentary techniques. Results highlighted that microbial diversity exhibited a specific pattern in each wetland. Bacteria were dominant over Archaea in both freshwater and saline systems. Bacterial and archaeal diversity were both higher in sediment than in water samples. Lago Chungará, Laguna de Piacota and Bofedal de Parinacota are freshwater wetlands located at high altitude (>4400 m) in the north of Chile. They support microbial communities closely related to psychrophilic bacteria (e.g. *Psychrobacter* sp., Pseudomonas congelans, Flavobacterium psychrolimnae) in water and Proteobacteria and Actinobacteria in sediment samples. Salar de Huasco and Salar de Ascotán are located further south at an altitude of 3800 m and exhibit a wide range of salinities (varying between freshwater to 120 gL<sup>-1</sup> of total dissolved salts). Microbial communities in these sites were characterized by bacteria tolerant to salt (e.g. halophilic Bacteria: Halomonas sp., halophilic Archaea: Halorubrum sp.). Cytophaga-Flavobacteria-Bacteroidetes was the most frequent group reported at the sites. In-depth studies focussing on the Salar de Huasco revealed a particular diversity of Archaea, characterized by a number of sequences related to uncultured groups and ammonia-oxidizing Crenarchaeota. Cyanobacteria from the Salar de Huasco were closely related to Cyanobacteria previously described from Antarctica. Isolates of halophilic bacteria and phototrophic bacteria displayed an elevated tolerance to different salt concentrations. The particular microbial diversity found in high altitude wetlands provides a new and exciting area of research.

#### ZUSAMMENFASSUNG

Die Phylogenie, Diversität und Funktion von mikrobiellen Gemeinschaften aus verschiedenen altiplanischen Feuchtgebieten wurde mit mehreren Techniken untersucht. Die Ergebnisse zeigen dass an jedem Standort die mikrobielle Diversität eine spezifische Struktur hat. Bakterien und Archaeen Diversität war höher im Sediment als in den Wasserproben. Lago Chungará, Laguna de Piacota und Bofedal de Parinacota sind auf über 4400 Metern Höhe gelegene Süßwasser-Feuchtgebiete in Nord-Chile. Die mikrobiellen Gemeinschaften waren im Wasser verschiedenen psychrophilen Bakterien (z. B. Psychrobacter sp., Pseudomonas congelans, Flavobacterium psychrolimnae) und im Sediment Proteobakterien und Actinobakterien ähnlich. Salar de Huasco und Salar de Ascotán liegen südlich auf 3800 m Höhe und die Salinität des Wassers schwankt in den verschiedenen Habitaten von Süßwasser bis 120 gL<sup>-1</sup> Salz gelöst im Wasser. Die mikrobielle Diversität an diesen Standorten ist charakterisiert durch salztolerante Bakterien (z.B. halophile Bakterien der Gattung *Halomonas* und halophile Archaeen der der CFB-Gruppe (Cytophaga-Flavobacterium-Halorubrum. Vertreter Bacteroidetes) waren in den Klonbibliotheken häufig. Im Salar de Huasco war die Diversität von Archaeen besonders hoch, und es wurden zahlreiche Sequenzen gefunden, die nicht kultivierten Archaeen und Ammoniak-oxidierenden Crenarchaeota ähnlich waren. Die Cyanobacterien aus dem Salar de Huasco waren sehr ähnlich mit Cyanobacterien aus der Antarktis. Isolate von halophilen Bakterien und phototrophen Bakterien zeigten eine hohe Toleranz gegenüber verschiedenen Salzkonzentrationen. Die besondere mikrobielle Diversität dieser Feuchtgebiete im Hochland der Anden bietet ein neues und spannendes Forschungsgebiet.

#### RESUMEN

La filogenia, diversidad y función de las comunidades microbianas fueron estudiadas en varios humedales altiplánicos con técnicas distintas y complementarias. Los resultados señalan que la diversidad microbiana exhibe un patrón específico en cada humedal. Bacteria fue dominante sobre Archaea en sistemas de agua dulce y salinos. La diversidad de Bacteria y Archaea fue mayor en las muestras de sedimento que en las de agua. El lago Chungará, la laguna de Piacota y el Bofedal de Parinacota son humedales de agua dulce ubicados a gran altura (>4400 m de altitud) en el norte de Chile. Estos sistemas contienen comunidades microbianas altamente relacionadas con bacterias psicrófilas en agua (por ejemplo, Psychrobacter sp., Pseudomonas congelans, Flavobacterium psychrolimnae) y en sedimentos Proteobacteria y Actinobacteria. El salar de Huasco y el salar de Ascotán están ubicados hacia el sur a una altura de 3800 m de altitud y muestra un amplio rango de salinidad (desde agua dulce hasta 120 gL<sup>-1</sup> de sales totales disueltas). Las comunidades microbianas en estos sitios están caracterizadas por ser tolerantes a la sal (por ejemplo, Bacteria halófila: Halomonas sp., Archaea halófila: Halorubrum sp.). Cytophaga-Flavobacteria-Bacteroidetes fue el grupo más frecuente en los sitios estudiados. Los estudios realizados en el Salar de Huasco revelaron una diversidad particular de Archaea caracterizada por un número de secuencias altamente relacionadas con grupos no cultivados y con Crenarchaea oxidadoras de amonio. Las cianobacterias del Salar de Huasco presentaron altas similitudes con las cianobacterias previamente descritas en la Antártida. Los aislados de bacterias halófilas y bacterias fototrófas muestran una alta tolerancia a la sal a distintas concentraciones. La particular diversidad microbiana encontrada en humedales altoandinos proporciona una nueva e interesante área de investigación.

## THESIS OUTLINE

Here, I use PCR-DGGE as a fingerprinting tool to compare microbial patterns from samples and clone libraries of 16S rRNA gene and *amoA* gene (Archaea and Bacteria) to infer phylogenetic relationships between the sequences. Bacteria and archaeal communities of Lago Chungará, Laguna de Piacota, Bofedal de Parinacota, Salar de Huasco and Salar de Ascotán are described using clone libraries of 16S rRNA gene (Chapter 3). Chapter 4 focuses on four sites of the Salar de Huasco and describes the composition of archaeal communities and the presence of archaeal *amoA*. Chapter 5 describes diversity and composition of photosynthetic bacteria including Cyanobacteria and phototrophic bacteria. Chapter 6 reports salt-tolerant enrichment cultures of ammonia oxidizing bacteria using 16S rRNA gene and bacterial *amoA* gene. Chapter 7 reports the growth of isolates of halophilic Bacteria at moderate and high concentrations of salt.

#### 1. INTRODUCTION

The aim of this thesis was the study of several diverse aspects of the microbial communities of wetlands located in the Chilean Altiplano, including phylogeny, diversity and function. The wetlands are located in the Altiplano, a high altitude plateau of the Andes mountain range.

# 1.1 Features of the Altiplano

# 1.1.1 Geomorphology

The north of Chile exhibits a particular and extreme relief. There is a 15000 m difference between the marine Atacama Trench in the Pacific Ocean (maximum depth 8065 m) and the summit of the Llullaillaco volcano (6723 m altitude) in the Andes, over a linear distance of less than 300 km. Within this region, several distinct morphostructural units have been identified, running from the west to the east (Chong, 1984; Charrier and Muñoz, 1997; Risacher et al., 2003); i) the Coast Range: a mountain chain (mean altitude 1500 m) located in the west, close to the Pacific Ocean, originally formed of marine sedimentary rocks of Mesozoic age; ii) the Central Depression: also known as the Atacama Desert. This region, of middle Tertiary to Holocene origin, is considered to represent the driest and oldest extant desert on Earth (Hartley et al., 2005). and has an altitude of 800 to 1400 m; iii) the Precordillera: the Domeyko Range is the highest part of the Precordillera (3500-4000 m altitude) and includes large copper ore deposits; iv) the Pre-Andean Depression: this large intramontane basin (altitude 2500 m) contains the Salar de Atacama, the largest evaporitic basin of Chile (3000 km<sup>2</sup>); v) the Western Cordillera (Chilean Altiplano): this elevated (4000 m) plateau is surrounded by numerous volcanoes reaching 6500 m elevation, that often delineate interior drainage basins that incorporate saline lakes and saline crusts; vi) the Bolivian Altiplano: this huge high plateau separates the Western Cordillera from the Eastern Cordillera, located in Bolivia, and includes the Salar de Uyuni, which is the largest evaporitic basin on Earth (surface area =  $10000 \text{ km}^2$ ).

In the current thesis, a series of water bodies were studied, all located in the Western Cordillera also referred to as the Chilean Altiplano. The Altiplano is located at latitudes between 15° and 27° S, at a mean altitude of 3700 meters above sea level. It is surrounded by volcanoes and mountains rising to 6700 meters, that occupy part of Peru, Bolivia and Chile, and cover an area 300 km wide and 1500 km long (Charrier and Muñoz, 1997). The Andes Range extends N-S, perpendicular to the zonal westerly airflow of the mid-latitudes, which creates distinct environmental gradients at meso- and micro-scales (Grosjean and Veit, 2005).

Together with the Himalayas, the Altiplano represents one of the largest plateaus in the world, and is the only plateau located in an active subduction zone (Charrier and Muñoz, 1997). Understanding the origin of the Altiplano plateau represents one of the most intriguing problems regarding the formation of mountains, and a series of models exist that attempt to explain their development. The origin of the Andes Range is related with the subduction of the Nazca Plate under the occidental region of South America. This process has also been associated with climate change during the Cenozoic (Lamb and Davis, 2003; Rech et al., 2006). The flat surface of the Altiplano was formed via a series of erosion processes and subsequent refilling by sedimentary and volcanic materials. Recent volcanism events have led to the presence of thermal waters and

geysers. Currently, the surface of the Altiplano includes a series of endorheic basins in the south (e.g., salares) and large lakes in the north (e.g., Lago Chungará, Lago Titicaca) (Charrier and Muñoz, 1997).

## 1.1.2 Climate

The Altiplano has distinctive meteorological conditions where the climatic variability has a strong impact on the availability of water resources in this semi-arid region. Paleoclimatic records indicate that environmental conditions in the Central Andes have undergone significant changes in the past (Garreaud et al., 2003). The present climate is influenced by the following factors:

- i) Reduced atmospheric pressure: at 4000 meters altitude atmospheric pressure in the Altiplano is 40% less than at sea level. The influence of low temperatures means that atmospheric pressures are actually 35% lower than at sea level (Aceituno, 1997).
- ii) Solar radiation: midday solar radiation reaching the surface of the Altiplano ranges from 600 to 1100 Wm<sup>-2</sup> during the year, (Hardy et al., 1998) and varies less than 30% from winter to summer. Due to the high altitude of the Altiplano, the atmosphere does not include the elements necessary to disperse solar radiation, resulting in an effective increase in radiation, particularly in the UV part of the spectra. On clear days, UV-B radiation levels in the Altiplano can be 20% higher than at the sea level (Cabrera et al., 1994). The reduced density of greenhouse gases (e.g. CO<sub>2</sub>, H<sub>2</sub>O) in the Altiplano makes the atmosphere more transparent to infrared wavelengths, resulting in a rapid cooling during the night (Aceituno, 1997).

iii) Temperature: average temperatures in the Altiplano are low. The region is characterized by extreme daily temperature cycles (e.g. -30°C at night and 5°C during the day in winter, and 5°C at night and 20°C in the day during summer: Fräre et al., 1975). iv) Precipitation: precipitation in the Altiplano largely results from Amazonian water vapor, occurring principally in the austral summer season (November to March), and especially on the western side of the Altiplano. Seasonal, intraseasonal (episodic) and interannnual variability is controlled by the amount of near-surface vapor (Garreaud et al., 2003). There is a clear daily cycle where maximum rainfall frequency and intensity occurs in the afternoon and early evening. Seasonal variations in rainfall are principally due to the solar cycle. Interannual precipitation variability is driven by strong climatic fluctuations varying from extremely dry to extremely wet austral summer conditions. This variability has been associated with the El Niño Southern Oscillation (ENSO). A series of studies have concluded that El Niño years (ENSO warm phase) tend to be dry and La Niña years (ENSO cold phase) are associated with wet conditions in the Altiplano. However, dry La Niña and wet El Niño years sometimes occur, revealing the complexity associated with climatic variation in the Altiplano (Garreaud et al., 2003). v) Evapotranspiration: evaporation levels are greater than precipitation, resulting in a negative water balance in the altiplanic endorheic basins. Between 75 and 90% of the precipitation inputs in the altiplanic basins is lost by natural evapotranspiration, and the rest is lost by runoff (Salazar, 1997).

#### 1.1.3 Flora and Fauna

Many of the flora and fauna of the Altiplano are endemic and many species face the risk of extinction (Muñoz et al., 1996; Glade, 1993; Benoit, 1989). The flora of the

northern Chilean Andes includes 865 plant species, of which 21% are endemic to Chile (Arroyo et al., 1997). Vegetation zonation is related with orographic effects (e.g. Luebert and Gajardo, 2005; Betancourt et al., 2000; Rundel and Palma, 2000; Arroyo et al., 1997). The upper altitudinal limit of vascular plants is 4500 m, and reflects an isotherm of 0°C. Between altitudes of 3800 and 4500 m, annual precipitation of 150 to 230 mm supports Andean steppe grasses (e.g. Festuca ortophylla, Jarava frigida, Stipa chrysophylla and Deyeuxia breviaristata) and cushion plants (Azorella compacta). Between 3200 to 3800 m, am annual precipitation of 70 to 150 mm supports Tolar shrubs (Parastrephia spp., Chuquiraga spp., Lampaya medicinalis, Junellia seriphioides and Fabiana spp.) and columnar cacti (Trichocereus atacamensis and Oreocereus leucotrichus). Between 2600 and 3200 m, a mean annual precipitation of 20 to 70 mm supports few annual plants, including salt-tolerant shrubs (Atriplex imbricata) and cushion cacti (Opuntia camachoi). There is a lack of vegetation at lower altitudes in the Atacama Desert due to the absence of rain.

The fauna of the Andean Altiplano is depauperate compared with the fauna located at lower altitudes. Amphibians, reptiles, fishes, birds and mammals all exhibit particular adaptations to conditions at high altitudes, low atmospheric pressure, high solar radiation and dryness in the Altiplano (Raggi, 1997). Approximately 150 species of birds and 31 species of mammals are reported in the Lauca Biosphere Reserve (UNESCO) located at an altitude of 4000 m (Rundel and Palma, 2000). From the six species of flamingos described globally, three inhabit this area: the Chilean flamingo (*Phoenicopterus chilensis*), the Andean flamingo (*Phoenicoparrus andinus*) and the James flamingo (*Phoenicoparrus jamesi*) (Raggi, 1997). The open *Festuca* grassland is

also habitat for several bird species including the lesser rhea (*Pterocnemia pennata*). Aquatic bird populations are particularly abundant in the Lago Chungará area. The giant coot (*Tagua gigante*), silvery grebe (*Podiceps occipitalis*), and Chilean teal (*Anas flavirostris*) are the most abundant species (Rundel and Palma, 2000). Within the mammalia, the Altiplano provides habitat for three South American camelidae: the wild species vicuña (*Vicugna vicugna*) and guanaco (*Lama guanicoe*) and the domesticated llama (*Lama glama*) and alpaca (*Lama pacos*). The natural predator of the camelidae is the Chilean puma (*Felis concolor*). Many of the mammals, reptiles, amphibia, birds and fishes found in the Altiplano are under danger of extinction (Glade, 1993).

## 1.2 Origin of high altitude altiplanic wetlands

During the Quaternary, large lakes occupied the center of the Altiplano and the small intravolcanic basins in the south. Two main lacustrine phases have been described: a humid pre-late glacial maximum in the Minchin period (30000-25000 yr BP) and a late glacial/early Holocene humid phase known as the Tauca (12000-10000 yr BP) (Servant and Fontes, 1978). Paleoecological studies and geochemical models have suggested that the Minchin and Tauca paleolakes were deep and saline (Risacher and Fritz, 1991). During the Tauca phase, plant diversity and primary production were higher than in the present – for example estimates of vegetation cover were between 50-80% in areas where contemporary vegetation cover is <5% (Latorre et al., 2002). Furthermore, an increased level of animal diversity (e.g. extinct horses) and initial indications of human occupation are both reported from this period (Núñez et al., 2002; Núñez, 1992). The extreme climatic variation during the last period of the Pleistocene including humid phases and

monsoonal precipitation has been revealed using several scientific methods including pollen profiles from wetlands, paleosols and archaeological sites (e.g., Núñez et al., 2002), plant macrofossils in rodent middens (Betancourt et al., 2000; Latorre et al., 2002) and sediment from closed-basin Altiplano lakes (e.g. Grosjean, 1994).

The water bodies currently found in the Altiplano are testimony to the existence of ancient paleolakes. As consequence of the arid conditions of the Holocene, paleolakes were restricted to several evaporitic endorheic basins currently found in the Altiplano. However, aridity was interrupted by humid periods during the mid-Holocene revealing a complex spatial and temporal pattern during this time (Moreno et al., 2007). Since 2000 yr BP until the present, arid conditions have prevailed, resulting in a decrease in the availability of water (Romero et al., 1997).

# 1.3 Ecology of altiplanic wetlands

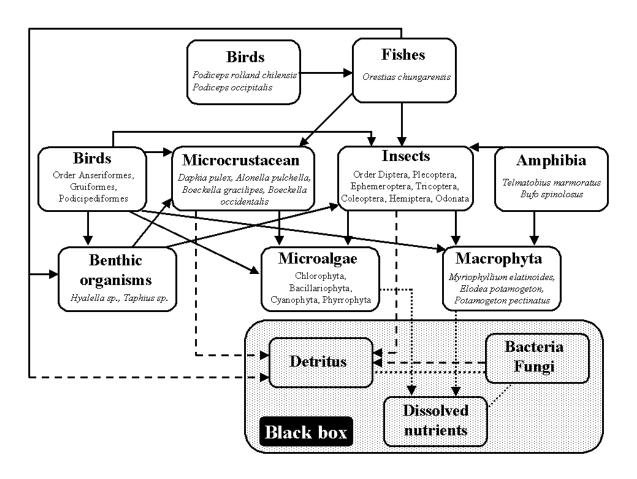
# 1.3.1 Description of altiplanic wetlands

Water availability in the Altiplano is the most important factor that determines and supports the Andean biota (Vila, 2002). Salares located in the Central Depression (Atacama Desert) represent relict wetlands (they receive no water), while the salares located in the Altiplano are hydrologically active and receive water inputs (Risacher et al., 1999). Three main sources of salt have been detected in inflow waters: atmospheric inputs through precipitation and dry fallout; stem from sea salts and desert dust (especially NO<sub>3</sub>-, Br and As), volcanic rock alteration, and brine recycling (Risacher et al., 2003). In the Chilean Altiplano (including the western Cordillera and the pre-Andean Depression) there are a total of 51 closed basins distributed along a 1000 km north-south

gradient along the Andes Range (Risacher et al., 2003). These closed basins are heterogeneous in terms of their physical, chemical and biological status, and have been classified as lake (1), lagoons (15) and salares (35). Lago Chungará, among the highest (4520 m) freshwater lakes in the world, is also the deepest (32 m) water body of the Chilean Altiplano. This lake, along with the Laguna del Negro Francisco (4110 m) located in the southern part of the Chilean Altiplano, have surface areas far larger than other water bodies (22.5 and 24.8 km<sup>2</sup> respectively) in the region. Laguna del Negro Francisco along with other altiplanic lagoons has a high salinity (maximum of total dissolved salts 328 gL<sup>-1</sup>). The salares with the greatest salinities have a maximum concentration of total dissolved salts of 340 gL<sup>-1</sup> (e.g. Salar de Atacama, Salar de Aguas Calientes). Although they are heterogeneous, they typically represent salt-saturated systems. Other aquatic systems of the Altiplano locally referred to as "bofedales" (peatlands, highland bogs) are located between 3200 to almost 5000 m in the north and central part of the Altiplano, and at elevations greater than 2800 m in the southern limit of the Altiplano (Squeo et al., 2006). Bofedales appear as green oases in valley bottoms, shallow basins and other areas of low relief in the arid landscape of the Altiplano (Villagrán and Castro, 2003). They play a critical role in sustaining a unique diversity of rare and endemic biota in the Andes (Squeo et al., 2006; Vila, 2002). These peatlands have areas from <1 to hundreds of hectares, and are formed mainly by association of members of Juncaceae, with the most common species being Oxychloe andina and Potasia clandestina (Squeo et al., 2006). Several bofedales in northern Chile are severely degraded and reduced in scale (e.g. Villagrán and Castro, 2003; Earle et al., 2003; Squeo et al., 2006). Some authors have proposed that this degradation is product of autoregulation processes of the local hydrological system (Earle et al., 2003). But this mechanism has not been studied in sufficient detail in other bofedales to permit a clear conclusion as to the reasons for the recent changes (Squeo et al., 2006).

The Altiplano is extremely sensitive to changes in effective moisture (precipitation minus evaporation). Even the smallest changes in the water budget could result in significant and amplified responses in the mostly saline and shallow lakes, via modifications of geomorphological forms and processes, vegetation changes and in other variations in the biogeochemical systems (Grosjean and Veit, 2005). The high water demand in the region (e.g. to support mining activities, groundwater extraction for lowland agriculture, urbanization) exceeds the availability of water. Mining operations have resulted in a decrease in the levels of many aquifers. Future proposals include the extraction of water from the Salar de Aguas Calientes, Laguna Tuyajto and Salar del Laco at levels of 1027 Ls<sup>-1</sup> over the next 20 years (La Nación, 2007). Paleo-research has revealed that the bulk of current groundwater resources comes from previous humid phases (pre-LGM, late-glacial and early Holocene). Hence, these resources are non-renewable and may be close to their limits (Grosjean and Veit, 2005).

Climate predictions estimate an increase of temperatures (>5°C) in the surface of the Altiplano and an increase of precipitation in the eastern part at the end of the XXI century. Therefore, a reduction of the Andean area capable to store snow due to the increase of the altitude of the 0°C isotherm would trigger an increase of the river volume flow, increase of basins water volume and a decrease in the water reserve (CONAMA, 2006).



**Fig. 1-1.** Intralacustrine trophic interactions in Lago Chungará, reproduced with permission of the author (Vargas, 2002).

## 1.3.2 Lacustrine trophic interactions

Figure 1-1 shows a conceptual model of the intralacustrine trophic interactions in Lago Chungará (Vargas, 2002). This model was prepared with the bibliographic information available about Lago Chungará and concludes that macrophytes represent a key component in the maintenance of the community structure. Macrophytes currently provide food, refugia, substrate and nesting sites for several taxa, including aquatic insects. The macrophyta belt around the lake supports most of the biota in the lake which would be affected directly by a possible decrease of the water volume. As primary producers, macrophytes are typically associated with herbivores: insects, microcrustacea

and mollusks which provide food for fish, amphibians, reptiles and birds, that together with the Aymara ethnic group of humans, represent the top predators of the system. The role of microorganisms and the means of nutrient recycling can be currently considered a black box in the Lago Chungará and other Altiplano water bodies.

#### 1.4 Comparison of Altiplanic wetlands with other similar aquatic systems

The water bodies located in the Altiplano are characterized by high altitude and their typically elevated salinities, and this distinguishes them from most other aquatic ecosystems.

High altitude lakes are located in upland areas of the Andes, North and Central America, East Africa, Asia and Europe. Most tropical high altitude lakes are located in the Andes Range, and exhibit an ecological continuity supporting both tropical and subantarctic flora and fauna (Vila and Mühlhauser, 1987). In the European Alps and Pyrenees lakes are found to a maximum altitude of 4800 m, and they are characterized by low ionic content and are typically extremely oligotrophic.

Saline water bodies are widely distributed in arid regions around the world. They include a variety of aquatic ecosystems: i) the Caspian Sea, Mono Lake and Dead Sea. These systems never totally desiccate but water levels may fluctuate considerably over long periods; ii) in arid regions many salt lakes are filled with water only episodically or only after episodic rain (e.g. Lake Eyre in Australia); iii) in semi-arid regions. Here annual rainfall patterns are typically predictable, and many salt lakes lack surface water in the dry season but are filled annually during the wet season (Williams, 2002). The salares described n the current study receive water inputs each year. However, due to

interannual variability in precipitation (principally reflecting the ENSO phenomena), some lagoons periodically become desiccated.

Saline water bodies vary with regard to their salt composition and can also be characterized according to their origin. Thalassohaline water bodies have a marine origin and their salt composition is the same than marine waters (NaCl dominated). They contrast with athalassohaline lakes that are rich in ions other than chloride or sodium. Studies in solar salterns (thalassohaline) show a decrease in microbial diversity at high salinities (e.g. Pedrós-Alió, 2005) and a typically low level of microbial diversity, e.g. in Maras Saltern, located at 3380 m in the Peruvian Andes from where only two groups of Archaea and one group of Bacteria were reported (Maturrano et al., 2006).

In terms of their geological origin and physical characteristics, the Andes are more similar to the Himalayas than to the European Alps (Kley and Eisbacher, 1999). Recent studies of Lake Chaka and Lake Quinghai, two athalassohaline lakes located on the Tibetan Plateau (NW China) at an altitude of 3200 m (Dong et al., 2006; Jiang et al., 2006) revealed similarities in the composition of microbial communities (e.g. dominance of *Cytophaga-Flavobacteria-Bacteroidetes* and *Proteobacteria*) with athalassohaline water bodies of the Altiplano. This similarity probably reflects common environmental conditions, as water bodies located in the Tibetan Plateau and the Altiplano are athalassohaline and located at high altitude, with similar abiotic conditions including UV-B radiation and negative water balances.

# 1.5 Microbial studies in altiplanic wetlands

Microbiological studies in the Altiplano are scarce and are dispersed throughout the literature. Initial surveys attempted to obtain cultures of microorganisms from soils and salt crusts of the Atacama Desert, because of the apparent similarity with the conditions of Mars (Cameron et al., 1966; Opfell and Zebal, 1967). In a solfataric pool in the Geysers del Tatio (4750 m) a methanogenic archaeon, Methanogenium tatii (Zabel et al., 1984) (reclassified as *Methanofollis tationis*: Zellner et al., 1999) was isolated. Several studies have been conducted in the Salar de Atacama. Strains of moderately halophilic bacteria have been analyzed by numerical taxonomy (Valderrama et al., 1991) and chemotaxonomic analysis (Márquez et al., 1993). Rivadeneyra et al. (1999) described the biomineralization of carbonates by Marinococcus albus and Marinococcus halophilus, two moderately halophilic Gram-positive bacteria isolated from Salar de Atacama. Campos (1997) reported that 35% of the isolates from different samples and sites in the Salar de Atacama were halophilic microorganisms. The moderate halophilic bacteria belonging to the genera Marinomonas, Vibrio, Alteromonas, Marinococcus, Acinetobacter and Micrococcus and the halotolerant bacteria were described as Bacillus, Pseudomonas-Deleya, Micrococcus, Acinetobacter and Staphylococcus. Also, members of Cyanobacteria were isolated: Anabaena, Gloeothece, Synechococcus, Gloecapsa, Nostoc and Oscillatoria. Cyanobacteria in the Laguna Tebenquinche (Salar de Atacama) were represented only by Oscillatoria (Zúñiga et al., 1991). Studies of microbial mats found in the Salar de Llamará revealed the presence of the Cyanobacteria Cyanothece, Synechococcus, Microcoleus, Oscillatoria, Gloeocapsa and Gloeobacter and the phototrophic bacteria *Chromatium* and *Thiocapsa* in different mats. All these studies used culture-dependent methods or microscopy to classify microbial communities.

The advent of molecular biological tools based on the comparison of DNA sequences obtained directly from environmental samples and not requiring prior cultivation, have permitted the identification of microorganisms from particular habitats (or inferred from their phylogenetic affiliations). The pioneer work of Woese et al. (1990) that utilized ribosomal RNA (rRNA) as a tool to classify taxa, described three domains of life: Bacteria (formerly eubacteria), Archaea (formerly archaebacteria) and Eukarya (formerly eukaryotes). rRNA has several advantages over alternative means of establishing phylogenetic relationships between very different organisms: The conserved nature of rRNA structure extends to the nucleotide sequence level, and signature sequences can be found in different domains and subdivisions (Woese, 1987). rRNA genes also appear to be free of artifacts of lateral transfer between phylogenetically distant organisms (Stackebrandt and Woese, 1981). There are three ribosomal RNAs: 5S, 16S/18S and 23S/28S. The 5S is too small to make phylogenetic inferences (120 bp), 23S/28S (23S in prokaryotes, 2900 bp) can provide a good indication of phylogenetic relationships between closely related taxa, but not between the more distantly related branches (Olsen and Woese, 1993). 16S/18S (16S in prokaryotes, 1500 bp) is widely used to infer relationships between prokaryotes. Ludwig et al., (1998) proposed the combined use of 23S and 16S rRNA genes to construct congruent phylogenies.

Several fingerprinting methods have been developed to describe microbial communities from environmental samples. Among them Denaturing Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE) (Muyzer et

al., 1993; Muyzer, 1999) and Terminal Restriction Fragment Length Polymorphism (T-RFLP) (Liu et al., 1997) are widely used in studies of microbial ecology.

Demergasso et al., (2004) used PCR-DGGE to describe the microbial communities in different altiplanic wetlands, and *Cytophaga-Flavobacteria-Bacteroidetes* and *Proteobacteria* were frequently found at the sites. This work provided an initial description of microbial diversity in the Altiplano water bodies but sequences retrieved from DGGE excised bands are short and difficult to obtain.

The work described in this thesis examines microbial diversity in water and sediment samples from altiplanic wetlands. A screening of physical, chemical and biological parameters in altiplanic wetlands led us to hypothesize that microbial communities play an important role in biogeochemical cycles within these systems. Furthermore, we expected that trophic interactions between different groups of Bacteria and Archaea would influence biogeochemical cycling in different systems. Due to the likelihood of nitrogen limitation in the water bodies located in the Altiplano (Vincent et al., 1984; 1985; Dorador et al., 2003), the presence and diversity of ammonia oxidizers (the group responsible for the first step of nitrification, the key step in the N cycle) was examined using 16S rRNA and amoA (Bacteria and Archaea) genes in several samples and with enrichment cultures. The presence of phototrophic bacteria and Cyanobacteria (pink-red and green layers in the surface of sediments) led us to question the role and composition of these two major groups of phototrophic microorganisms (i.e. microorganisms that use light as an energy source). As salares are saline water bodies, we also were interested in the composition of the archaeal communities, because this group is abundant in hypersaline waters. Another aspect that we studied was the tolerance of Bacteria to salt. We examined bacterial growth and the composition of ammonia oxidizers and phototrophic bacteria in enrichment cultures at a range of different salt concentrations.

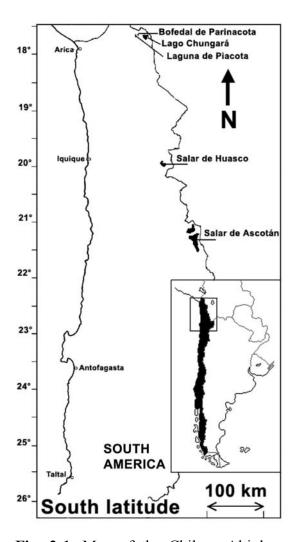
#### 2. MATERIALS AND METHODS

# 2.1 Site description and sampling

# 2.1.1 Altiplanic wetlands

Microbial diversity was examined at five different and contrasting wetlands situated at latitudes between 18° and 21°S in the Chilean Altiplano (Figs. 2-1, 2-2), and altitudes between 3700 and 4500 m. Precipitation occurs mainly in the northern part of the Altiplano and to a lesser degree in the south.

Lago Chungará (Chun) is the most southern of the intertropical Andean lakes and at the highest altitude (Dorador et al., 2003; Mühlhauser et al., 1995). Bofedal de Parinacota (Par) represents a shallow and small wetland system known locally as "bofedales" (peatlands) (Squeo et al., 2006). Laguna de Piacota (Pia) is a small lagoon located adjacent to Parinacota village (Vila, 2006). Salar de Huasco (Hua) and Salar de Ascotán (Asc) (Chong, 1984; Risacher et al., 2003) are two salt-flats situated further south. Some morphometric, physical and chemical characteristics of these aquatic ecosystems are shown in Table 2-1.



**Fig. 2-1.** Map of the Chilean Altiplano indicating the study sites.

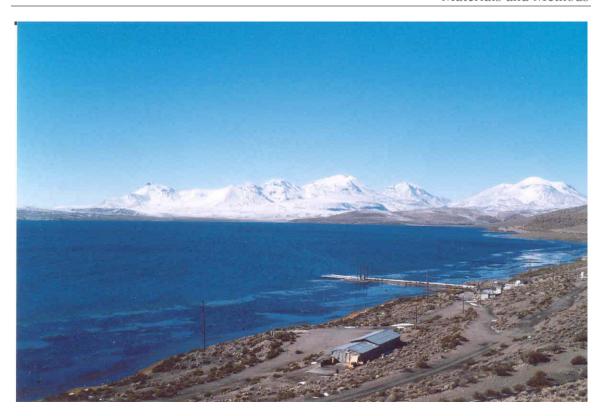


Fig. 2-2 (A). Lago Chungará



Fig. 2-2 (B). Bofedal de Parinacota



Fig. 2-2 (C). Salar de Ascotán

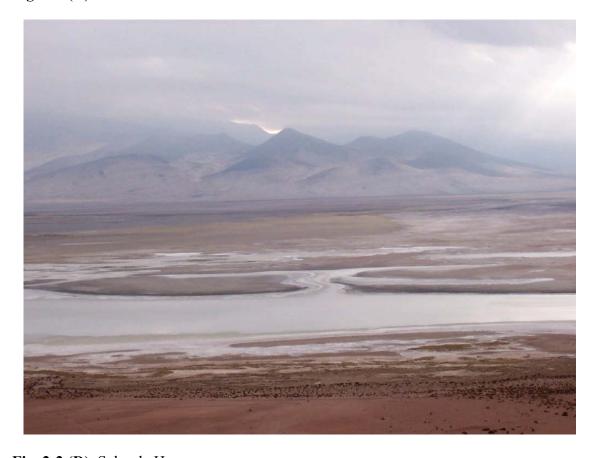


Fig. 2-2 (D). Salar de Huasco

**Table 2-1.** Morphometric and physicochemical characteristics of the studied wetlands in the north of Chile. Physical, morphometric and total dissolved salts data were taken from (Risacher et al., 2003), conductivity, nutrients, pH and ion concentrations were determined in this study.

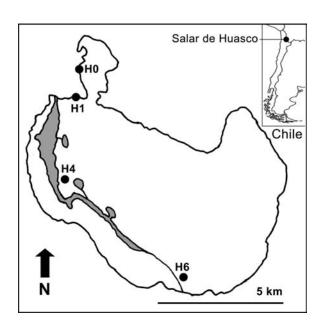
Characteristics	Sites				
	Lago Chungará	Bofedal de Parinacota	Laguna de Piacota	Salar de Huasco	Salar de Ascotán
Location	18°14'S, 69°09'W	18°11'S, 69°19'W	18°11'S, 69°15'W	20°18'S, 68°50'W	21°32'S, 68°22'W
Site of sampling	Shore	Shore	Shore	Shore, site H1	Shore, site Cebollar
Altitude (m)	4520	4300	4400	3800	3722
Maximum depth (m)	34	≤3	≤3	≤3	≤3
Area basin (km²)	273	100	nd	1572	1757
Area water (km <sup>2</sup> )	22.5	nd	nd	2.5	18
Air temperature annual mean (°C)	1.9	4.2	8.4	5	5.8
Precipitation (mm year <sup>-1</sup> )	338	321	256	150	125
Evaporation (mm year <sup>-1</sup> )	1230	1260	nd	1260	1630
Conductivity (µScm <sup>-1</sup> )	1500-2600	550	600	43200	45300
Total dissolved salts min (mgL <sup>-1</sup> )	47	108	nd	108	89
Total dissolved salts max (mgL <sup>-1</sup> )	1633	784	nd	113093	119853
pH	9-10	8-9	8-9	7.5-9	7-10
Total nitrogen µgNL <sup>-1</sup>	1253	772	nd	6399	1699
Total phosphorus μgPL <sup>-1</sup>	821	465	nd	9594	11311
$S-SO_4^{2-}mgL^{-1}$	90	35	nd	6535	1337
Anions	HCO <sub>3</sub> >SO <sub>4</sub> <sup>2</sup> ->Cl>NO <sub>3</sub>	HCO <sub>3</sub> >SO <sub>4</sub> <sup>2</sup> >Cl	nd	HCO <sub>3</sub>	Cl <sup>-</sup> >HCO <sub>3</sub> <sup>-</sup> >SO <sub>4</sub> <sup>2</sup> -
Cations	$Mg^{2+}>Na^{+}>Ca^{2+}>K^{+}$	$Na^{+}>Mg^{2+}>Ca^{2+}$	nd	Na <sup>+</sup> >Ca <sup>2+</sup> >Mg <sup>2+</sup>	$Na^{+}>Ca^{2+}>Mg^{2+}$

nd: no determined

During the southern hemisphere winter of 2003 (June), water samples from each of these wetlands were collected for physicochemical measurements. Temperature was recorded with a digital Hanna HI thermometer, pH with a Hanna HI 8314 meter, and conductivity with an YSI 33 meter. Total nitrogen, phosphorus and sulfate were analyzed according to Standard Methods (APHA, 1999).

#### 2.1.2 Salar de Huasco

Water and sediment samples were collected in the austral summer of 2005 (January) at four different sites from the Salar de Huasco (20°18'S, 68°50'W), a saline wetland located at 3800 meters altitude in the Chilean Altiplano (Fig. 2-3).



**Fig. 2-3.** Map indicating the location of the Salar de Huasco and four study sites (H0, H1, H4, H6). Greyed areas indicate the presence of permanent lagoons.

This athalassohaline water body was formed during the Pleistocene and evolved into an evaporitic basin, due to high rates of evaporation and erosion (Chong, 1984). Although several streams flow into the salar, River Collacagua represents the principle inflow (Risacher et al., 1999). The salar exhibits high spatial heterogeneity, represented by a mosaic bofedales (peatlands), of streams,

shallow permanent and non-permanent lagoons and salt crusts, with a gradient in salt concentration from north to south. The catchment has a total area of 1572 km<sup>2</sup>. The surface area of the salar extends to ca. 50 km<sup>2</sup>, with open water representing only 2.5 km<sup>2</sup>

(Risacher et al., 2003). In the salar we distinguished 6 sampling sites: H0, H1, H2, H3, H4, H5 and H6. For this study we collected samples from H0, H1, H4 and H6 because they can be considered representative of the salar as a whole (Fig. 2-4). A summary of some morphometric and physico-chemical characteristics is given in Table 2-2. The sampling sites can be described from north to south as follows: a) H0 is a stream surrounded by abundant macrophytes e.g. *Oxychloe andina* (Squeo et al., 2006) and aquatic ferns (mostly *Azolla* sp.) and is characterized by large amounts of organic matter in the sediments; b) H1 is a permanent lagoon with low salinity; c) H4 is a shallow, anoxic hypersaline lagoon (approximately 3 cm deep at the time of sampling) with no vegetation; d) H6 is a lagoon with fluctuating water level and high salinity located in the south of the salar. Sites H2 and H5 represent hypersaline permanent lagoons and site H3 is a stream.

**Table 2-2.** Physico-chemical characteristics of water samples from the four sites in Salar de Huasco.

Characteristics	Sites					
Chai acteristics	H 0	H 1	H 4	Н 6		
Location	20°15'32", 68°52'25"20°16'08", 68°52'29"20°17'41", 68°53'00"20°19'43", 68°50'19"					
Altitude (m)	3799	3795	3789	3789		
Type	Stream	Lagoon	Lagoon	Lagoon		
Conductivity (µScm <sup>-1</sup> )	607	645	63100	13740		
Total dissolved salts (gL <sup>-1</sup> )	0.42	0.46	64.93	9.38		
Dissolved oxygen (mgL <sup>-1</sup> )	6.9	10.3	0	8.4		
рН	7.7	8.7	8.2	8.8		
$N-NO_3^- (\mu g L^{-1})$	55.5	53.5	60	30		
$P-PO_4^{3-}(\mu g L^{-1})$	40.3	20.3	3905	807		
Cations	$Ca^{2+}>Na^{+}>K^{+}>Mg^{2+}$	$Ca^{2+}>Na^{+}>K^{+}>Mg^{2+}$	$Mg^{2+}>Ca^{2+}>K^+>Na^+$	$Ca^{2+}>Mg^{2+}>Na^{+}>K^{+}$		



Fig. 2-4. Sampling sites at Salar de Huasco.

# 2.2 DNA extraction from environmental samples

Environmental DNA was extracted from water and sediment samples from each site. Water samples were filtered at the site onto 0.2 μm, 25 mm diameter filters (Supor 200, Pall). The filtered volume varied between 0.05 and 1 L depending on the amount of suspended solids in the samples. Filters and sediment samples were maintained at –20°C for a few days until subsequent DNA extraction in the lab. DNA from filters and sediments (0.25 g) was extracted with the Ultra Clean Soil DNA Isolation Kit (MoBio Lab., Inc.). DNA from cultures was extracted with the same kit.

# 2.3 PCR amplifications

## 2.3.1 Bacterial and archaeal 16S rRNA genes

Oligonucleotide primers Eub9-27F and Eub1542R (Stackebrandt and Liesack, 1993) were used to PCR-amplify eubacterial 16S rDNA. NitA and NitB primers were used to amplify 16S rDNA from ammonia oxidizers of the Betaproteobacteria as described (Voytek and Ward 1995) and NOC1-NOC2 to amplify gamma-AOB (Ward et al. 2000). Fragments of cyanobacterial 16S rDNA were amplified with a nested PCR approach using PCR products from eubacterial 16S rDNA as template and the following set of primers: CYA106F, CYA359F, CYA781R(a) and CYA781R(b) (Nübel et al., 1997). Fragments of archaeal 16S rRNA genes were amplified in a nested PCR approach according to (Jurgens et al., 2000). First, fragments of 1500 bp were obtained with primers Ar4F and Un1492R. Two primer pairs were used to amplify 16S rDNA from Archaea using the first round PCR products as templates in a nested PCR with: i) Ar3F-Ar9R (Jurgens et al., 2000) and ii) Arc21F-Arc958R (DeLong, 1992). For amplifications of Bacteria and Archaea, the PCR reaction contained 10×PCR-buffer with 2 mM MgCl<sub>2</sub> (Roche), 200 mM dNTP mixture (Gibco), 1 pmol of each primer, 2.5 U Taq polymerase (Roche), 10-100 ng template DNA and water to a final volume of 50 μl.

## 2.3.2 AmoA gene fragments

The bacterial *amo*A gene was amplified by PCR with primers *amo*A-1F and *amo*A-1R according to Rotthauwe et al. (1997) and the archaeal *amoA* gene with primers CrenAmo1F and CrenAmo1R (Könneke et al. 2005).

# 2.4 Denaturing Gradient Gel Electrophoresis (DGGE) analysis

DGGE was performed according to Muyzer et al. (1993) with PCR products of eubacterial 16S rDNA generated with the primers P2/P3. PCR products were applied onto 7.5% polyacrylamide gels containing a linear gradient of 30-60% denaturant where 100% denaturant was defined as 7M urea and 40% formamide. DGGE was carried out in the BioRad D Gene System (BioRad) at 60°C, 200 V for 6 hours. Gels were stained with SYBR Gold nucleic acid gel stain (Molecular Probes). In order to find relationships between communities in the different samples, a matrix was constructed from the distribution pattern of the bands in different samples, and cluster analyses (UPGMA) were conducted using the Multivariate Statistical Package (MSVP version 3.12d; Kovach Computing Services, Wales, UK). Bands were excised and re-amplified for sequencing (Chapter IV).

# 2.5 Cloning and 16S rDNA sequence analysis

Clone libraries of bacterial and archaeal 16S rDNA PCR products were generated from Chun, Par, Asc and Hua water and sediment samples, and Pia sediment samples (Chapter 3) and from water and sediment samples of four sites at Salar de Huasco (Chapters 4, 5). Samples for cloning were selected according to the band pattern in order to represent best the total diversity of the microbial communities found in DGGE analysis. Purified amplicons were cloned into pCR-Blunt vector (Invitrogen) according to the manufacturer's instructions. 96 clones per sample were picked for environmental samples and 24 for cultures (Chapter 6), and inserts were amplified with M13F/R primers. Cycle sequencing was performed with the BigDye Terminator Cycle Sequencing

Kit v3.1 and analyzed in an automated capillary sequencer (model 3100 Gene Analyzer, Applied Biosystems). Sequences were checked for chimeras using Chimera check from the RDP II (http://www.cme.msu.edu/rdp).

Rarefaction curves (Simberloff, 1972) were determined by RARFAC program (http://www.icbm.de/pmbio/downlist.htm) and used to evaluate if a sufficient number of clones were screened to estimate total diversity in each clone library. We used the Shannon-Weaver index (H') to estimate diversity of clones following:  $H'=\Sigma pi(\ln pi)$ where pi is the relative abundance of the phylotype i (Krebs, 1998). Total number of phylotypes in each clone library was estimating by calculating the non-parametric richness estimators S<sub>ACE</sub> and S<sub>Chaol</sub>. Based on the frequency with which different phylotypes occurred, coverage C<sub>ACE</sub> was calculated in order to estimate the proportion of phylotypes in the sample which is represented in the library (Chao, 1984; 1987). The analyses performed via web interface available were the at http://www.aslo.org/lomethods/free/2004/0114a.html (Kemp and Aller, 2004).

# 2.6 Phylogenetic analysis

The 16S rDNA sequences were analyzed by BLAST search (http://www.ncbi.nlm.nih.gov/blast) to determine the closest relatives present in the database. Phylogenetic affiliations were inferred with the classifier tool in RDP II (http://www.cme.msu.edu/rdp). Sequences were aligned using the alignment tool of the ARB package (http://www.arb-home.de) and a maximum likelihood analysis in the program PhyML (Guindon et al., 2005) with GTR substitution model (generalized time reversible) and 100 bootstrap re-samplings was calculated. Topologies of the trees were

confirmed with a Neighbor-Joining tree calculated from a distance matrix by the method of Jukes and Cantor in MEGA 3 (Kumar et al., 2004). Sequences not included in the ARB database were obtained from GenBank. Sequences with similarities >99% (Chapters 3, 5, 6) and >97% (Chapter 4) were considered to represent the same phylotype.

#### 2.7 Enrichment cultures

# 2.7.1 Enrichment cultures of ammonia oxidizing bacteria

Fresh samples of water and sediment were collected at four sites (H0, H1, H4 and H6) from the Salar de Huasco. On collection they were inoculated into mineral media with 10 mM NH<sub>4</sub>Cl (Koops et al. 2006) at five different salt concentrations (10, 200, 400, 800 and 1,400 mM NaCl) and pH 8 adjusted with 10% NaHCO<sub>3</sub>. Because of the high concentration of Li, As and B at the sites (Risacher et al. 2003) we added LiCl (0.5 mM), NaAsO<sub>2</sub> (0.5 mM) and HBO<sub>3</sub> (0.2 mM) to the media. Growth was controlled through nitrite production every two weeks and the positive cultures were transferred four times into fresh media. Incubation temperature was maintained at ±30°C.

# 2.7.2 Halophilic medium

We used three different media to cultivate halophilic microorganisms: i) HYM medium (halophilic denitrifying Bacteria) (Tomlinson et al., 1986), ii) *Halorubrum* medium (Rodriguez-Valera et al., 1983) and iii) *Halobacterium* medium (Oren, 2006). Low concentrations of LiCl<sub>2</sub>, NaAsO<sub>2</sub> and HBO<sub>3</sub> were added to the media because these elements are commonly found at the sites (Risacher et al., 1999). Cultures were maintained in the dark at 37°C. 15 ml tubes were filled with 4 ml medium and 1 ml

sample. After three weeks, small aliquots (100 µl) of liquid cultures were transferred to solid media. Morphologically distinct colonies were streaked four times on agar plates. In HYM cultures, bottles were filled with fresh media and sealed with serum caps to ensure anaerobic conditions. After noticeable amounts of gas were formed, fractions of the culture were transferred to fresh media four times and colonies were subsequently streaked on agar plates of HYM media (Tomlinson et al., 1986). Descriptions of the media are given in Table 2-3. Unique colonies were selected for DNA extraction.

**Table 2-3.** Composition of the media used in this study. HYM, Halophilic denitrifying Bacteria.

Ingredient (gL <sup>-1</sup> )	НҮМ	Halorubrum medium	Halobacterium medium
Yeast extract	5	5	10
Casein acid	2	-	7.5
NaCl	175	175	250
$MgSO_46H_2O$	20	-	20
KNO <sub>3</sub>	5	-	-
KCl	5	-	2
$MgCl_2 6H_2O$	-	20	-
$K_2SO_4$	-	5	-
CaCl <sub>2</sub> 2H <sub>2</sub> O	0.1	0.1	0.1
Na <sub>3</sub> Citrate 3H <sub>2</sub> O	-	-	3
FeCl <sub>3</sub>	-	-	36 mg
$MnCl_2$	-	-	0.36 mg
LiCl	1 ml (0.05 mM)	1 ml (0.05 mM)	1 ml (0.05 mM)
$NaAsO_2$	1 ml (0.05 mM)	1 ml (0.05 mM)	1 ml (0.05 mM)
$HBO_3$	0.1 mg	0.1 mg	0.1 mg
pН	7.4	6.8	7.2-7.4
Total dissolved salts	206	200	272

# 2.7.3 Enrichment cultures of phototrophic bacteria

Sediment samples were inoculated at the sites into Pfennig's medium at pH 7.2 with 1% NaCl (Imhoff, 2006b). Bottles were maintained at room temperature (~24°C) for three weeks until pink-red coloration appeared. Aliquots of positive enrichment cultures

were subsequently transferred to agar shakes under natural illumination and unique pink or purple colonies were picked and inoculated into serial dilutions. This procedure was repeated at least 4 times until pure colonies were obtained (assessed via microscopy).

To test the tolerance of anoxygenic phototrophic bacteria to salt, a repeat set of samples were taken in April 2006 and inoculated in to modified Pfennig's medium containing increased salt concentrations (Caumette et al., 1988). Fresh samples were inoculated directly into agar shakes following the method described above at 0, 5, 10 and 15% salt (a stock solution of NaCl and MgCl<sub>2</sub>×6 H<sub>2</sub>O 6:1).

# 3. COMPARATIVE ANALYSIS OF BACTERIAL AND ARCHAEAL COMMUNITIES IN DIFFERENT HIGH ALTITUDE WETLANDS IN NORTHERN CHILE

#### 3.1 ABSTRACT

The diversity of prokaryotes inhabiting water and sediments of five different aquatic habitats of the Chilean Altiplano was studied by PCR-DGGE and 16S rDNA clone libraries. Lago Chungará, Bofedal de Parinacota and Laguna de Piacota are located at latitude of 18°S, and 4500-4300 m above sea level, and have conductivity values ranging between 500 and 2600 µScm<sup>-1</sup>. Located further south at 3700 m, the hypersaline salares Ascotán and Huasco both have conductivity values >43000 µScm<sup>-1</sup>. The microbial community composition was highly variable between the different wetlands, but also between water and sediment samples. Each of the environments supported a unique community of Bacteria and Archaea, revealing a differentiation between the high altitude lake (Lago Chungará), freshwater wetlands (Bofedal de Parinacota and Laguna de Piacota) and saline wetlands (Salar de Huasco and Salar de Ascotán). From a total of 16 clone libraries 836 clone sequences from Bacteria and Archaea were obtained. The Cytophaga-Flavobacteria-Bacteroidetes (CFB) group was the most frequent in all samples (24-94% of clones). The following bacterial phyla were recorded: Proteobacteria (alpha, beta, gamma and delta subgroups), Firmicutes, Actinobacteria, Planctomycetes, Verrumicrobia, Chloroflexi, Cyanobacteria, Acidobacteria, Chlorobi, Deinococcus-Thermus and Candidate Divisions WS3, OP8, TM6 and TG3, Archaeal diversity was lower than bacterial diversity and was represented by Methanobacteria, Halobacteria and Crenarchaeota. Altogether the investigated habitats have unique

microbial communities not found elsewhere on this planet and many representative groups have counterparts in other extreme habitats, noticeably in cold and saline habitats like Qinghai Lake in the Himalaya and some Antarctic lakes.

#### 3.2 INTRODUCTION

Northern Chile is geographically characterized by seven distinct morphostructural units located from west to east: the Coast Range, the Central Depression, the Precordillera, the pre-Andean Depression, the Western Cordillera, the Altiplano and the Eastern Cordillera (Risacher et al., 2003). The Western Cordillera and the Altiplano, surrounded by numerous volcanoes, are of Miocene to Holocene age and reach an altitude of 6000 m. More than 50 water bodies are located along a 1000 km north-south transect in the Andes Range (Risacher et al., 2003). The Altiplano wetlands can be considered as "extreme environments" where organisms encounter extreme climatic variation over various temporal scales (diurnal, seasonal and inter-seasonal). High solar radiation, negative water balance (e.g. precipitation rates of 50-300 mm y-1 versus evaporation rates of 600 to 1200 mm y-1) (Klohn, 1972; Risacher et al., 2003), extreme day to night variation in temperature (e.g. -10 to +25 °C), a wide range of salinity conditions (from freshwater to saturated saltwater in the same basin) are some examples of the extreme environmental features of the Altiplano. Lake shores may freeze during the night and melt during the day due to the influence of solar radiation. During the last decade, water volumes in wetlands in this region (e.g. Lago Chungará and Río La Gallina) have decreased, with an associated increase in salinities (Dorador et al., 2003; Earle et al., 2003). Three principle groups of water bodies can be defined: high altitude lakes (e.g. Lago Chungará), freshwater wetlands (Laguna de Piacota and Bofedal de Parinacota) and saline wetlands (e.g. Salar de Huasco and Salar de Ascotán) (Fig. 2-1).

Biogeochemical processes in high altitude wetlands are influenced by basin geomorphology, high solar radiation, low temperatures and oxygen deficiency. Together with nutrient availability, these factors control the biological productivity (Vincent et al., 1984; 1985). Photosynthetic primary production reached a maximum of 4.65 mgCm<sup>-3</sup>h<sup>-1</sup> at 3 m depth in Lago Chungará (Mühlhauser et al., 1995), and was possibly limited by nutrient availability (Dorador et al., 2003). Generally, high altitude lakes are considered to be nutrient limited, and microorganisms could play an essential role in the maintenance of food webs (Yuhana, 2005) and in biogeochemical transformations, rock weathering and leaching of minerals (Petsch et al., 2001).

Currently, little is known on the composition and functional diversity of bacterial communities in these extreme environments. Microbial communities in many high altitude lakes, as in other freshwater systems, are characterized by a predominance of *Cytophaga-Flavobacteria-Bacteroidetes* (CFB) and *Proteobacteria* (Glöckner et al., 2000; Liu et al., 2006; Weidler et al., 2007), both groups having the capability of adaptation to low temperature (Glatz et al., 2006) and to UV-B radiation (Fernández Zenoff et al., 2006). In the case of saline lakes, microbial communities are usually dominated by halophilic Archaea, CFB and Proteobacteria (Bowman et al., 2000; Demergasso et al., 2004; Humayoun et al., 2003). A recent study examining microbial community structure in sediments of the saline high altitude (3196 m) lake Qinghai from the Qinghai-Tibetan plateau in China, highlighted the predominance of low G+C Grampositive bacteria in anoxic sediments, and the similarity of the microbial community composition with other similar systems (Dong et al., 2006). In another study on the

athalassohaline Lake Chaka, located at 3214 m in China, CFB dominated in water samples, while low G+C Gram-positive bacteria dominated in sediments (Jiang et al., 2006). In both lakes, archaeal diversity was markedly lower than bacterial diversity. In the Chilean Altiplano, a survey in Salar de Ascotán and Laguna Miscanti (Demergasso et al., 2004) has also revealed a predominance of CFB and Proteobacteria in water samples. The same study described CFB, Alpha- and Betaproteobacteria and high G+C Grampositive bacteria in Salar de Llamará and Salar de Atacama, both located in the Atacama Desert at lower altitude (<2350 m) using PCR-DGGE. The goal of the present study was to describe microbial diversity in water and sediment of five representative, but contrasting wetland habitats of the Chilean Altiplano using 16S rDNA clone libraries. Considering the contrasting chemical characteristics of the studied sites, we expected to reveal elevated levels of microbial diversity.

# 3.3 RESULTS

# 3.3.1 Physicochemical parameters

The sampling sites had diverse physicochemical properties (Table 2-1). The lowest conductivity (500-700 μScm<sup>-1</sup>) was recorded in Bofedal de Parinacota and Laguna de Piacota, whereas in Salar de Ascotán and Salar de Huasco the values were two orders of magnitude higher (43200-45300 μScm<sup>-1</sup>). Nitrogen and phosphorus concentrations were lowest in Lago Chungará and Bofedal de Parinacota, and highest in the two salares. The ionic composition in these systems is different: Lago Chungará is dominated by Mg<sup>2+</sup>/HCO<sub>3</sub>-, Bofedal de Parinacota and Salar de Huasco by Na<sup>+</sup>/HCO<sub>3</sub>-, and Salar de Ascotán by Na<sup>+</sup>/Cl<sup>-</sup> (Risacher et al., 2003).

# 3.3.2 Bacterial diversity in water samples

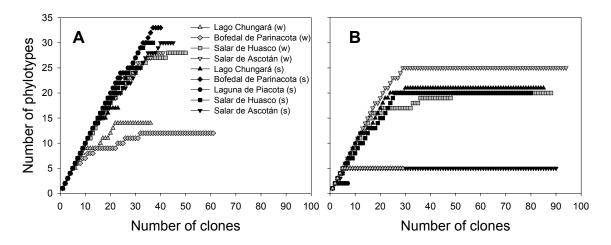
Four clone libraries (Chun, Par, Hua and Asc) were analyzed (a total of 187 clones). The observed numbers of phylotypes ranged between 12 and 28, but the total number of phylotypes estimated by S<sub>Chao1</sub> was higher in all libraries. C<sub>ACE</sub> fluctuated between 49 and 64%. Shannon diversity index values were lowest in Par (H'=1.7) and highest in Asc (H'=3.2) (Table 3-1). Rarefaction curves showed saturation in all bacterial clone libraries from water samples (Fig. 3-1A).

**Table 3-1.** Total number of clones, number of phylotypes observed, species richness estimator  $S_{Chao1}$ , coverage  $C_{ACE}$  and Shannon-Weaver Diversity Index (H'), for clone libraries from the studied wetlands.

Site	Sample	Abbreviated name	Clone Library	Total number of clones	Number of phylotypes observed		Coverage C <sub>ACE</sub> (%)	H'
Lago Chungará	Water	Chun-w	Bacteria	36	14	21	64	2.2
Bofedal de Parinacota	Water	Par-w	Bacteria	61	12	20	67	1.7
Salar de Huasco	Water	Hua-w	Bacteria	50	28	61	62	3.1
Salar de Ascotán	Water	Asc-w	Bacteria	41	28	70	49	3.2
Lago Chungará	Sediment	Chun-s	Bacteria	23	17	43	43	2.7
Bofedal de Parinacota	Sediment	Par-s	Bacteria	40	33	109	30	3.4
Laguna de Piacota	Sediment	Pia-s	Bacteria	31	25	275	26	3.1
Salar de Huasco	Sediment	Hua-s	Bacteria	37	30	104	32	3.3
Salar de Ascotán	Sediment	Asc-s	Bacteria	45	30	99	47	3.2
Lago Chungará	Water	Chun-w	Archaea	29	5	9	-	1.1
Salar de Huasco	Water	Hua-w	Archaea	88	20	36	74	2.0
Salar de Ascotán	Water	Asc-w	Archaea	94	25	101	40	1.7
Lago Chungará	Sediment	Chun-s	Archaea	85	21	49	33	1.4
Laguna de Piacota	Sediment	Pia-s	Archaea	7	2	3	86	0.4
Salar de Huasco	Sediment	Hua-s	Archaea	80	20	50	46	2.0
Salar de Ascotán	Sediment	Asc-s	Archaea	90	5	6	78	0.4

Most of the phylogenetic groups recovered are common between sites (CFB, *Alphaproteobacteria*, *Actinobacteria*) but some groups were found only in fresh (*Firmicutes*) or saline water bodies (*Deltaproteobacteria*, *Betaproteobacteria*,

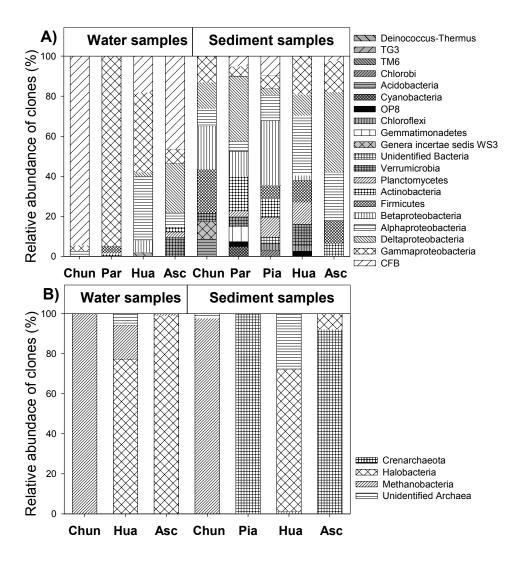
*Planctomycetes, Verrumicrobia, Deinococcus-Thermus*). Sequences were assigned to the following bacterial groups (Fig. 3-2A and 3-3):



**Fig. 3-1.** Rarefaction analysis of seven clone libraries from water and nine from sediment samples for Bacteria (A) and Archaea (B).

- (i) *Alphaproteobacteria*. Sequences related to this group were retrieved from Chun (1 clone), Hua (16 clones) and Asc (3 clones). Sequences from Hua and Asc formed a cluster closely related to the Rhodobacteraceae family.
- (ii) *Betaproteobacteria*. Only one sequence was found in Hua, and had 98% similarity with an uncultured Comamonadaceae retrieved from bottled mineral water (Loy et al., 2005).
- (iii) *Gammaproteobacteria*. 2 clones from Hua formed a cluster related to an uncultured bacterium retrieved from water of Lake Bonney in Antarctica (Glatz et al., 2006). Clones from Par formed two clusters, one closely related to *Acinetobacter* spp. (5 clones) and the other one to *Psychrobacter* spp. (47 clones). Sequences from Par (6 clones), Hua (14 clones) and Chun (1 clone) clustered together with *Pseudomonas* spp.. Three clones from Asc and one from Hua clustered with *Marinobacter* spp..

(iv) *Deltaproteobacteria*. Sequences of this group were retrieved only from Asc (10 clones) and Hua (1 clone). They formed two clusters, one with sequences from Asc and related to *Desulfotignum phosphitoxidans*, a sulfate-reducing bacterium isolated from marine sediments with phosphite as sole electron donor (Schink et al., 2002) and another with sequences from Asc and Hua, which was related to uncultured Deltaproteobacteria from Mono Lake in USA (GenBank information).



**Fig. 3-2.** Composition of clone libraries from Lago Chungará (Chun), Bofedal de Parinacota (Par), Laguna de Piacota (Pia), Salar de Huasco (Hua) and Salar de Ascotán (Asc) of Bacteria (A) and Archaea (B) in water and sediment samples.

- (v) Cytophaga-Flavobacteria-Bacteroidetes (CFB) was the dominant group in libraries from Chun (34 clones) and Asc (19 clones) and the second dominant group in Hua (9 clones). Sequences of Chun and Hua formed a cluster related to Flavobacterium spp.. Three clones from Hua were closely related to Psychroflexus torquis, previously isolated from Antarctica (Bowman et al., 1998). Another group of 4 sequences from Asc, Hua and Chun clustered together with Sphingobacterium spp. and with uncultured Bacteroidetes retrieved from Ikaite tufa columns in Greenland, an alkaline, low temperature and low salinity environment (Schmidt et al., 2006). 9 clones from Asc clustered together and had similarities between 94-98% with uncultured CFB retrieved from Lake Bonney in Antarctica (Glatz et al., 2006).
- (vi) *Verrumicrobia*. A single sequence from Asc was 94% similar to an uncultured *Verrucomicrobium* retrieved from a hypersaline selenium-contaminated evaporation pond in San Joaquin Valley in California, USA (de Souza et al., 2001).
- (vii) *Firmicutes*. Only two clones from Par were phylogenetically related to this group. Both had 98% similarity with a *Carnobacterium* sp. isolated from Antarctica and *Exiguobacterium* sp. (GenBank information).
- (viii) *Planctomycetes*. A single sequence from Asc had low similarity (87%) with an uncultured planctomycete retrieved from permeable shelf sediments from the South Atlantic Bight (Hunter et al., 2006).
- (ix) *Deinococcus-Thermus*. A single sequence of Hua had 92% similarity with *Truepera radiovictrix*, a radiation-resistant bacterium isolated from hot springs in the Azores (Alburquerque et al., 2005).

(x) *Actinobacteria*. A single sequence of this group was retrieved from Par and was 98% similar with *Arthrobacter sulfureus* (Koch et al., 1995). Another three clones from Asc had 72% similarity with this group.

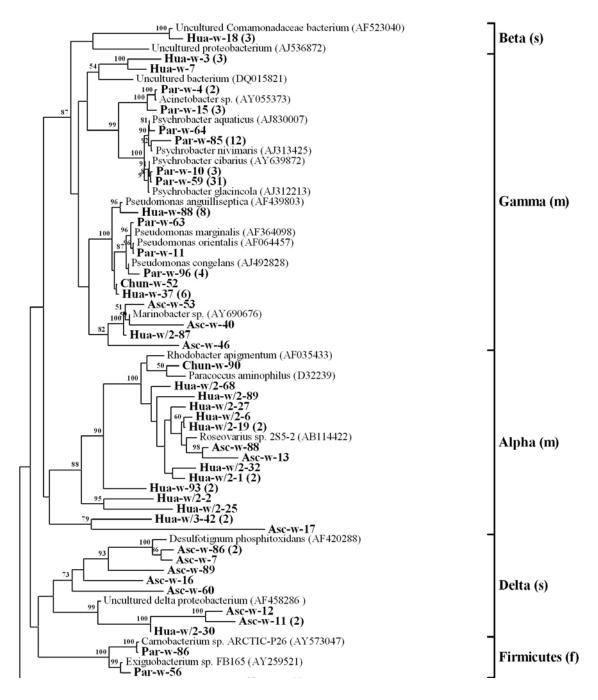
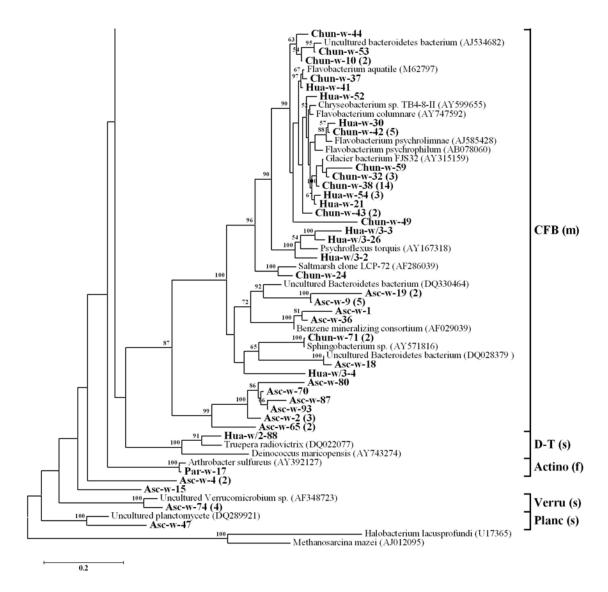


Fig. 3-3.



**Fig. 3-3** Phylogenetic tree inferred from partial 16S rDNA sequences (≥900 bp) of phylotypes of Bacteria in water using maximum likelihood analysis. The numbers of clones identical with each phylotype are shown in brackets. Clone sequences from this study are in bold and coded as follows for the example of Hua-w/2-68: Hua, Salar de Huasco; w, water sample, 2, plate number; 68, clone number. For the phylogenetic groups the origin of the sequences is shown: freshwater (f), saline water (s), mixed (m). Bootstrap values of >50% (for 100 pseudoreplicates) are shown. Scale bars indicate 0.2 substitutions per site. *Methanosarcina mazei* and *Halobaterium lacusprofundis* were used as outgroup. Abbreviations: *Proteobacteria* (alpha, beta, gamma and delta subgroups); CFB, *Cytophaga-Flavobacteria-Bacteroidetes*; D-T, *Deinococcus-Thermus*; Actino, *Actinobacteria*; Verru, *Verrumicrobia*; Planc, *Planctomycetes*.

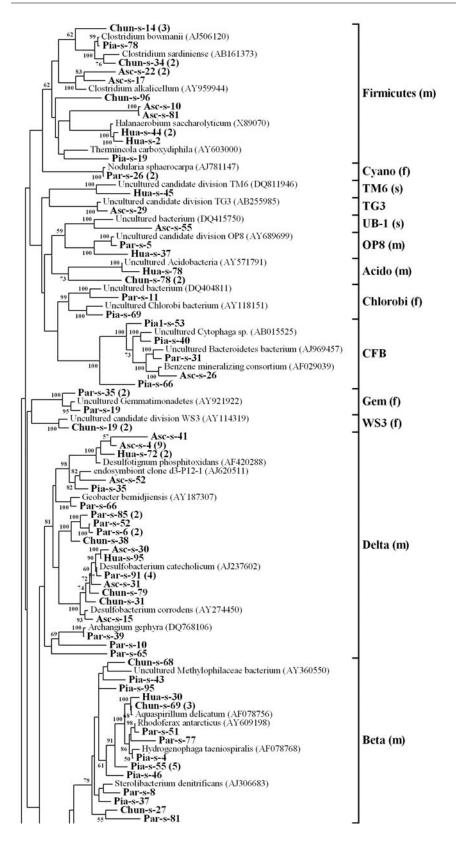
# 3.3.3 Bacterial diversity in sediment samples

A total of 176 sequences were retrieved in five clone libraries from sediment samples. The number of phylotypes was higher than in water samples (between 17 and 33). S<sub>Chao1</sub> indicated higher richness for all the libraries, and C<sub>ACE</sub> values fluctuated between 26 to 47%. The Shannon diversity index was highest in Par (H'=3.4) and lowest in Chun (H'=2.7). Libraries of Pia and Par did not reach saturation in rarefaction curves, indicating an underestimation of taxonomic richness in sediment samples (Fig. 3-1A). Groups common to sediment samples, from both fresh and saline water bodies were CFB, *Proteobacteria* (alpha, beta, gamma and delta subgroups), *Firmicutes*, *Planctomycetes*, *Verrumicrobia*, *Chloroflexi* and the candidate division OP8. *Actinobacteria*, candidate division WS3, *Gemmatimonadetes*, *Cyanobacteria*, *Acidobacteria* and *Chlorobi* were only recorded from freshwater sites. Also within the more common groups, specific clusters for freshwater or saline water bodies could be distinguished. The clones were classified as follows (Fig. 3-2A and 3-4):

- (i) *Alphaproteobacteria*. This group was dominant in clone libraries from Hua (11 clones) and was present in libraries from Asc (11 clones), Pia (4 clones), Par (2 clones) and Chun (2 clones). Two clusters were formed with sequences from Hua and Asc; one was related with an uncultured iodide-oxidizing bacterium retrieved from brines in Japan (Amachi et al., 2005), while the other did not have clear relations with any published sequence. Three clones from Pia, 1 from Huasco and 1 from Chun clustered together with *Rhodobacter sphaeroides*, a phototrophic purple nonsulfur bacterium (Okubo et al., 2005).
- (ii) Betaproteobacteria. This group was dominant in Pia (10 clones). One clone of Hua and another from Chun formed a cluster together with Aquaspirillum delicatum. Two

clones from Par were closely related to *Rhodoferax antarcticus*. Individual clones from Pia and Par formed a separate cluster related to *Sterolibacterium denitrificans*, a member of *Rhodocyclales* (Tarlera and Denner, 2003).

- (iii) *Gammaproteobacteria*. Three Hua clones and four Asc clones clustered with uncultured bacteria and *Halomonas glaciei*, a psychrophilic bacterium isolated from ice in Antarctica (Reddy et al., 2003). A further cluster formed with sequences from Asc and Hua was closely related to *Chromatiales*.
- (iv) *Deltaproteobacteria*. This group dominated the libraries of Par (13 clones) and Asc (18 clones). 10 clones from Asc and one from Hua were related to *Desulfotignum phosphitoxidans* (Schink et al., 2002). One sequence from Asc and one from Pia formed a cluster together with, an endosymbiont of a worm (*Olavius crassitunicatus*) found in sediments along the Peruvian coast (Blazejak et al., 2005). One sequence from Par had 95% similarity with *Geobacter bemidjiensis*, a bacterium capable of Fe(III) reduction (Holmes et al., 2004). Sequences from Chun, Par, Hua and Asc formed a cluster related to *Desulfobacterium* spp. a genus of sulfate-reducing bacteria. Three sequences from Par grouped together with *Archangium gephyra*, a member of Myxococcales.
- (v) *Cytophaga-Flavobacteria-Bacteroidetes* (CFB). Sequences of this group were retrieved from Par (2 clones), Pia (3 clones) and Asc (1 clone), and clustered with uncultured *Cytophaga* retrieved from deep-sea sediment (Li et al., 1999) and uncultured *Bacteroidetes* from hydrothermal vents (GenBank description).
- (vi) *Verrumicrobia*. Sequences were obtained from Chun (1 clone), Par (2 clones) and Hua (4 clones) and were similar to uncultured Verrumicrobia retrieved from farm soil, anoxic marine sediment and Arctic Ocean.



**Fig. 3-4**. Phylogenetic tree inferred from partial 16S rDNA sequences (≥900 bp) of phylotypes of Bacteria in sediments. Characteristics of the tree are the same as described in Fig. 3-3. Abbreviations: Cyano, *Cyanobacteria*; UB, unidentified Bacteria; Acido, *Acidobacteria*; Gem, *Gemmatimonadetes*.

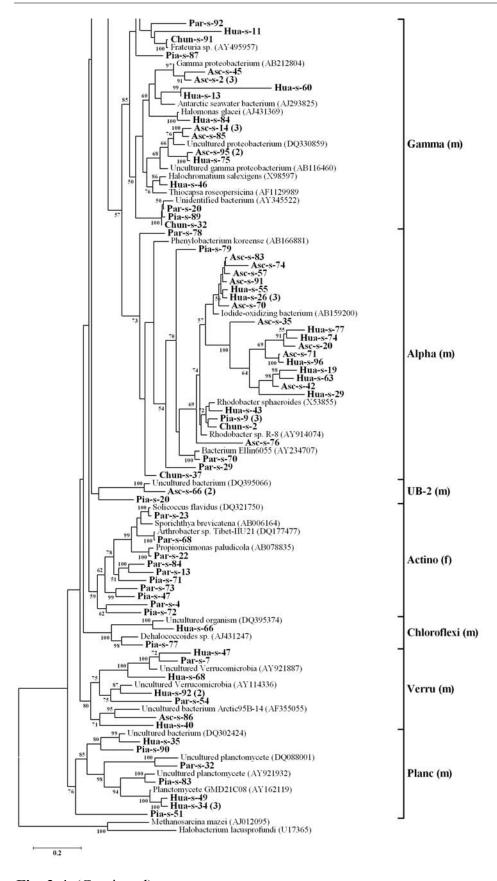


Fig. 3-4. (Continued)

- (vii) *Firmicutes*. This group was the most abundant in Chun (5 clones). One cluster was formed with five clones from Chun, one from Pia and two from Asc and was closely related to *Clostridium* spp. Clones from Chun, Asc and Hua were related to *Halanaerobium saccharolyticum*, a halophilic anaerobic bacterium (Rainey et al., 1995). One clone from Pia was 84% similar with *Thermincola carboxydiphila*, an anaerobic, thermophilic and alkalitolerant bacterium isolated from a hot spring in Lake Baikal in Russia (Sokolova et al., 2005).
- (viii) *Planctomycetes*. Members of this group were found in Par (1 clone), Pia (3 clones) and Huasco (4 clones). The clonal sequences were ≤96% similar to their closest relatives in the database which were retrieved from diverse environments including pasture soil (Sangwan et al., 2005), and the Sargasso Sea (Zengler et al., 2002).
- (ix) *Actinobacteria*. Sequences related to this group were found in Par (7 clones) and Pia (3 clones). Three clones from Par grouped together with *Propionicimonas paludicola* (Akasaka et al., 2003), *Sporichthya brevicatena*, isolated from soil samples from Japan (Tamura et al., 1999) and *Solicoccus flavidus*. Other clones from Par and Pia did not have any clear relation with available sequences in the database.
- (x) *Chlorobi*. One sequence from Pia was 96% similar to an uncultured bacterium from a benzene-degrading consortium (Ulrich and Edwards, 2003) and one sequence of Par was 92% similar to an uncultured bacterium from heavy metal contaminated soil (Abulencia et al., 2006).
- (xi) *Chloroflexi*. One sequence from Pia belonged to the Chloroflexi group and had a 94% similarity with *Dehalococcoides* sp., a bacterium used for bioremediation due to its capacity for chlororespiration (Löffler et al., 2000). Another sequence from Hua grouped with an uncultured organism retrieved from deep-sea coral (GenBank information).

- (xii) *Acidobacteria*. One clone from Chun and a single sequence from Hua were related to this group. This sequence had 95% similarity with a sequence from hydrocarbon-contaminated soil in Antarctica (Saul et al., 2005).
- (xiii) *Cyanobacteria*. One sequence from Par was similar to *Nodularia spumigena*, a planktonic toxin producing cyanobacterium isolated from the Baltic Sea (Lyra et al., 2005).
- (xiv) *Gemmatimonadetes*. Three clones from Par were related to uncultured bacteria from this group. It was recently shown that this group dominated soil cores taken from the Atacama Desert (Drees et al., 2006).
- (xv) Candidate divisions. Two clones (Par, Hua) were similar to clonal sequences of the candidate division OP8 (Opsidial Pool clone) described from Yellowstone Hot Springs (Hugenholtz et al., 1998). One clone of Asc had 96% similarity to the TG3 group (Hongoh et al., 2006). One sequence of Hua was 95% similar with TM6 candidate division retrieved from mangrove soil in Korea. Two clones from Chun were 93% similar with a clone from candidate division WS3 retrieved from anoxic marine sediments (Freitag and Prosser, 2003).
- (xvi) Unidentified Bacteria (UB). Sequences with low similarity (<80%) to known subdivisions of Bacteria were considered as "unidentified". Cluster UB-1 was formed with a clone from Asc and cluster UB-2 was formed with two clones from Asc and Pia respectively.

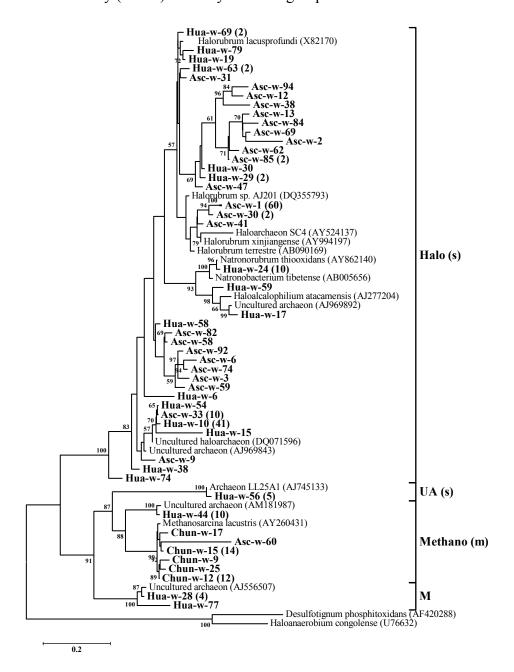
# 3.3.4 Archaeal diversity in water

A total of 211 archaeal 16S rDNA sequences were obtained from water samples collected from Chun, Hua and Asc. 5, 20 and 25 phylotypes were recorded from Chun, Hua and Asc respectively, and richness estimators were higher than the observed

numbers of phylotypes. Coverage  $C_{ACE}$  was estimated at 40% in Asc and 74% in Hua.  $C_{ACE}$  was not calculated for Chun because this index is designed for libraries with rare phylotypes ( $\leq$ 10 clones). The Shannon diversity index varied between 1.1 and 2.0 in Chun and Hua respectively (Table 3-1). All libraries reached an asymptote in the rarefaction analysis (Fig. 3-1B). Sequences were affiliated to the following groups (Fig. 3-2B and 3-5):

- i) *Halobacteria*. 67 clones from Hua and 94 clones from Asc clustered into this group. Several clusters could be distinguished. One formed with clones from Hua and Asc was related to *Halorubrum lacusprofundis*, a halophilic and psychrophilic archaeon isolated from Deep Lake in Antarctica (Holmes et al., 1990). Another group of sequences from the salares grouped together with *Halorubrum terrestre* and *Halorubrum xinjiangense*. Related to these clusters, sequences of Asc and Hua formed another group possibly related to *Halorubrum*. Only clones from Hua showed any similarity with *Natronobacterium thiooxidans*, an extremely halophilic and neutrophilic archaeon isolated from hypersaline lakes in Altai, Russia (Soronkin et al., 2005) and *Haloalcalophilium atacamensis* isolated from the Salar de Atacama (GenBank description). A large amount of clones was without clear relations to cultured *Halobacteria*.
- ii) *Methanobacteria*. This group was found in all water samples. 29 clones from Chun and one from Asc formed a cluster related to *Methanosarcina lacustris*, a psychrotolerant methanogen isolated from an anoxic lake (Simankova et al., 2001). Clones from Hua formed two different clusters, one together with an uncultured archaeon retrieved from sediments of Lake Kinneret in Israel (Schwarz et al., 2007) and another with one sequence from temperate anoxic soil of a rice field in Italy (Wu et al., 2006).

iii) Unidentified Archaea. One clone of Hua was considered "unidentified" because of low similarity (<80%) with any archaeal group.

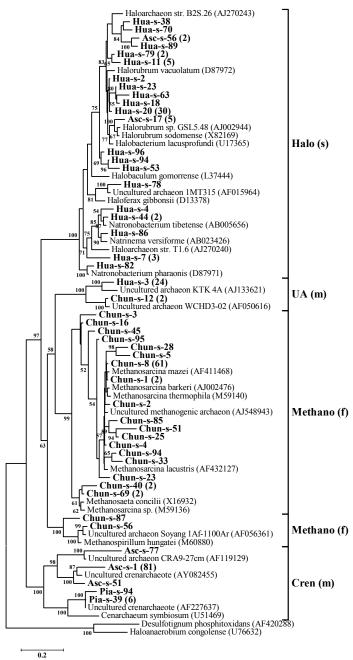


**Fig. 3-5**. Phylogenetic tree inferred from partial 16S rDNA sequences (≥900 bp) of phylotypes of Archaea in water. Characteristics of the tree are the same as described in Fig. I-4. *Desulfotignum phosphitoxidans* and *Haloanaerobium congolense* were used as outgroup. Abbreviations: Halo, Halobacteria; UA, unidentified Archaea; Methano, Methanomicrobia.

# 3.3.5 Archaeal diversity in the sediments

A total of 262 sequences were obtained from four clone libraries of the sediment samples from Chun, Pia, Hua and Asc. The number of phylotypes ranged between 2 in Pia and 21 in Chun. S<sub>Chao1</sub> showed higher values than observed phylotypes numbers. C<sub>ACE</sub> was 33% in Chun and 86% in Par. The Shannon diversity index was lowest in Pia and Asc (H'=0.4) and higher in Chun (H'=1.4) and Hua (H'=2.0) (Table 2-1). The sequences were classified in the following group (Fig. 3-2B and 3-6):

- i) *Halobacteria*. As found in the water samples, only sequences from the two salares Asc and Hua were related to this group. 7 clones from Asc and 44 from Hua were related to *Halorubrum*. Most sequences from Hua were similar to *Halorubrum lacusprofundis* (Holmes et al., 1990). Another 8 clones from Hua formed a cluster with *Natronobacterium*.
- ii) *Methanobacteria*. 82 clones from Chun clustered with *Methanosarcina* sp.. Two sets of clones from Chun were similar to *Methanosaeta concilii* (Patel and Sprott, 1990) and *Methanospirillum hungatei*, respectively.
- iii) *Crenarchaeota*. One clone from Asc was 95% similar with an uncultured archaeon retrieved from deep-sea sediments (Vetriani et al., 1999) and described as a member of the Marine Benthic Group C of uncultured Crenarchaeota (Schleper et al., 2005). 81 clones from Asc had 97% similarity with a sequence from a ZnS-forming biofilm in a mine drainage system in USA (Labrenz and Banfield, 2004). Seven clones from Pia formed a cluster similar to the non-thermophilic crenarchaeon *Cenarchaeum symbiosum*, a symbiont of *Axinella mexicana* (Preston et al., 1996).
- iv) Unidentified Archaea. 24 clones from Hua and 2 clones from Chun were considered "unidentified" because of the low similarity (<80%) with archaeal groups.



**Fig. 3-6.** Phylogenetic tree inferred from partial 16S rDNA sequences (≥900 bp) of phylotypes of Archaea in sediments. Characteristics of the tree are the same as described in Fig. 3-3 and 3-5. Abbreviation: Cren, Crenarchaeota

#### 3.4 DISCUSSION

Microbial diversity in high altitude wetlands of the Chilean Altiplano varies considerably and reflects site and sample characteristics (e.g. water or sediment). At all of the study sites, both freshwater (Chun, Par, Pia) and saline (Hua, Asc) bacterial diversity

was higher than archaeal diversity. Many sequences had a similarity lower than 95% with their closest cultured relative (80% of the phylotypes in sediment and 50% in water samples), but most of them showed a high similarity at phylum level. In this study, we showed the presence of several different groups besides those previously described as dominant in other high altitude lakes (CFB, *Proteobacteria*) and that are common in freshwater and saline habitats (Weidler et al., 2007; Dong et al., 2006; Jiang et al., 2006; Yuhana, 2005).

In sediment samples the number of clone sequences analyzed here may not completely describe bacterial diversity, considering the expected high phylotype richness indicated by the non-parametric estimator  $S_{Chao1}$  and the low coverage  $C_{ACE}$  (<47%) (Table 3-1). In water but not in sediment samples, rarefaction curves reached a strong asymptote indicating an underestimation of the bacterial phylotype richness in sediment (Fig. 3-1A). For archaeal clone libraries rarefaction curves reached a strong asymptote in all the samples but the richness estimator S<sub>Chaol</sub> was higher than the observed number of phylotypes. Non-parametric richness estimators such as S<sub>Chao1</sub> have been widely used to describe microbial diversity (Hughes et al., 2001; Kemp and Aller, 2003; 2004), but may underestimate true diversity if used with small sample size or unevenly distributed communities (Curtis et al., 2006). In our study, especially with bacteria from sediment samples, most of the phylotypes occurred only once in a library, and thus these would not yield stable estimates of phylotype richness (Kemp and Aller, 2004). Therefore, a higher number of clones would improve the determination of microbial diversity in sediment samples.

Bacteria of the *Cytophaga-Flavobacteria-Bacteroidetes* group, which are common in all libraries from this study, have been frequently observed in freshwater

(Kirchman, 2002), as well as in aquatic environments with high salinity (Bowman et al., 2000; Humayoun et al., 2003), marine waters (Kirchman, 2002) and high-altitude, cold environments (Dong et al., 2006; Jiang et al., 2006; Liu et al., 2006; Demergasso et al., 2004; Glöckner et al., 2000). Usually they are involved in the degradation of organic matter (Abell et al., 2005; Kirchman, 2002). In Salar de Ascotán we found a cluster that was more related to Flexibacter-Cytophaga than to Flavobacteria and revealed the existence of potentially new species of CFB. It has been demonstrated that isolates of Cytophaga from high-altitude wetlands in the Argentinean Altiplano were more resistant to UV-B radiation than bacteria isolated from areas at sea level, an ability that might aid survival in the Altiplano with its intensive solar radiation (Fernández Zenoff et al., 2006). A previous study using PCR-DGGE demonstrated the dominance of CFB in water of Salar de Ascotán located in the Altiplano and Salar de Atacama and Salar de Llamará, both located in the Atacama Desert (Demergasso et al., 2004). Here, we confirm these results and extend the observation of CFB to altiplanic wetlands. Recently, the presence and potential phototrophic function of proteorhodopsin in marine Flavobacteria has been reported (Gómez-Consarnau et al., 2007). The degradation of organic matter and possible phototrophy may allow these organisms to be more successful in many environments including high altitude wetlands.

Using a biogeographical approach by considering the spatial distribution of prokaryotic taxa at local, regional and continental scales (Ramette and Tiedje, 2006), it might be possible to find patterns of diversity according to the environmental conditions of the sites. At a local scale, the freshwater habitats (Chun, Pia, Par) are located in the north of the Chilean Altiplano where precipitation occurs with higher intensity, and water bodies exhibit low salt concentration and high water volume compared with the salares.

Both salares exhibit permanent and non-permanent shallow lagoons, and water volumes depend on the amount of precipitation in the summer season mainly influenced by effects of El Niño (wet years) or La Niña (dry years) (Garreaud et al., 2003). The climatic variability in the Altiplano represented in the water volume and salinity of the sites studied here, could affect the diversity (types of microbial groups) and composition (distribution of the microorganisms in time and space) of the microbial communities.

In conclusion, each water body exhibited a unique microbial diversity pattern in concordance with the heterogeneity of the sampled sites (lake, freshwater wetlands, saline wetlands). Altogether the investigated habitats have unique microbial communities not found elsewhere. Many representative groups have counterparts in other extreme habitats, noticeably in cold and saline habitats like Qinghai Lake in Himalaya and some Antarctic lakes. Apparently the unique circumstances of high altitude and irradiation together with elevated salinity and cold and fluctuating temperatures, but also the geographical isolation from comparable habitats have selected specifically adapted microbial communities not found elsewhere on this planet.

# 4. DIVERSITY OF ARCHAEA IN ENVIRONMENTAL SAMPLES FROM SALAR DE HUASCO

#### 4.1 ABSTRACT

Archaeal communities were analyzed from four representative sites of the Salar de Huasco, a high-altitude (3800 m), saline, wetland located in the Chilean Altiplano, using DGGE and clone libraries of 16S rDNA PCR products. Samples from a tributary stream (H0) and three shallow lagoons (H1, H4, H6), were analyzed. Archaeal diversity was higher in sediment than in water samples. Euryarchaeota were recovered from all samples and most sequences were related to the uncultured groups MBG-D, Group III (*Thermoplasmata* and relatives) and TMEG. Between 40-50% of the clones were highly related with methanogenic Archaea. One cluster (Hua-4) that was identified from sediment samples was related to Euryarchaeota, but with no clear affiliation to any previously described groups. Crenarchaeota clustering in the Group I.1b dominated the clone library of water from the stream site (H0). Sequences from sediment samples were affiliated to Crenarchaeota of the Marine Group I.1a, MBG-B, MBG-C and three clusters (Hua-1, Hua-2, Hua-3) had no clear affiliation with described groups. Ammonia oxidizing Crenarchaeota were detected in the water sample of H0, by amoA sequences closely related to Nitrosopumilus maritimus and Cenarchaeum symbiosum, providing evidence for the presence of archaeal ammonia oxidation in a high altitude, cold, saline wetland.

#### 4.2 INTRODUCTION

Archaea are widely distributed in both extreme (e.g., hot springs, hydrothermal vents, solfataras, salt lakes, soda lakes, sewage digesters, rumen) and non-extreme (e.g.,

ocean, lakes, soil) environments (Chaban et al., 2006). The domain Archaea consists of two major phyla, Crenarchaeota and Euryarchaeota. In addition two further phyla have been proposed: Korarchaeota (Barns et al., 1994; 1996) and Nanoarchaeota (Huber et al., 2002). With the advent of molecular techniques, an immense number of 16S rDNA sequences of "uncultured Archaea" have been retrieved in clone libraries from different environments (Schleper et al., 2005). To date, approximated 40% of the 16S rDNA sequences of Archaea from environmental samples deposited in GenBank are considered "uncultured" or "unidentified" because they do not exhibit close similarity with cultured groups. Several of these uncultured archaeal groups have been defined (DeLong, 1998; Vetriani et al., 1999; Takai et al., 2001; Schleper et al., 2005). For Euryarchaeota, Group II (marine plankton, anaerobic digestor), Group III (marine sediments, marine plankton) (DeLong, 1998), Marine Benthic Group C (MBG-C, deep sea sediments), Marine Benthic Group D (MBG-D, deep sea sediments, salt marsh sediment) (Vetriani et al., 1999) and South Africa gold mine euryarchaeotic group (SAGME-1, SAGME-2; Takai et al., 2001) have been frequently reported from several terrestrial and marine environments (e.g., Inagaki et al., 2003; Shao et al., 2004; Sørensen et al., 2005; Sørensen and Teske, 2006; Kendall et al., 2007).

The current study site, the Salar de Huasco, is one of the representative wetlands of the Chilean Altiplano due to the almost complete absence of anthropogenic perturbation and the existence of distinct microhabitats within the same basin. Abiotic conditions in the Altiplano, including low temperatures (mean annual temperature <5°C), low atmospheric pressure (40% lower than that at sea level), high solar radiation (<1100 Wm<sup>-2</sup>), climate variation at different time-scales (daily, annual, interannual) and negative water balance shape the biota in these water bodies (Vila and Mühlhauser, 1987).

Microbiological studies in the arid northern Chile have focused mainly on salares located in the Atacama Desert, e.g., Salar de Llamará (Demergasso et al., 2003), and particularly in the Salar de Atacama, from where a new species of *Halorubrum* was isolated (Lizama et al., 2002). The genus *Halorubrum* has been frequently detected at different salinities in the Salar de Atacama (Demergasso et al., 2004). In the present study, DGGE and clone libraries of 16S rDNA were used to describe archaeal diversity at four contrasting sites of the Salar de Huasco. The presence of ammonia oxidizing Crenarchaeota was tested in clone libraries made from PCR products of the ammonia monooxygenase gene *amoA*.

#### **4.2 RESULTS**

# 4.2.1 Archaeal distribution between sites and samples

The dendrogram constructed from banding patterns of the DGGE with 16S rDNA PCR products from water and sediment samples using UPGMA cluster analysis (Fig. 4-1: see Materials and Methods for sampling and analytical details), shows one cluster that largely consists of sediment samples, but also includes the water sample from site H6. The pattern of bands from water samples collected from different sites within the Salar de Huasco showed no clear grouping, indicating that they were only distantly related. For example, water samples from H4 and H1 were less than 60%, and the water sample from H0 less than 50% similar with water samples from all other sites.

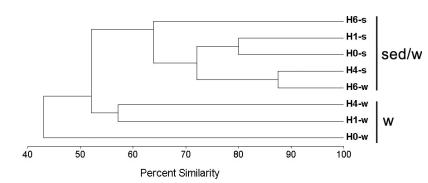
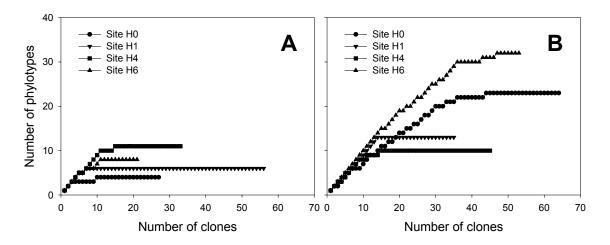


Fig. 4-1. UPGMA clustering of DGGE band patterns of archaeal 16S rDNA from water (w) and sediment (sed) samples of the four sites in Salar de Huasco.



**Fig. 4-2.** Rarefaction curves of archaeal 16S rDNA clone libraries from water (A) and sediment (B).

#### 4.2.2 Construction of 16S rDNA clone libraries and estimation of archaeal richness

Clone libraries of 16S rDNA were made from the following sites: H0, H1, H4 and H6. In total, 137 (water) and 197 (sediment) clones were obtained from all samples. Rarefaction curves from the four sites (Figs 4-2A and 4-2B) show saturation at low phylotype numbers (between 4 and 11) in water samples and higher phylotype numbers in sediment samples (between 10 and 32). The richness estimators S<sub>ACE</sub> and S<sub>Chao1</sub> (Chao, 1984; Chao, 1987) provide estimates of the total number of phylotypes between 5 to 44 and 23 to 107 in water samples and between 23 to 107 and 18 to 91 in sediment samples respectively (Table 4-1). The Shannon diversity index indicated an increased archaeal diversity in sediment (1.4-3.0) compared to water samples (0.7-1.7).

# 4.2.3 Phylogenetic analysis of archaeal communities in water

*Crenarchaeota*. Four phylotypes from H0 (100% of the library) (Figs 4-3 and 4-4) were clustered in Group I.1b of the *Crenarchaeota* (DeLong, 1998; Schleper et al., 2005) containing sequences from soil, sediment, freshwater and subsurface. These sequences

were 97% similar to an uncultured crenarchaeon retrieved from a radioactive thermal spring in the Alps (Weidler et al., 2006).

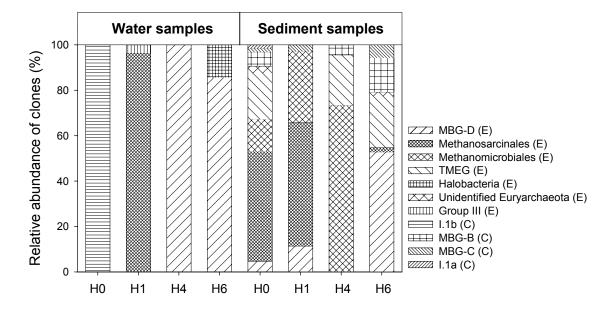
**Table 4-1.** Number of clones and phylotypes, richness estimators  $S_{ACE}$  and  $S_{Chao1}$ , number of bands in DGGE and Shannon diversity (H') in libraries of 16S rDNA from water and sediment.

Site	Clone library	Number of clones in library	Number of phylotypes observed	Predicted value of S <sub>ACE</sub>	Predicted value of S <sub>Chao1</sub>	Shannon diversity index (H')	Number of bands in DGGE
Н0	Water	27	4	5	4	0.7	16
H1	Water	56	6	8	7	1.1	4
H4	Water	33	11	44	26	1.6	10
H6	Water	21	8	17	10	1.7	7
Н0	Sediment	64	23	34	30	2.8	10
H1	Sediment	35	13	23	18	2.1	10
H4	Sediment	45	10	36	32	1.4	9
Н6	Sediment	53	32	107	91	3.0	9

Euryarchaeota. This group dominated the libraries from sites H1, H4 and H6. Most of the sequences from site H1 were affiliated to *Methanosaeta*. The most frequent clone had 98% similarity with *Methanosaeta concilii*, a mesophilic methanogenic euryarchaeon that produces methane from acetate (Eggen et al., 1989). The clone Hualw362 was highly similar (>95%) with *Methanosarcina barkeri* and *M. lacustris*. A considerable proportion of clones (1.8% of H1, 100% of H4 and 85.7% of H6) were not affiliated with any cultured representative. The phylotype Hua6-w15 had 97% similarity with an uncultured haloarchaeon retrieved from the Great Western Salt works solar saltern (California, USA: Bidle et al., 2005). The clone Hua1-w46 was affiliated with sequences inhabiting wastewater sludge and lake sediment (Chan et al., 2005) belonging to Group III of the Euryarchaeota (*Thermoplasmata* and relatives) previously described by DeLong (1998). 11 phylotypes from H4 and 6 from H6 clustered with the Marine Benthic Group D (MBG-D), described primarily with sequences from subsurface marine sediments (Vetriani et al., 1999). The most frequent clone from site H6 (phylotype Hua6-

w21) had 97% similarity with the clone BCMS-5 described from prawn farm sediments in China (Shao et al., 2004). The two most abundant clones from site H4 (phylotypes Hua4-w4 and Hua4-w21) were 89% similar with a group of clones from an endoevaporitic microbial mat from Eilat solar saltern in Israel (Sørensen et al., 2005).

The topology of the tree was confirmed by the independent treeing methods described in Materials and Methods.



**Fig. 4-3.** Composition of clone libraries of 16S rDNA from water and sediment samples. Affiliation of the phylogenetic groups is indicated for Euryarchaeota (E) and Crenarchaeota (C). Group designations: MBG, Marine Benthic Group (B, C, D) (Vetriani et al., 1999); TMEG, Terrestrial Miscellaneous Euryarchaeotic Group (Takai et al., 2001); Group III, Marine Group I.1a/b (DeLong, 1998).

# 4.2.4 Phylogenetic analysis of archaeal communities in sediment

*Crenarchaeota*. Sequences related to this group represented ca. 10% of the total number of clones (Figs 4-3 and 4-5), but were apparent at all sites. Most *Crenarchaeota* sequences were retrieved from H6, where they constituted 21% of the total clones. Clone Hua0-s31 was affiliated with the marine plankton group I.1a (DeLong, 1998) at 92%

similarity with the uncultivated marine crenarchaeote *Cenarchaeum symbiosum* (Fig. 4-5), a symbiont of the marine sponge *Axinella mexicana* (Preston et al., 1996).

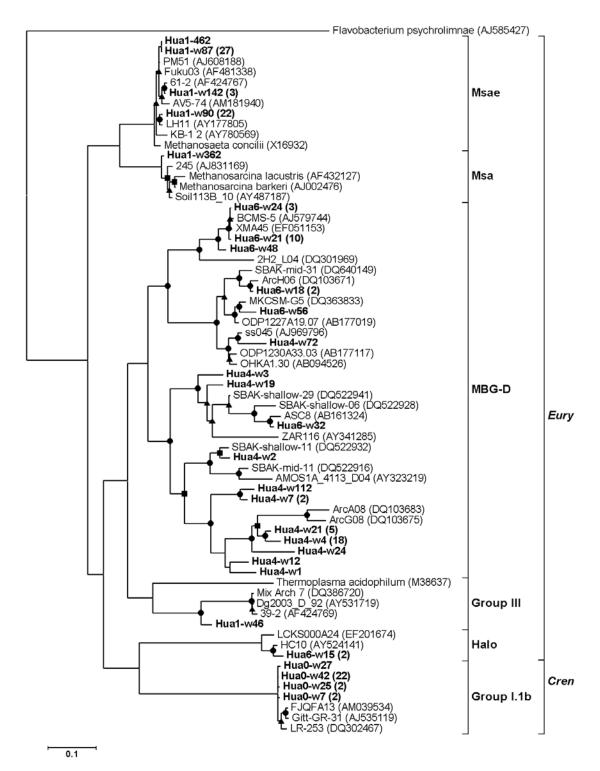


Fig. 4-4.

**Fig. 4-4.** Phylogenetic tree based on partial 16S rDNA sequences (~700 bp) of phylotypes of Archaea in water inferred by maximum likelihood analysis. Group designations: Msae, *Methanosaeta*; Msa, *Methanosarcina*; MBG-D, Marine Benthic Group D (Vetriani et al., 1999); Group III (*Thermoplasmata* and relatives: DeLong, 1998); Halo, Halobacteria; Group I.1b (DeLong, 1998). The scale bar represents 10% nucleotide sequence difference. Symbols on the branches indicate bootstrap values as follows: ● >80%; ■ 60-80%; ▲ 40-60%. Clone sequences from this study are coded as follows (example of Hua1-w90): Hua1, Salar de Huasco, site H1; w, water sample; 90, clone number. One representative clone for each phylotype is shown, and the total number of clones in brackets. *Flavobacterium psychrolimnae* (AJ585427) was used as outgroup.

Clones from H0, H4 and H6 were affiliated to the Marine Benthic Group B, that includes clones from deep-sea sediments (CRA8-27cm, APA3-11cm) (Vetriani et al., 1999), microbial mats associated with methane seeps (clone Bscra3) (Tourova et al., 2002) and deep-sea hydrothermal vents (clones pMC2A36, C1\_R043 and VC2.131) (Takai and Horikoshi, 1999; Reysenbach et al., 2000; Teske et al., 2002). The Marine Benthic Group C (Vetriani et al., 1999) was represented in the libraries of H0, H1 and H6.

Euryarchaeota. Many clones from sediment samples were affiliated with methanogenic Archaea (<60% of the clones in samples from H0, H1 and H4 and 2% in H6). The Methanomicrobia group was the most frequent representative in clone libraries (Fig. 4-5), and was composed of two distinct clusters: Methanosarcinales and Methanomicrobiales. Clones similar to Methanomicrobiales were found in samples from H0, H1 and H4. They grouped together with sequences retrieved from water samples of Lake Valkea Kotinen in Finland (Jurgens et al., 2000) and from sediment of Lake Soyang in Korea (Go et al., 2000). Methanosarcinales were found in the libraries from H0 and H1 and only one clone from H6. The most frequent clone from H1 (phylotype Hua1-s2) was 97% similar to Methanosaeta concilii (Eggen et al., 1989), while the most abundant clone

from H0 (phylotype Hua0-s82) was 98% similar to *Methanomethylovorans hollandica*. This methanogen was previously isolated from freshwater sediment and utilizes dimethyl sulfide as carbon and energy source (Lomans et al., 1999). Two more phylotypes from H0 were phylogenetically associated with *Methanosarcina lacustris*, a psychrotolerant methanogen isolated from an anoxic lake (Simankova et al., 2001) and *M. barkeri*. The clone Hua0-s95 was 94% similar with *Methanolobus oregonensis*, isolated from anoxic, subsurface sediments of a saline, alkaline aquifer near Alkali Lake in Oregon, USA (Liu et al., 1990).

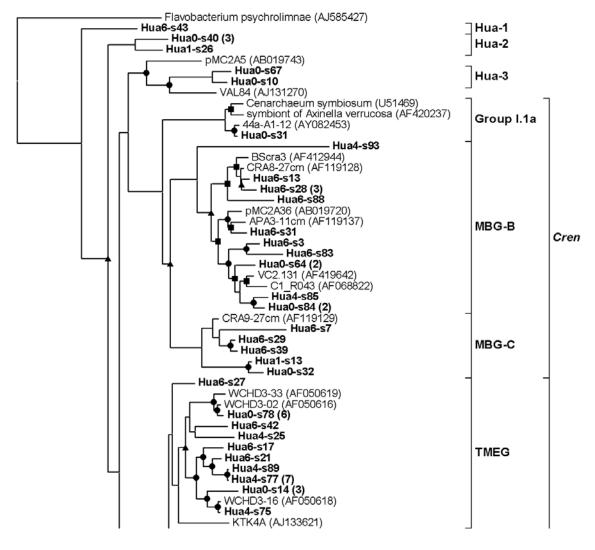


Fig. 4-5.

**Fig. 4-5.** Phylogenetic tree based on partial 16S rDNA sequences (~700 bp) of phylotypes of Archaea in sediment inferred by a maximum likelihood analysis. Characteristics of the tree as in Fig. 4-4. Abbreviations: Hua-1, Hua-2, Hua-3, Hua-4: Salar de Huasco clusters; Group I.1a, Marine Group I.1a (DeLong, 1998); MBG-B, MBG-C, Marine Benthic Groups B and C (Vetriani et al., 1999), TMEG, Terrestrial Miscellaneous Euryarchaeotic Group (Takai et al., 2001); Mmic, Methanomicrobia.

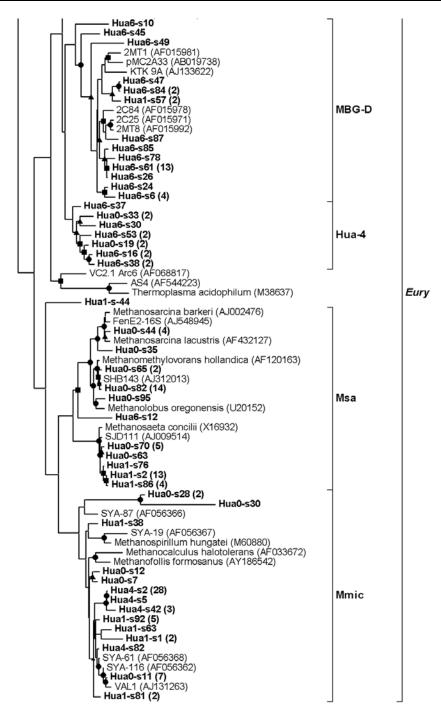


Fig. 4-5. (Continued)

0.1

A number of clones from H0, H4 and H6 were affiliated with the Terrestrial Miscellaneous Euryarchaeotic Group (TMEG) (Takai et al., 2001) formed by sequences retrieved from several very diverse sources, including hydrocarbon contaminated sediments (Dojka et al., 1998), a subsurface gold mine in South Africa (Takai et al., 2001) and methane hydrate-bearing deep sediments on the Pacific Ocean Margin (Inagaki et al., 2006).

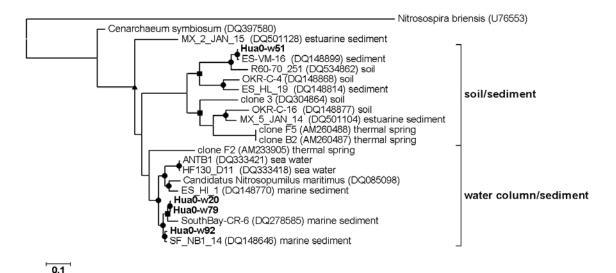
The H6 clone library was dominated by sequences related to the Marine Benthic Group D (Vetriani et al., 1999). The Huasco-specific cluster Hua-4 contained seven phylotypes from H0 and H6, and was distantly related with *Thermoplasma acidophilum* (81% similarity) and MBG-D.

Unidentified Archaea. The clone Hua6-s43 (Hua-1) was distantly related with available sequences and its first hit in BLAST (83% similarity) was the clone SBAK-shallow-04 described from sediments of Skan Bay in Alaska (Kendall et al., 2002) as "unaffiliated Euryarchaeota". The group Hua-2 comprised the clones Hua0-s40 and Hua1-s26 and was distantly related (84% similarity) with the clone 69-1 described from wastewater sludge (Williams et al., direct submission to GenBank database, 2001). Clones Hua0-s10 and Hua0-s67 (Hua-3) were distantly related to the clone VAL84 (Jurgens et al., 2000) and clone pMC2A5 described from deep-sea hydrothermal vents (Takai and Horikoshi, 1999). The topology of the tree (Fig. 4-5) was confirmed by the independent treeing methods described in Materials and Methods.

### 4.2.5 Ammonia oxidizing Archaea

PCR products of archaeal *amoA*, the gene coding for ammonia monooxygenase subunit A, were obtained only from water of site H0. In a clone library produced from

these products, four clones were affiliated to sequences retrieved from soil, sediment and water (Fig. 4-6).



**Fig. 4-6.** Phylogenetic tree from Crenarchaeota *amo*A gene sequences (~600 bp) of clones from water of site H0 in Salar de Huasco using maximum likelihood analysis. Symbols on the branches indicate bootstrap values: ● >80%; ■ 60-80%; ▲ 40-60%. *Nitrosospira briensis* (U76553) was used as outgroup.

A maximum likelihood tree with the most similar sequences of *amoA* selected from GenBank, showed that three sequences from Salar de Huasco clustered together with archaeal *amoA* sequences from marine sediment in a group that was associated with the ammonia oxidizer *Nitrosopumilus maritimus* (Könneke et al., 2005). One clone (Hua0-w51) clustered with sequences retrieved from soil and sediment. The similarities with *N. maritimus* ranged between 72 to 80% for the nucleotide sequences and between 84 to 96% for the amino acid sequences (Table 4-2). When compared with *amoA* from the yet uncultivated marine crenarchaeon *Cenarchaeum symbiosum* (Preston et al., 1996, Hallam et al., 2006), the similarities of nucleotide and amino acid sequences ranged between 74 to 90% and 81 to 94%, respectively. In BLAST searches, nucleotide sequences from Salar de Huasco shared more than 95% (>98% amino acid sequences)

identity with *amoA* of uncultured Crenarchaeota retrieved from marine sediments (Francis et al. 2005).

**Table 4-2.** Sequence similarity of Crenarchaeota *amo*A sequences from Salar de Huasco compared with *Cenarchaeum symbiosum* (CS, accession numbers DQ397580 and ABK77038) and *Nitrosopumilus maritimus* (NM, accession numbers DQ085098 and AAZ38768) and the first hit in BLAST search for nucleotide and protein sequences.

		First hit in BLAST				rity (%)
Clone	Type	Similarity (%)	Clone name	Habitat	CS	NM
Hua0-w20	Nucleotide	96	SF_NB1_14 (DQ148646)	Marine sediment	80	89
Hua0-w51	Nucleotide	99	ES-VM-16 (DQ148899)	Estuarine sediment	72	74
Hua0-w79	Nucleotide	95	SF_NB1_14 (DQ148646)	Marine sediment	79	88
Hua0-w92	Nucleotide	98	SF_NB1_14 (DQ148646)	Marine sediment	80	90
Hua0-w20	Protein	98	SF_NB1_3 (AAZ81136)	Marine sediment	96	94
Hua0-w51	Protein	100	ES-VM-6 (AAZ81389)	Estuarine sediment	84	81
Hua0-w79	Protein	99	SF_NB1_8 (AAZ81141)	Marine sediment	90	86
Hua0-w92	Protein	98	SF_NB1_14 (AAZ81147)	)Marine sediment	94	92

## 4.3 DISCUSSION

The diverse Archaeal community from the Salar de Huasco consists of phylotypes largely related to uncultured Archaea that have been retrieved from an extremely diverse set of environments. Most were only distantly related to cultured strains, and we found that only those sequences related to methanogens (Methanosarcinales and Methanomicrobiales) were similar to cultivated Archaea. The results from the DGGE as well as phylogenetic analyses of clone libraries indicated that archaeal diversity had a specific pattern in each of the sites, and that marked differences were demonstrated between water and sediment samples from each site. Since the work of DeLong (1998) who classified the uncultured Archaea retrieved from 16S rDNA analysis according to the environment where they were recovered, new groups have been reported (e.g. Vetriani et al., 1999; Takai et al., 2001; Inagaki et al., 2003; Shao et al., 2004; Sørensen et al., 2005; Sørensen and Teske, 2006; Kendall et al., 2007) highlighting the widespread character of Archaea.

Sequences classified as members of the Marine Benthic Group (A, B, C, D) (Vetriani et al., 1999) were originally found in deep-sea sediment and at hydrothermal vents but have subsequently been detected in many other environments. Sequences related to MBG-D were often found in water from sites H4 and H6 (high salinity sites) and sediment samples from sites H0, H4 and H6. This group was also found in an endoevaporitic microbial mat of a solar saltern in Israel (Sørensen et al., 2005), reflecting the likely importance of MBG-D in saline environments. MBG-B belonging to *Crenarchaeota* have a cosmopolitan distribution in marine subsurface environments (Inagaki et al., 2003, Biddle et al., 2006; Sørensen and Teske, 2006). The possible role of this group and the Miscellaneous Crenarchaeotal Group in the oxidation of methane without assimilation of methane-carbon in marine subsurface sediments was postulated by Biddle et al. (2006).

We found MBG-B only in sediment samples and also other uncultured Crenarchaeota groups (MBG-C, TMEG), but we did not identify any clones related to ANME groups (anaerobic methane oxidation). If members of MBG-B are involved in methane oxidation in marine sediments, there is no guarantee that their physiology will be similar with other 16S rDNA sequences related to this group (Achenbach and Coates, 2000). However, considering the high diversity of uncultured Archaea in the Salar de Huasco, we cannot discount the important role of Archaea in biogeochemical cycles like nitrogen or carbon cycles. Group I of Crenarchaeota was reported from both water and sediment of samples collected at site H0 (Group I.1b in water and Group I.1a in sediment). Accordingly, we found sequences related to archaeal *amo*A at this site, extending the occurrence of archaeal ammonia oxidizers to high altitude, cold and moderate saline environments, emphasizing the apparent ubiquity of this group.

Methanotrophic Archaea dominated the 16S rDNA clone libraries of sediment samples from sites H0, H1 and H4, which are a likely indicator of elevated methanogenic activity. In sediments, the sequences clustered with four genera of Methanosarcinales: *Methanosarcina*, *Methanosaeta* (aceticlastic methanogens), *Methanothylovorans* and *Methanolobus* (methylotrophic organisms) highlighting the diverse substrates used by methanogens in these environments. Considering the high sulfate concentrations reported in Salar de Huasco, sulfate reduction could be expected, especially in sites H4 and H6 (Risacher et al., 1999). Future studies designed to determine whether competition for H<sub>2</sub> or other substrates exists between methanogens and sulfate-reducing bacteria would be useful.

Salares located in the Altiplano are testimony of ancient water bodies that have undergone temporal succession and are now found as evaporitic basins (Chong, 1984). The Salar de Huasco currently reflects this long-term evolution with considerable spatial variability in abiotic environments. During this study, we selected four sites contrasting in salinity conditions, and our results reveal that archaeal diversity was clearly distinct between the sites and samples (water and sediment). Estimates of richness indicators demonstrated a large number of phylotypes and high diversity (H') in the most saline sites in water samples. However, there is not a clear relation between richness and diversity with salinity in sediment samples (Table 4-1).

H6 exhibited the greatest archaeal diversity of the different sites both with regard to water and sediment samples (Fig. 4-3). At this site we found only one phylotype affiliated to Halobacteria (89% similarity to *Haloferax volcanii*). The highest total salt concentration was found at site H4 (65 gL<sup>-1</sup>), but previous studies have reported salinities >113 gL<sup>-1</sup> at site H5 (Risacher et al., 1999). The major factors that determine the presence

of Halobacteriales in nature are total salt concentrations (>50-200 gL<sup>-1</sup>), the ionic composition of the salts and the availability of nutrients (Oren, 2006). Divalent cations have an important ecological relevance in the establishment of Halobacteriales and were dominant in the water of the Salar de Huasco: the greatest Mg<sup>2+</sup> concentration was recorded at site H4 (0.15 M), while the greatest Ca<sup>2+</sup> concentration was reported at site H6 (0.09 M). These values are considerably low in comparison with the Dead Sea, an athalassohaline water body where Halobacteriales dominate (Oren, 2002). The consequences of water level fluctuations e.g., effects on primary productivity and biological community structure, have been described for altiplanic wetlands (Squeo et al., 2006a, Squeo et al., 2006b, Vila and Mühlhauser, 1987) and will also impact the community dynamics of microbial communities. Therefore, we suggest that the absence of Halobacteria in the Salar de Huasco is due to water-level fluctuations and a subsequent reduction in salt concentration during the sampling period.

The high diversity of uncultured Archaea found in Salar de Huasco (most related to marine environments) together with the unique environmental conditions that combine fresh and saline waters, make this high altitude wetland an excellent example of the widespread character of Archaea. Future studies that develop the genomics of uncultured Archaea (e.g., Schleper et al., 2005) or provide new insights in cultivation based in the hypothesis that uncultured Archaea have specific adaptations to low energy availability (Valentine, 2007) are likely to be useful for the understanding of the ecological role of Archaea in this specialized environment.

# 5. DIVERSITY AND COMPOSITION OF PHOTOSYNTHETIC BACTERIAL COMMUNITIES IN SALAR DE HUASCO

# 5.1 CYANOBACTERIAL COMMUNITIES IN ENVIRONMENTAL SAMPLES 5.1.1 ABSTRACT

We examined the diversity of cyanobacteria in water and sediment samples from four representative sites of the Salar de Huasco using DGGE and analysis of clone libraries of 16S rDNA PCR products. Salar de Huasco is a high altitude (3800 m) saline wetland located in the Chilean Altiplano. We analyzed samples from a tributary stream (H0) and three shallow lagoons (H1, H4, H6) that contrasted in their physicochemical conditions and associated biota. 78 phylotypes were identified in a total of 268 clonal sequences from seven clone libraries of water and sediment samples. Oscillatoriales were frequently found in water samples from sites H0, H1 and H4 and in sediment samples from sites H1 and H4. Pleurocapsales were found only at site H0, while Chroococcales were recovered from sediment samples of sites H0 and H1, and from water samples of site H1. Nostocales were found in sediment samples from sites H1 and H4, and water samples from site H1 and were largely represented by sequences highly similar to Nodularia spumigena. Cyanobacterial communities from Salar de Huasco are unique, and a number of clone sequences were related to sequences and clusters previously described from Antarctic environments.

### **5.1.2 INTRODUCTION**

In terms of their morphology and phylogenetics, cyanobacteria are one of the most diverse groups of prokaryotes (Waterbury, 2006). Their ecological tolerance, (e.g.

to a broad range of temperatures, high salinities, adaptations to light) contribute to their competitive success in a variety of environments, both as planktonic or benthic organisms (Cohen and Gurevitz, 2006). Cyanobacteria can dominate primary production in some environments including microbial mats (Stal, 1995) and some extreme environments, such as Antarctic permafrost aquatic systems (Jungblut et al., 2005).

Cyanobacteria are currently placed into five orders: Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales and Stigonematales. Members of the Chroococcales and Oscillatoriales are dispersed throughout the phylogenetic tree, indicating that these two orders at least do not represent coherent evolutionary lineages (Waterbury, 2006).

Recent studies in wetlands located in the Chilean Altiplano described high microbial diversity and high spatial variability of the microbial communities (Demergasso et al., 2004, Chapter 3). The athalassohaline water bodies located in this area are subject to extreme conditions including high UV radiation, low temperatures, negative water balance and variable salt concentration. Little information is available on cyanobacterial diversity in Andean salares, with the exception of a study examining the microbial mats of the Salar de Llamará, located in the Atacama Desert (Demergasso et al., 2003). This study revealed the presence of *Cyanothece* sp., *Synechococcus* sp., *Microcoleus* sp., *Oscillatoria* sp., *Gloeocapsa* sp. and *Gloeobacter* sp. in different mats. *Oscillatoria* sp. were also revealed to be a dominant component of the cyanobacterial community of the Laguna Tebenquinche in the Salar de Atacama (Zúñiga et al., 1991).

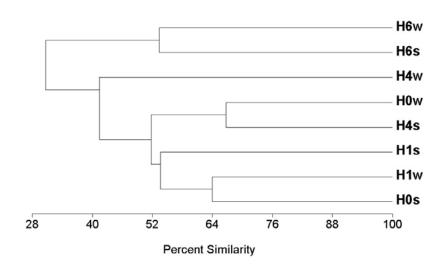
Salar de Huasco is an Andean salar (Chong, 1984) located at 3800 m altitude that exhibits high spatial variability in distinct microniches. Using 16S rDNA clone libraries

and PCR-DGGE, we examined cyanobacterial community structure in water and sediment samples collected from four different sites within the Salar de Huasco.

# **5.1.3 RESULTS**

## 5.1.3.1 Composition of cyanobacterial communities in Salar de Huasco

We used cluster analysis (UPGMA) of DGGE bands in order to determine similarities in the cyanobacterial composition between the samples and sites. Samples of water and sediment from site H6 clustered together, but other samples did not show any clear grouping reflecting sample type or site (Fig. 5-1). The number of DGGE bands and clonal sequence diversity was higher in sediment than in water samples (Table 5-1), except for the sample H1w. Previous (September 2002, March 2003, September 2003) microscopic observations of water samples from the same sites detected *Oscillatoria* sp. in sites H0, H1 and H6, *Anabaena sp.* in H1 and *Spirulina* sp. in H6 (Vila et al., unpublished).



**Fig. 5-1.** UPGMA clustering of DGGE band patterns of 16S rDNA from water and sediment samples of the four sites in Salar de Huasco.

**Table 5-1.** Summary of data obtained from DGGE and cyanobacterial 16S rDNA clone libraries.

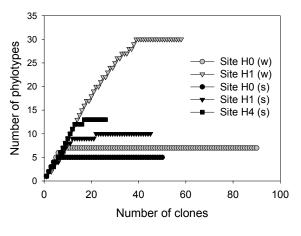
DGGE			16S rDNA clone library				
Sample	Number of bands	Shannon diversity index (H')	Number of clones	Number of phylotypes	Coverage (%)	S <sub>Chao1</sub>	Shannon diversity index (H')
H0w	5	1.61	90	7	95.55	11.13	0.59
H1w	11	2.40	57	29	64.91	67.45	3.01
H4w	7	1.95	7	6	nd	nd	nd
H6w	6	1.79	7	7	nd	nd	nd
H0s	14	2.64	50	5	98.00	5.16	0.90
H1s	8	2.08	44	10	88.63	20.50	1.55
H4s	10	2.30	27	14	59.25	43.26	2.21
H6s	9	2.20	nd	nd	nd	nd	nd

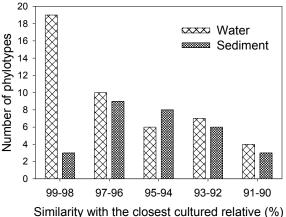
nd: not determined

# 5.1.3.2 Cyanobacterial 16S rDNA clone library

Four clone libraries of water (sites H0, H1, H4 and H6) and three of sediment samples (sites H0, H1 and H4) were constructed. From the water samples 147 clones were obtained and grouped into 49 phylotypes. Sequence analysis of clones from sites H4 and H6 revealed a large number of unspecific sequences related to Bacteria (92% of the clones of H4 and 90% of H6). These libraries were subsequently excluded from rarefaction analyses. We obtained 121 clones in 29 phylotypes from sediment samples (Table 5-1). Rarefaction analysis revealed saturation in all libraries at a number of phylotypes between 6 and 14, except for the sample H1w (29 phylotypes) (Fig. 5-2). In addition, coverage indicated that more than 59% of total diversity was detected in the clone libraries. The richness estimator  $S_{Chao1}$  was higher than the number of observed phylotypes in all libraries but almost identical for one sample (H0s). Generally, diversity was higher in sediment than in water samples, but the highest diversity was observed in the water sample of site H1 (Table 5-1). A BLAST search was used to find similarities of

the phylotypes with sequences in GenBank. Most phylotypes from water samples had a high similarity (98-99%) with their closest cultured relatives. In sediment samples, most were 96-97% similar. A significant proportion of the phylotypes were less than 95% similarity to their closest cultured relatives (Fig. 5-3).





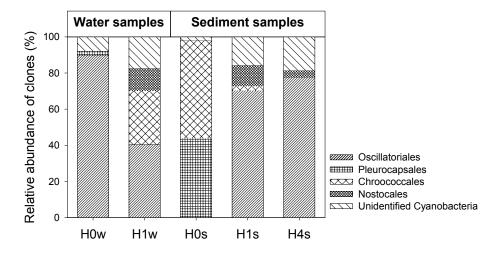
**Fig. 5-2.** Rarefaction analysis of 16S rDNA clone libraries of cyanobacteria from water (w) and sediment samples (s) of sites H0, H1 and H4.

**Fig. 5-3.** Percent similarity of the phylotypes with their closest cultured relatives.

## **5.1.3.3** Phylogenetic diversity

Cyanobacterial communities were distinct in each of the four sites from the Salar de Huasco. The sequences were mainly related to described phylotypes retrieved from microbial mats of Antarctic, marine and freshwater environments. We detected cyanobacteria from 4 orders Oscillatoriales, Nostocales, Pleurocapsales, and Chroococcales (Fig. 5-4).

In addition, between 8-18% and 2-19% of unidentified cyanobacteria were found in water and sediment samples, respectively. In detail, the following cyanobacteria were identified at the different sites (Fig. 5-4):



**Fig. 5-4**. Composition of the cyanobacterial 16S rDNA clone libraries from water (w) and sediment (s) samples of the sites H0, H1 and H4.

In water samples from site H0, the clone library was dominated by Oscillatoriales but Chroococcales and Pleurocapsales were dominant in sediment samples, Oscillatoriales were the abundant group in water and sediment samples from the other sites, and Pleurocapsales were only found at H0. Nostocales were identified at H1 (water and sediment) and from sediment at H4.

78 phylotypes, defined to have 99% similarity between the clones, were grouped into 12 clusters with distinct phylogenetic affiliation (Table 5-2, Fig. 5-5). Clusters A, B, D, G and H were formed at <97% similarity with the closest relatives in GenBank (underlined clones). Most sequences with lower similarity with their closest relatives from GenBank were retrieved from the site H1 and were distributed across the 12 defined clusters.

Phylotype H1w-93 was distantly related (91%) to the planktonic *Limnothrix* sp. (cluster A). Cluster B included the phylotypes H4s-42 and H1w-72 that grouped with the phylotype 16ST17, previously described from Antarctic environments (Taton et al., 2006a) and with the benthic *Geitlerinema carotinosum*. Cluster D included the

phylotypes H0s-1 and H1w-27 related to the unicellular *Chamaesiphon subglobosus*. Cluster G included two phylotypes from water samples (site H4) that were 95% similar to members of the Chroococcales. Cluster H consisted of two groups, one formed with phylotypes from sites H1 and H6 and distantly related with their first hit in BLAST (<92%). The second group was formed with two phylotypes from water samples from H1 that showed 95% similarity to *Merismopedia glauca*.

Clusters C, J, K, and L were affiliated to the Oscillatoriales, cluster E to the Nostocales and cluster F to the Pleurocapsales (Fig. 5-5, Table 5-2). Cluster C was comprised of phylotypes from sites H0, H1 and H6 that were related to the benthic, filamentous cyanobacterium *Phormidium*. Phylotype H1w-15 had 98% similarity with Phormidium inundatum SAG 79.79 isolated from thermal waters in France (Marquardt and Palinska, 2007). A further two phylotypes from water samples (site H0) had 96-99% similarity with the clone Fr147 retrieved from microbial mats of Lake Fryxell in Antarctica (Taton et al., 2003). Three phylotypes from sediment and one from water samples all collected at site H1, clustered together with 93-99% similarity to *Phormidium* sp. ETS.05 previously isolated from thermal springs in Italy (Berrini et al., 2004). Most of the phylotypes from site H0 water samples formed a separate group inside cluster C, with similarities between 96-99% with Microcoleus vaginatus and Phormidium sp. NIVA-CYA 203, both isolated from terrestrial environments from Arctic Norway (Rudi et al., 1997). Sequences from Lake Fryxell in Antarctica (Taton et al., 2003) and the clone 173-2 retrieved from soil crusts in the Colorado Plateau in USA (Gundlapally and Garcia-Pichel, 2006) are also part of this sub-cluster which has been described as Cluster I (Taton et al., 2003).

**Table 5-2.** Description of the clusters in the phylogenetic tree and phylotypes. Percent similarity with closest relatives and closest cultured relatives in GenBank are shown.

Cluster	Salar de Huasco phylotypes	Closest GenBank entry (% similarity)	Closest cultured relatives (% similarity)	Habitat of closest relative
A	H1w-93	Limnothrix sp. CENA 110 (EF088338) (91%)		Waste stabilization pond, Brazil
В	H4s-42 , H1w-72	Uncultured cyanobacterium clone A206 (DQ181671) (92-96%)	Geitlerinema carotinosum AICB 37 (AY423710) (92-95%)	Microbial mat, Lake Ace, Vestfold Hills, Antarctica
C	H1w-15	Phormidium inundatum SAG 79.79 (AM398801) (98%)		Thermal water, France
	H1s-30, H1w-77, H1s-79, H1s-38	Phormidium sp. ETS-05 (AJ548503) (93-99%)		Thermal mud, Euganean thermal springs, Italy
	H0w-44, H0w-51	Uncultured Antarctic cyanobacterium clone Fr147 (AY151731) (96-99%)	Phormidium uncinatum SAG 81.79 (AM398780) (93%)	Microbial mat, Lake Fryxell, McMurdo Dry Valleys, Antarctica
	H0w-87	clone 173-2 (AJ871976) (97%)	Microcoleus vaginatus PCC 9802 (97%)	Biological soil crust, Colorado Plateau, USA
	H0w-63, H0w-79, H0w-1	Uncultured cyanobacterium clone G1-1_9 (EF438215) (96-99%)	<i>Phormidium</i> sp. NIVA-CYA 203 (Z82792) (96-99%)	Epilithon, Douglas River, Ireland
	H1s-3	Phormidium cf. terebriformis KR2003/25 (AY575936) (96%)		Hot spring, Lake Bogoria, Kenya
	H1w-20	Phormidium pseudopristleyi ANT.ACEV5.3 (AY493600) (98%)		Microbial mat, Lake Ace, Vestfold Hills, Antarctica
	H4w-78, H4w-28, H4w-90	<i>Phormidium</i> sp. UTCC 487 (AF218376) (96-98%)		Canada, Artic
D	H0s-1	Uncultured cyanobacterium clone SepB-17 (EF032663) (97%)	Chamaesiphon subglobosus PCC 7430 (AY170472) (97%)	River biofilm, Cloghoge River, Irland
	H1w-7	Nodularia spumigena strain NSLA02A4 (AF268008) (93%)		Lake Alexandrina, SA, Australia
E	H4s-37	Aphanizomenon cf. gracile 271 (AJ293125) (97%)		Lake Norre, Denmark
	H4s-56	Anabaena cylindrica PCC 7122 (AF247592) (95%)		Japan
	H1s-29	Cyanospira rippkae (AY038036) (97%)		Soda lake Magady, Kenya
	H1w-78	<i>Tolypothrix</i> sp. PCC 7415 (AM230668) (97%)		Soil, greenhouse, Stockholm, Sweden

	H1w-18	Nodularia spumigena strain NSLA02A4 (AF268008) (99%)		Lake Alexandrina, SA, Australia
	H1w-86	Nodularia spumigena strain BY1 (AF268004) (99%)		Baltic Sea
	H1w-59	Nostoc sp. 8941 (AY742448) (97%)		Gunnera dentata, New Zealand
	H1s-24	Calothrix sp. BECID30 (AM230685) (94%)		Rock surface, Baltic Sea, Finnland
F	H0s-57	Uncultured cyanobacterium clone TAF-A202 (AY038730) (92%)	<i>Dermocarpella</i> sp. PCC 7326 (AJ344559) (91%)	Epilithon, River Taff, UK
	H0s-2, H0s-58, H0w-42, H0s-6	Uncultured cyanobacterium clone TAF-A202 (AY038730) (94-98%)	<i>Pleurocapsa</i> sp. CALU 1126 (DQ293994) (94-98%)	Epilithon, River Taff, UK
G	H4w-85, H4w-67	Uncultured cyanobacterium clone SC3-19 (DQ289927) (95%)	Gloeothece sp. KO68DGA (AB067580) (95%)	Sediment, South Atlantic Bight
Н	H6w-40, H1s-69, H6w-77	Uncultured bacterium clone MSB-2E11 (EF125441) (92%)	Symploca sp. VP642c (AY032934) (91%)	Mangrove soil
	H1w-14, H1s-52, H1s-53	Uncultured bacterium clone MSB-2E11 (EF125441) (93%)	Gloeothece membranacea PCC 6501 (X78680) (91-92%)	Mangrove soil
	H1w-3	Synechocystis PCC6805 (AB041938) (97%)		
	H1w-19	Merismopedia glauca B1448-1 (X94705) (95%)		Microbial mat, Norderney Island, Germany
I	H1w-4	Gloeocapsa sp. PCC 73106 (AF132784) (94%)		
	H1s-95, H1w-80	Uncultured cyanobacterium clone GPENV127 (DQ512831) (97-95%)	Synechocystis sp. PCC 6308 (AB039001) (97-95%)	Gorompani warm spring, Assam, India
	H1w-31	Cyanobacterium stanieri PCC 7202 (AM258981) (98%)		Microbial mat, Euganean thermal springs, Italy
J	H4s-45	Oscillatoria sp. CCAP 1459/26 (AY768396) (98%)		
	H1w-44	Halomicronema excentricum str. TFEP1 (AF320093) (93%)		Microbial mat, Eilat artificial ponds, Israel
	H1w-5	Leptolyngbya sp. 0BB32S02 (AJ639894) (93%)		Bubano basin, Imola, Italy
	H4s-26, H1w-92	Uncultured cyanobacterium clone Ct-3-39 (AM177427) (93%)	Halomicronema sp. SCyano39 (DQ058860) (92%)	Coral reef sediments, Heron Island, Australia
	H1w-35	Leptolyngbya nodulosa UTEX 2910 (EF122600) (93%)		

	H1w-65	Leptolyngbya sp. CCMEE6011 (AY790838) (95%)		Travertine rock, Narrow Gauge Lower Terrace, Yellowstone National Park, USA
	H4s-20, H4s-33, H4s-19, H6w-1, H4s-24, H4s-15, H1w-13	Leptolyngbya sp. 0BB30S02 (AJ639892) (95-98%)		Bubano basin, Imola, Italy
	H1w-53	Leptolyngbya antarctica ANT.ACEV6.1 (AY493589) (98%)		Microbial mat, Lake Ace, Vestfold Hills, Antarctica
	H1w-1	Oscillatoria sp. CCMEE 416 (AM398781) (98%)		Marble Point, Antarctica
	H4w-62, H1w-8, H4s-66	Leptolyngbya sp. 0BB24S04 (AJ639893) (97-98%)		Bubano basin, Imola, Italy
K	H1w-71	Uncultured cyanobacterium clone G1-1_58 (EF438248) (97%)	Leptolyngbya sp. 0BB19S12 (AJ639895) (90%)	Epilithon, Douglas River, Ireland
	H1w-82	Leptolyngbya frigida ANT.LH70.1 (AY493574) (99%)		Microbial mat, Lake Reid, Larsemann Hills, Antarctica
	H1w-79	Uncultured cyanobacterium clone RJ004 (DQ181705) (99%)	Leptolyngbya antarctica ANT.LH18.1 (AY493607) (99%)	Microbial mat, Lake Reid, Larsemann Hills, Antarctica
	H1w-27	Filamentous thermophilic cyanobacterium tBTRCCn 302 (DQ471445) (96%)	Oscillatoria sp. OH25 (AF317508) (96%)	Zerka Ma'in thermal springs, Jordan
L	H4s-61	Uncultured cyanobacterium clone RJ037 (DQ181715) (93%)	Leptolyngbya antarctica ANT.FIRELIGHT.1 (AY493590) (92%)	Microbial mat, Lake Reid, Larsemann Hills, Antarctica
	H4s-31	Uncultured Antarctic cyanobacterium clone Fr285 (AY151759) (94%)	Leptolyngbya antarctica ANT.FIRELIGHT.1 (AY493590) (93%)	Microbial mat, Lake Fryxell, McMurdo Dry Valleys, Antarctica
	H4s-18, H6w-73	Leptolyngbya antarctica ANT.FIRELIGHT.1 (AY493590) (97-99%)		Microbial mat, Lake Firelight, Bolingen Islands, Antarctica

Sequences from this sub-cluster within cluster C have a particular 11-nucleotide insertion, first described for Antarctic and Artic species (Nadeau et al., 2001), and also lately found in Antarctic clone libraries (Taton et al., 2003). We found this insertion in the phylotypes H0w-1, H0w-87, H0w-79 and H0w-63. The phylotype H1w-20 was 98% similar to *Phormidium pseudopriestleyi* ANT.ACEV5.3, isolated from Lake Ace in Antarctica (Taton et al., 2006b) and was included in a cluster related to saline environments (Taton et al., 2006a).

Three phylotypes of water samples from site H4 formed a separate group: clones H4w-78 and H4w-28 were 96-98% similar with *Phormidium* sp. UTCC 487, isolated from benthic substrate in Canadian Arctic (Casamatta et al., 2005). Clone H4w-90 was 99% similar with *Phormidium* sp. OL S6, previously isolated from a microbial mat in the North Sea. Both *Phormidium* species formed one cluster (Marquardt and Palinska, 2007). Cluster E was affiliated to the Nostocales and contained phylotypes from sites H1 and H4. Two sediment phylotypes from site H4 were >95% similar to members of the Nostocaceae. Another set of phylotypes from H1 grouped together with *Nodularia*. Phylotypes H1w-18 and H1w-86 were 99% similar to two strains of *Nodularia* spumigena, described as a planktonic, toxic, bloom-forming cyanobacterium with heterocysts and high 16S rRNA gene sequence similarity with other members of the genus ranging from 98.5–100% (Moffit et al., 2001; Lyra et al., 2005), and with the clone A180 retrieved from microbial mats of Lake Ace in Antarctica (Taton et al., 2006a). Further studies are necessary to determine if *Nodularia* in Salar de Huasco produces nodularin, a hepatotoxin produced via a nodularin synthetase (Lyra et al., 2005) and has gas vesicles. These studies could include analysis of the *nif*H gene and toxin production (Palinska et al., 2006).

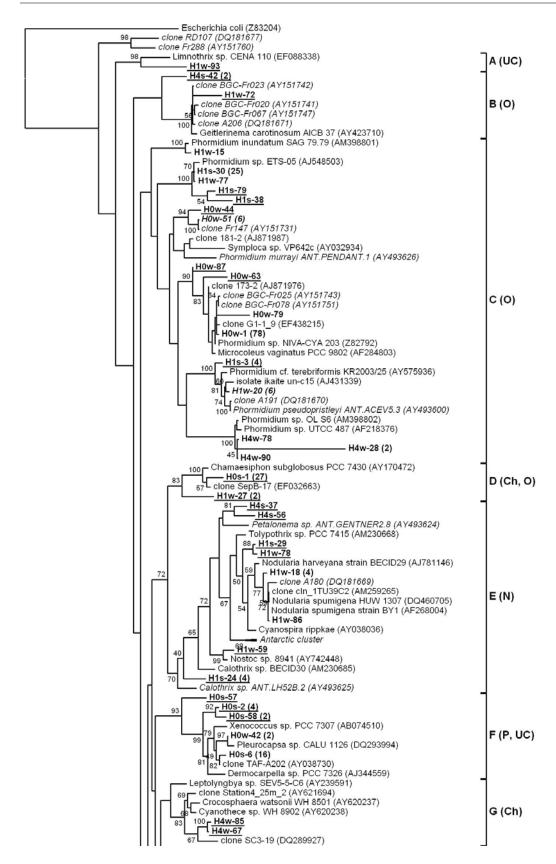


Fig. 5-5.

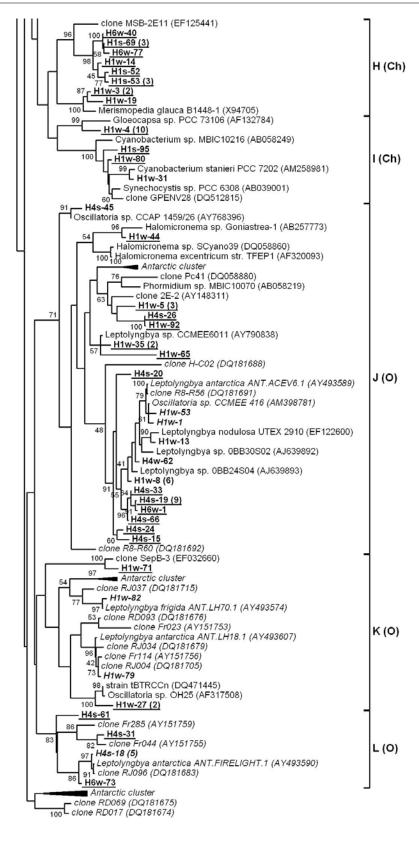


Fig. 5-5. (Continued)

0.1

**Fig. 5-5.** Phylogenetic tree based on partial 16S rDNA sequences (~660 bp) calculated by maximum likelihood analysis. The scale bar represents 10% nucleotide sequence difference. Bootstrap values greater than 40% are shown. Clone sequences from this study are in bold and coded as follows (example of H0w-42): H, Salar de Huasco, site H0; w, water sample; 42, clone number. Underlined clones represent sequences with <97.5% similarity to the closest relatives in BLAST. Clones in italics had >98% similarity with their closest relatives retrieved from Antarctica. The number of clones in each phylotype is shown in brackets. Phylogenetic affiliations of the clusters are indicated as follows: UC, Unidentified Cyanobacteria; O, Oscillatoriales; N, Nostocales; P, Pleurocapsales; Ch, Chroococcales. *Escherichia coli* (Z83204) was used as outgroup.

Phylotype H1w-59 had 97% similarity with *Nostoc* sp. 8941 isolated from *Gunnera dentata* in New Zealand (Svenning et al., 2005). In the same cluster E, the phylotype H1s-24 showed 94% sequence similar to *Calothrix* sp. ANT.LH52B.2, isolated from Lake Bruehwiler in Antarctica. This species was considered as a new phylotype (Taton et al., 2006b).

Cluster F, affiliated to the Pleurocapsales, only contained phylotypes from site H0. Sequence similarity of the clones of this cluster ranged between 92 to 98% with clone TAF-A202 retrieved from sediment samples from epilithon of river Taff in the UK (O'Sullivan et al., 2002). Clone H0w-42 had 98% similarity with *Pleurocapsa* sp. CALU 1126 (GenBank information).

Cluster I was affiliated to the Chroococcales and only included sequences from site H1. The phylotype H1w-31 had 98% similarity with *Cyanobacterium stanieri* PCC 6308 (GenBank information).

Cluster J included members of the Oscillatoriales and was formed with phylotypes from sites H1, H4 and H6 (Fig. 5-5, Table 5-2). Phylotype H4s-45 was 98% similar with *Oscillatoria* sp. CCAP 1459/26 (GenBank information). Phylotype H1w-44 was 93% related to *Halomicronema excentricum* str. TFEP1, a new filamentous benthic genus isolated from microbial mats in artificial ponds from Eilat in Israel (Abed et al., 2002). Three phylotypes (H1w-5, H4s-26, H1w-92) clustered together but at low

similarity, and their affiliation inside the Oscillatoriales was unclear. Phylotypes H1w-35 and H1w-65 clustered with *Leptolyngbya* sp. CCMEE6011 isolated from dry travertine rocks in the Yellowstone National Park in USA (Norris and Castenholz, 2006). Two phylotypes from Site H1 water samples (H1w-53, H1w-1) were highly similar (<98%) with Antarctic strains and sequences of clone libraries of 16S rDNA. Phylotypes H1w-13, H4w-62 and H1w-8 had 98% similarity with *Leptolyngbya* sp. 0BB24S02 and *Leptolyngbya* sp. 0BB24S04, isolated from Bubano basin in Imola, Italy (Castiglioni et al., 2004). Another set of phylotypes exhibited similarity values lower than 97% with the strains described above.

Cluster K contained phylotypes of the water sample from site H1. Phylotype H1w-82 was 99% similar with *Leptolyngbya frigida* ANT.LH70.1, isolated from Lake Reid and considered as a new strain from Antarctica (Taton et al., 2006b). Another phylotype (H1w-79) was highly similar (99%) with clone RJ004 from a cluster hitherto unique for Antarctic environments (Taton et al., 2006a).

Cluster L was formed by 4 phylotypes retrieved of sediment samples from sites H4 and water samples from site H6. They grouped together with clones and one strain recovered from Antarctica. Phylotype H4s-18 was 99% similar with *Leptolyngbya antarctica* ANT.FIRELIGHT.1 that was considered unique for Antarctica (Taton et al., 2006b).

### 5.1.4. DISCUSSION

Cyanobacterial diversity in the Salar de Huasco exhibited a pattern similar to that described from Antarctic microbial mats. The sequences from Salar de Huasco that were related to samples from the Antarctic were highly similar to those retrieved from Lake

Fryxell, a productive freshwater lake in the Taylor Valley in McMurdo Dry Valley, Antarctica (Taton et al., 2003), with microbial mats from the saline lakes Ace and Reid and with the freshwater lakes Bruehwiler and Firelight located in East Antarctic (Sabbe et al., 2004; Taton et al., 2006a; Taton et al., 2006b). Most of the sequences from this study, including those from water samples, were related to benthic cyanobacteria. We found no clear differences between cyanobacterial communities from water and sediment samples (Fig. 5-1). Microscopic analysis of the planktonic communities in Salar de Huasco revealed the dominance of diatoms at the high salinity sites, but Chlorophyta and to a lesser extent Cyanobacteria dominated the sites with low salt concentration (Vila et al., unpublished). Nevertheless, we found highly diverse cyanobacterial communities, spread over four of the five taxonomic orders: Oscillatoriales, Chroococcales, Pleurocapsales and Nostocales. The community at site H1 was the most diverse and had the highest number of phylotypes. Sequences related to *Nodularia spumigena*, a planktonic, usually toxic and bloom forming cyanobacterium and other Nostocales with heterocysts (e.g. *Nostoc* sp.) were found among them. This raises the possibility of cyanobacterial nitrogen fixation at this site, which as water bodies located in the Altiplano are limited by nitrogen is of clear importance (Vincent et al., 1984; 1985, Dorador et al., 2003).

Sequences retrieved from site H0 had the lowest diversity. Those from sediment samples formed two groups related to Pleurocapsales and to Chrooccocales, while sequences from water samples grouped into the same cluster related to *Phormidium* (cluster C, Fig. 5-5). These sequences have an 11 bp insertion, firstly considered as a signature for endemism of Arctic and Antarctic Oscillatoriales (Nadeau et al., 2001), but also sequences from other non-polar environments may have this insertion (Taton et al., 2003) including sequences from the Salar de Huasco, as found in the present study.

In general, sequences from the present study had low similarity with their closest relatives in GenBank. Threshold values of 97.5% have frequently been used to distinguish between cyanobacterial species (Taton et al., 2003; 2006b). Because 16S rDNA sequences with 97.5% of similarity likely correspond to DNA-DNA hybridization values of less than 70%, these sequences probably represent two distinct species (Stackebrandt and Göbel, 1994). If we consider 97.5% as a threshold value, 90% of the sequences from sediments and 59% from water samples could be considered as new phylotypes (Fig. 5-3).

Based on morphological data, endemism of cyanobacteria in Antarctic habitats has been discarded and cyanobacteria appear to have cosmopolitan distribution (Vincent, 2000; Taton et al., 2003). Conversely, molecular tools have revealed evidence for a bipolar distribution of Antarctic and Arctic cyanobacteria (Comte et al., 2007) and the existence of some clusters endemic for Antarctica (Taton et al., 2003; Jungblut et al., 2005; Taton et al., 2006a; Taton et al., 2006b). The current study has revealed a high microdiversity of cyanobacterial communities in different compartments of the Salar de Huasco, an almost unexplored water body in the Chilean Altiplano. Futhermore, it has shown the presence of some clusters that have been considered up to now as new or endemic for Antarctic habitats. Because the Chilean Altiplano is geographically well separated from both polar regions, their presence in high altitude Altiplano wetlands may be indicative of their adaptation to cold habitats worldwide. This is a likely conclusion, because these organisms, or their sequences, have been obtained exclusively from cold habitats. However, cyanobacteria are adapted to a wide range of environmental conditions and all those representatives not specifically adapted to the cold may either be tolerant to the cold temperatures or restricted to growth during warmer period of the wetland habitats. Most strikingly, a number of sequences (and isolates) related to those from warm or hydrothermal waters were found. If these, despite some sequence difference, have similar physiological properties as their counterparts from warmer habitats, they may apparently not be well adapted to conditions in the Altiplano wetlands. Alternatively, they may not actually be adapted to the elevated temperatures, but their presence reflects in the Altiplano reveals that they are actually relatively insensitive to temperature extremes.

# 5.2 ANOXYGENIC PHOTOTROPHIC BACTERIA AND EVIDENCE OF ROSEOBACTER-LIKE SEQUENCES

## **5.2.1 ABSTRACT**

Phototrophic bacteria were investigated in Salar de Huasco, a cold, high altitude (3800 m), saline wetland located in the Chilean Altiplano using cultivation methods and clone libraries of 16S rDNA. 11 isolates were obtained and their 16S rDNA sequences were related to *Thiocapsa roseopersicina*, *Ectothiorhodospira* sp., *Rhodovulum* sp., and *Rhodobacter* sp. A separate set of samples was used to examine the tolerance of anoxygenic phototrophic bacteria to salinity. Our results demonstrated that the growth of red-pink colonies was notably related to salinity at the sites. Phylogenetic analyses revealed that most isolates and clones were associated with the *Alpha-*, *Beta-* and *Gammaproteobacteria*, but some sequences were related to *Chloroflexi*. Within the *Alphaproteobacteria*, a distinct cluster was identified with less than 94% similarity with a *Roseobacter* clade, a marine group of aerobic anoxygenic phototrophic bacteria.

### **5.2.2 INTRODUCTION**

Phototrophic prokaryotes (*i.e.* organisms that have the ability to use light as energy source) are distributed widely in the biosphere. Two major groups are defined as phototrophic bacteria: the oxygenic cyanobacteria and the anoxygenic purple and green phototrophic bacteria (Imhoff, 1988). It is likely that during the initial stages of the evolution of the biosphere, these bacteria were responsible for the entire global photosynthetic fixation of carbon (Overmann and Garcia-Pichel, 2006). Photosynthetic prokaryotes are distributed in five phylogenetic lineages: *Chlorobi*, *Chloroflexi*, *Cyanobacteria*, *Proteobacteria* and *Firmicutes*. With the exception of *Cyanobacteria*, phototrophic bacteria perform anoxygenic photosynthesis (Overmann and Garcia-Pichel, 2006).

Many obligate aerobic species have a purple bacterial type of photosynthetic apparatus (AAnP, aerobic anoxygenic phototrophs) and are widely distributed in freshwaters, meromictic lakes, marine environments (e.g. microbial mats, water, hydrothermal vents), hot springs and other environments (Yurkov and Beatty, 1998). Three marine genera: Erythrobacter, Citromicrobium, Roseobacter, seven freshwater genera: Erythromicrobium, Roseococcus, Roseateles, Porphyrobacter, Acidiphilium, Erythromonas, Sandaracinobacter and two soil genera: Craurococcus, Paracraurococcus have been taxonomically described (Yurkov, 2006). These bacteria contain bacteriochlorophyll a, and form a significant and diverse component of the marine bacterioplankton community (Béjà et al., 2002; Oz et al., 2005).

Anoxygenic phototrophic bacteria are of particular interest because of i) the simple molecular architecture and variety of their photosystems, ii) the elevated diversity within the group, which has implications on the reconstruction of the phylogeny and

evolution of photosynthesis, and iii) because the importance of these taxa in global biogeochemical cycles, e.g. carbon and sulfur (Overmann and Garcia-Pichel, 2006).

Purple and green anoxygenic phototrophic bacteria are widely distributed in planktonic and benthic environments, and are also found in environments with high salt concentrations. The degree of dependency and the tolerance of microorganisms to salt reflect physiological differences in the capacity to adapt to different salinity conditions. It also reflects different ecological distributions. These different degrees are defined as: nonhalophilic (up to 0.2 M NaCl for optimum growth); slightly halophilic (0.2 to 1.0-1.2 M); moderately halophilic (1.0-1.2 to 2.0-2.5 M); extremely halophilic (more than 2.0-2.5 M) and halotolerant (more than 3 M NaCl) (Imhoff, 1993; Imhoff, 1986).

The Salar de Huasco is a high-altitude (3800 m) wetland located in the Chilean Altiplano with a maximum salinity of 113 gL<sup>-1</sup> (Risacher et al., 1999). It is subjected to high UV-B radiation, low temperatures and variable climatic conditions. Numerous continental evaporitic deposits (salares) are distributed throughout northern Chile and can be classified according to their geographical location, origin and chemical properties. Those located at high altitude receive water inputs directly from precipitation during the austral summer, in contrast to those located in the Atacama Desert where precipitation is almost absent during the year (Chong, 1984; Garreaud, 2003; Risacher et al, 2003). Few microbiological surveys have been conducted in these systems. Demergasso et al. (2003) described the photosynthetic communities in microbial mats from the Salar de Llamará located in the Atacama Desert. Filamentous cyanobacteria dominated the mats but in the purple layer, cells related to *Chromatium* sp. and *Thiocapsa* sp. were identified with microscopical observations. Here we describe phototrophic bacteria from four different sites in the Salar de Huasco using culture and culture-independent methods.

### **5.2.3 RESULTS AND DISCUSSION**

## 5.2.3.1 Growth and salt tolerance of anoxygenic phototrophic bacteria

From a total of ten enrichment cultures, six exhibited growth of anoxygenic phototrophic bacteria from different sediment types and site locations. Red-pink coloration of the enrichments was found in bottles inoculated with colored or black sediments (Table 5-3). Microscopic observations and 16S rDNA analysis were used to identify *Thiocapsa* sp. in samples from the sites H1, H4 and H6, *Rhodobacter* sp. in sites H0 and H1 (0.43-0.46 gL<sup>-1</sup> total dissolved salts), and *Ectothiorhodospira* sp. and *Rhodovulum* sp. in site H6 (9.4 gL<sup>-1</sup> total dissolved salts) (Table 5-4).

**Table 5-3.** Enrichment cultures of phototrophic bacteria, noting the presence (+) or absence (-) of growth.

Name	Site	Description	Growth	Identification of the isolates
H0a	H0	gravel sediment	+	Rhodobacter sp.
H0b	H0	thin sediment	-	-
H1a	H1	black sediment	+	Rhodobacter sp.
H1b	H1	red-brown sediment	+	Thiocapsa sp.
H4a	H4	black sediment	+	Thiocapsa sp.
H4b	H4	black and grey sediment	-	-
H4e	H4	green salt crust	-	-
Н6а	Н6	grey sediment	-	-
H6b	Н6	grey and green sediment	+	Thiocapsa sp., Rhodovulum sp.
Н6с	Н6	grey sediment	+	Ectothiorhodospira sp.

Rhodobacter and Rhodovulum are purple non-sulfur bacteria of the Rhodobacter-group (α-3 Proteobacteria). They are characterized by the presence of carotenoids and their extreme metabolic versatility and flexibility (Imhoff, 2006a). Members of this group have been isolated from freshwater and marine environments. Thiocapsa roseopersicina has low salt requirement (<1%) but is quite tolerant to higher salt concentrations. It is a member of the Chromatiaceae (Gammaproteobacteria) and a widely distributed species

recorded from marine coastal habitats, wastewater treatment systems and sediments (Imhoff, 2006b; Imhoff, 2001). *Ectothiorhodospira* (Ectothiorhodospiraceae) are halophilic and alkaliphilic purple sulfur bacteria that are found in alkaline environments with saline or hypersaline conditions (Imhoff, 2006c).

**Table 5-4.** Growth (+) of phototrophic bacteria cultures in media containing various salt concentrations (0, 5, 10 and 15% of NaCl:MgCl<sub>2</sub>×6H<sub>2</sub>0 6:1).

		% salt concentration				
Sample	Site	0	5	10	15	
H0-1	Н0	-	+	-	+	
H0-2	H0	+	+	-	-	
H1-1	H1	+	+	+	+	
H1-2	H1	+	+	-	-	
H4	H4	-	-	+	+	
H6-1	Н6	-	-	+	+	
H6-2	Н6	+	+	-	-	
H6-3	Н6	+	+	+	+	
H6-4	Н6	-	+	+	+	

The phototrophic bacteria from the Salar de Huasco were tolerant to elevated salt concentrations (Table 5-4). Under different salt concentrations, pink or purple colonies grew from isolates collected at all sites, but the level of growth was related to the salinity of both the collection site and the culture medium. Sites H0 and H1 are located in the northern part of the Salar de Huasco, and have salt concentrations similar to freshwater. Accordingly, colonies were detected at salt concentrations lower than 5%, except for a single sample from site H1. Site H4 exhibited the highest salt concentration (64.93 gL<sup>-1</sup> total dissolved salts, 63100 μScm<sup>-1</sup>) and colonies were able to grow at salinities between 10 and 15%. *Halorhodospira halophila* grows optimally at 15% NaCl but can grow at up to 30% NaCl (Imhoff and Süling, 1996). Also some other genera of Alpha- and Gammaproteobacteria exhibit high salt tolerance (e.g., *Halochromatium, Thiohalocapsa*,

Rhodothalassium, Rhodovibrio and Rhodovulum can growth at up to 20% NaCl). Considering that colonies of phototrophic bacteria from site H4 grew only at high salt concentrations and the paucity of investigations examining the microbial ecology of these specialized environments, the potential to find new halophilic species at this site is high.

Phototrophic bacteria from site H6 where salt concentration was lower than site H4 but higher than at H0 and H1 (9.38 gL<sup>-1</sup> total dissolved salt, 13740  $\mu$ Scm<sup>-1</sup>) grew over a wide range of salinities (from 0 until 15%) (Table 5-4).

## 5.2.3.2 Phylogenetic relationships of isolates and environmental clones

We constructed a phylogenetic tree using clonal sequences from water and sediment samples from site H1 described a previous study (Chapter 3) that were affiliated with sequences of phototrophic bacteria. We also included sequences of a clone library of 16S rDNA made during the current study from sediment samples taken at site H4.

Isolates and clones were affiliated with *Alpha-*, *Gamma-* and *Betaproteobacteria* and *Chloroflexi* (Fig. 5-6, Table 5-5, Table 5-6). Two clones from sediment samples (Hua4-s-79, Hua-s-66) had low similarity (92-89%) with sequences described as *Chloroflexi*, indicating the possibility of the existence of new members of this group in Salar de Huasco. Clone Hua4-s-49 was 99% similar to *Rhodoferax antarcticus*, a moderately psychrophilic betaproteobacterium previously isolated from the water column of the permanently frozen lake Fryxell in Antarctica. This strain (Fryx1) differs in morphology and DNA-DNA hybridization with another phylogenetically closely related *Rhodoferax antarcticus* strain (AB) (Jung et al., 2004; Madigan et al., 2000).

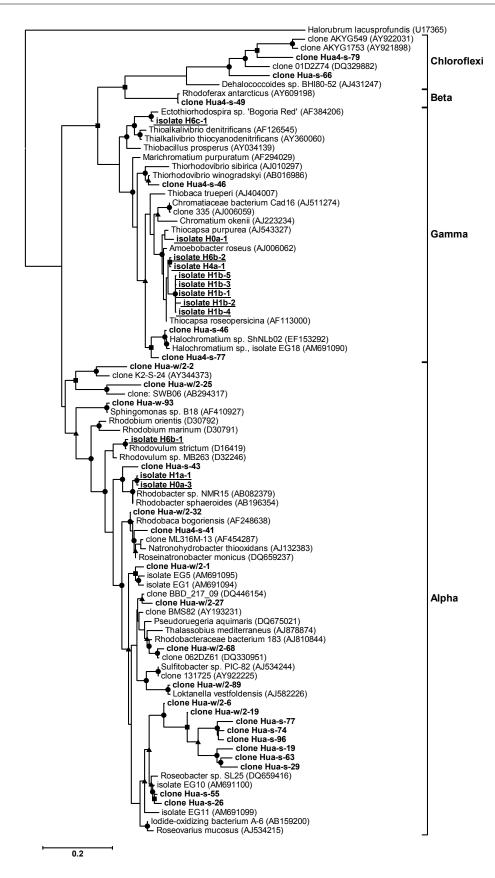


Fig. 5-6.

**Fig. 5-6.** Phylogenetic tree of isolates and clones related to phototrophic bacteria from Salar de Huasco based on partial 16S rDNA sequences (~800 bp) calculated by maximum likelihood analysis. Clone sequences are in bold and coded as follows for the example of Hua-w/2-25: Hua, Salar de Huasco; w, water sample, 2, plate number; 25, clone number or s, sediment sample (Chapter 3). Clones sequences Hua4 were retrieved in the current study. The scale bar represents 10% nucleotide sequence difference. Symbols on the branches indicate bootstrap values: ● >80%; ■ 60-80%; ▲ 40-60%. Underlined clones represent isolates. *Halorubrum lacusprofundi* (U17365) was used as outgroup.

Sequences similar to *Rhodoferax antarcticus* (97.9%) were also reported from benzene-contaminated groundwaters in the UK (Fahy et al., 2006), indicating that this group can exist in habitats which are in great contrast with those from where this strain had been initially described.

**Table 5-5.** Identification of the isolates using 16S rDNA sequence comparison with GenBank entries.

Culture name	Closest relative in BLAST	Similarity (%)	Phylogenetic affiliation
H6c-1	Ectothiorhodospira sp. 'Bogoria Red' (AF384206)	98	Gammaproteobacteria
H0a-1	Thiocapsa roseopersicina strain 1711 (AF113000)	98	Gammaproteobacteria
H6b-2	Thiocapsa roseopersicina strain 1711 (AF113000)	99	Gammaproteobacteria
H4a-1	Thiocapsa roseopersicina strain 1711 (AF113000)	98	Gammaproteobacteria
H1b-5	Thiocapsa roseopersicina strain 1711 (AF113000)	99	Gammaproteobacteria
H1b-3	Thiocapsa roseopersicina strain 1711 (AF113000)	99	Gammaproteobacteria
H1b-1	Thiocapsa roseopersicina strain 1711 (AF113000)	99	Gammaproteobacteria
H1b-2	Thiocapsa roseopersicina strain 1711 (AF113000)	98	Gammaproteobacteria
H1b-4	Thiocapsa roseopersicina strain 1711 (AF113000)	99	Gammaproteobacteria
H6b-1	Rhodovulum sp. ShRb01 (EF153294)	98	Alphaproteobacteria
H1a-1	Rhodobacter sphaeroides ATCC 17029 (CP000578)	98	Alphaproteobacteria
H0a-3	Rhodobacter sphaeroides strain ATCC 17023 (DQ342321)	99	Alphaproteobacteria

Most isolates from sites H1, H4, and H6 (Fig. 5-6, Table 5-5) were highly similar (98-99%) with the gammaproteobacterium *Thiocapsa roseopersicina* strain 1711 (Jonkers et al., 1999). This species is found in illuminated anoxic marine ecosystems and shallow, brackish lagoons, sometimes causing red colorations of the water (Caumette, 1988). *T. roseopersicina* is tolerant to oxygen and can grow chemoorganotrophically or

chemolithotrophically (Imhoff, 2001). We did not recover any clones similar to this species from the clone libraries. Isolate H6c-1 from site H6 was 98% similar to *Ectothiorhodospira* sp. "Bogoria Red" published only in Genbank, and 92-94% similar with the type strains *Ectothiorhodospira shaposhnikovlii*, DSM 243 and *Ect. mobilis* DSM 237 (Imhoff and Süling, 1996). Clone Hua4-s-46 had 97% similarity with *Thiorhodovibrio winogradskyi* (Overmann et al., 1992) part of the marine branch of Chromatiaceae (Imhoff, 2006b). Another two clones were related to Chromatiaceae: Huas-46 and Hua4s-77 had 98-95% similarity with isolates described as *Halochromatium* sp.

Most clones were affiliated with Alphaproteobacteria (Fig. 5-6, Table 5-6). The clones clustered with  $\alpha$ -3 Proteobacteria (*Rhodobacter* and relatives, purple non sulfur bacteria) including the isolates H6b-1, H1a-1 and H0a-3, which were closely related to Rhodovulum and Rhodobacter respectively (Fig. 5-6, Table 5-5). Eight clones from water and sediment samples formed a separate cluster related to Roseobacter, a widely distributed oceanic group capable of aerobic anoxygenic photosynthesis (Wagner-Döbler and Biebl, 2006; Selje et al., 2004; Algaier et al., 2003). These clones were <98% similar with their closest relatives in GenBank (Table 5-6) and similarities with Roseobacter denitrificans ranged between 93-94% and less than 92% with freshwater aerobic anoxygenic phototrophs. This represents the first report of Roseobacter-like sequences in non-marine environments, and has important ecological implications because this group plays an important role for the global carbon and sulfur cycle and the climate, since they have the trait of aerobic anoxygenic photosynthesis, oxidize the greenhouse gas carbon monoxide, and produce the climate-relevant gas dimethylsulfide during the degradation of algal osmolytes (Wagner-Döbler and Biebl, 2006). Further studies based on specific 16S rDNA primers for this group (Selje et al., 2004) and functional genes coding for the photosynthetic reaction center complex (e.g, *puf*L and *puf*M) would permit a detailed examination of the existence of *Roseobacter*-like sequences in Salar de Huasco and to gain a more detailed understanding of the ecological role of phototrophic bacteria.

**Table 5-6.** Identification of clones from water and sediment samples of Salar de Huasco. Clones designated as Hua4 were retrieved in this study. The remaining clones were taken from Chapter 3.

Clone name	Closest relative in BLAST	Closest cultured relative	Similarity (%)	Phylogenetic affiliation
Hua4-s-79	uncultured Chloroflexi bacterium (DQ329882)	Dehalococcoides sp. BHI80-52	89	Chloroflexi
Hua-s-66	clone ctg_BRRAA08 (DQ395380)		92	Chloroflexi
Hua4-s-49	Rhodoferax antarcticus (AY609198)		99	Betaproteobacteria
Hua4-s-46	Thiorhodovibrio winogradskyi (AB016986)		97	Gammaproteobacteria
Hua-s-46	Halochromatium sp. ShNLb02 (EF153292)		98	Gammaproteobacteria
Hua4-s-77	Halochromatium sp., isolate EG18 (AM691090	)	95	Gammaproteobacteria
Hua-w/2-2	unidentified bacterium (AY344373)	Rickettsia prowazekii (M21789)	92/86	Alphaproteobacteria
Hua-w/2-2:	5 uncultured bacterium (AB294317)	Mesorhizobium sp. GWS-BW-H238 (AY332116)	90/86	Alphaproteobacteria
Hua-w-93	Sphingomonas sp. B18 (AF410927)		99	Alphaproteobacteria
Hua-s-43	uncultured sludge bacterium A41 (AF234761)	Rhodobacter sphaeroides (AM696296)	97/96	Alphaproteobacteria
Hua-w/2-32	2 Roseinatronobacter monicus (DQ659237)		97	Alphaproteobacteria
Hua4-s-41	Roseinatronobacter monicus (DQ659237)		97	Alphaproteobacteria
Hua-w/2-1	isolate EG1 (AM691094)		97	Alphaproteobacteria
Hua-w/2-2'	7 clone BBD_217_09 (DQ446154)	Roseovarius nubinhibens ISM (AF098495)	98/97	Alphaproteobacteria
Hua-w/2-68	8 clone 062DZ61 (DQ330951)	Roseobacter sp. Ber2107 (AM180476)	98/97	Alphaproteobacteria
Hua-w/2-89	9 Loktanella vestfoldensis (AJ582226)		99	Alphaproteobacteria
Hua-w/2-6	Roseobacter sp. SL25 (DQ659416)		98	Alphaproteobacteria
Hua-w/2-19	9 isolate EG10 (AM691100)	Roseobacter sp. SL25 (DQ659416)	98/97	Alphaproteobacteria
Hua-s-77	isolate EG5 (AM691095)		96	Alphaproteobacteria
Hua-s-74	isolate EG10 (AM691100)		95	Alphaproteobacteria
Hua-s-96	isolate EG11 (AM691099)		98	Alphaproteobacteria
Hua-s-19	clone BMS13 (AY193156)	Maritimibacter alkaliphilus (DQ915443)	95	Alphaproteobacteria
Hua-s-63	clone 131725 (AY922225)	Sulfitobacter sp. PIC-82 (AJ534244)	97	Alphaproteobacteria
Hua-s-29	clone BMS82 (AY193231)	Rhodobacteraceae bacterium ROS8 (AY841782)	95	Alphaproteobacteria
Hua-s-55	isolate EG10 (AM691100)		98	Alphaproteobacteria
Hua-s-26	isolate EG10 (AM691100)		97	Alphaproteobacteria

# 6. SALT TOLERANCE OF ENRICHMENT CULTURES OF AMMONIA OXIDIZING BACTERIA FROM SALAR DE HUASCO

### 6.1 ABSTRACT

We analyzed ammonia-oxidizing bacteria (AOB) populations using enrichment cultures from several contrasting sites located in the Salar de Huasco, a high altitude, saline, neutral pH water body located in the Chilean Altiplano. Samples were inoculated in mineral media with 10 mM NH<sub>4</sub><sup>+</sup> at five different salt concentrations (10, 200, 400, 800 and 1400 mM NaCl). Growth of beta-AOB was not clearly related with either site or media salinity. Low diversity (up to 3 phylotypes per enrichment) of beta-AOB was detected using 16S rDNA and *amo*A clone libraries. In total, five and six phylotypes were found and were related to *Nitrosomonas marina*, *N. europaea/Nitrosococcus mobilis*, *N. communis* and *N. oligotropha* clusters. Sequences related to *N. halophila* were frequently found at all salinities. No gamma-AOB and ammonia-oxidizing Archaea were found in enrichment cultures.

## **6.2 INTRODUCTION**

The Salar de Huasco is an athalassohaline, high altitude (3800 m) salt-flat with neutral pH located in the Chilean Altiplano. This system exhibits high spatial and temporal variability with contrasting water salinities from freshwater to salt-saturated brines (Risacher et al., 1999). Salt-flats located in the Altiplano (locally called "salares") can have high nutrient concentrations especially at the most saline sites (Chapter 3). In altiplanic wetlands, microbial diversity is dominated by Bacteria instead of Archaea and exhibits a specific pattern according to the type of water body. These salares support an

increased diversity relative to lakes and peatlands (locally referred to as bofedales) (Chapter 3).

Chemolithoautotrophic ammonia oxidizing bacteria (AOB) are involved in the aerobic oxidation of ammonia to nitrite, the initial stage of nitrification and a key component of the nitrogen cycle. In N-limited aquatic systems AOB populations compete with heterotrophs and benthic algae for reduced nitrogen (Bernhard and Peele, 1997; Risgaard-Petersen et al., 2004; Geets et al., 2006). Nitrogen limitation has been reported in Lago Titicaca (Vincent et al., 1984; 1985) and in Lago Chungará (Dorador et al., 2003) both located in the tropical Andes, and this phenomenon is likely to occur in other water bodies in the region. Nitrification and denitrification rates in Lago Titicaca varied largely between years. Lago Titicaca experiences low levels of oxygen saturation due to the high altitude of the lake, which in turn favours hypolimnentic anoxia, and thus denitrification (Vincent et al., 1985).

16S rRNA gene sequence analysis shows that AOB are phylogenetically diverse. *Nitrosococcus oceani* and *Nitrosococcus halophilus* belong to *Gammaproteobacteria* and members of *Nitrosomonas* (including *Nitrosococcus mobilis*) and *Nitrosospira* (including *Nitrosolobus* and *Nitrosovibrio*) are affiliated with *Betaproteobacteria* (Purkhold et al., 2000; 2003). AOB have been detected in a variety of environments including soils, marine, estuarine, salt lakes and freshwater systems (e.g. Bothe et al., 2000; Koops et al., 2006). Clone libraries of 16S rDNA in the hypersaline Mono Lake revealed the existence of sequences related to *Nitrosomonas europaea* and *Nitrosomonas eutropha* which exhibit high levels of salt tolerance (Ward et al., 2000). In estuarine systems, dominance of *Nitrosospira* at marine sites and prevalence of *Nitrosomonas oligotropha* and *Nitrosomonas* sp. Nm143 in freshwater and intermediate sites have been reported

(Bernhard et al., 2005; Freitag et al., 2006), together with the loss of diversity of AOB with the increase of the salinity along a salinity gradient in Plum Island Sound estuary and Schelde estuary (Bernhard et al., 2005; Bollmann and Laanbroek, 2002). In the present study, we used enrichment cultures at different salt concentrations to characterize populations of ammonia oxidizers from Salar de Huasco and to examine their tolerance to salt.

# **6.3 RESULTS**

# **6.3.1 Enrichment cultures**

C:N rates of water samples were determined for the sites H0, H1, H4 and H6 in Salar de Huasco (Table 6-1). Sites H0 (C:N=4.3) and H6 (C:N=5.8) do not show N limitation as defined by Hecky et al. (1993).

**Table 6-1.** Nutrient concentrations and C:N ratios in water samples of four sites in Salar de Huasco.

Site	Total dissolved salts (gL <sup>-1</sup> )	C:N	N-NH <sub>4</sub> <sup>+</sup> (μΜ)	N-NO <sub>3</sub> - (μM)	P-PO <sub>4</sub> <sup>3-</sup> (μΜ)
H0	0.47	4.33	0.28	1.22	0.58
H1	0.39	11.78	1.33	7.74	0.92
H4	42.77	10.55	17.89	nd	25.28
Н6	66.99	5.82	42.44	nd	18.69

nd: not detected

C:N rates detected in sites H1 (C:N=11.8) and H4 (C:N=10.6) indicating potential limitation of N. Ammonia concentrations at the sites were 0.28, 1.33, 17.9 and 42.4  $\mu$ M in H0, H1, H4 and H6 respectively. In the enrichment cultures, nitrite production was detected in eight of nine samples at different salt concentrations (Table 6-2).

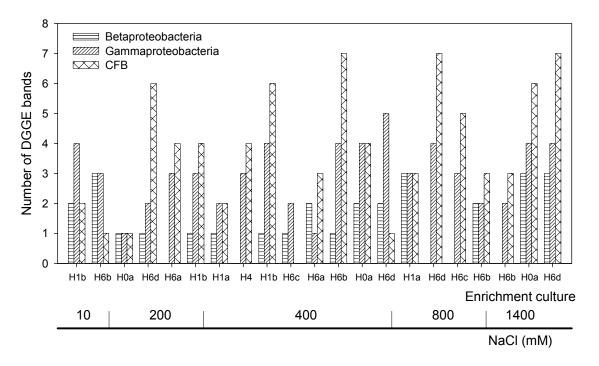
**Table 6-2.** Enrichment cultures of AOB from samples of Salar de Huasco at different salt concentrations. The presence (+) or absence (-) of nitrite accumulation are indicated. The enrichment used for molecular analysis and the ammonia oxidizers associated determined from 16S rDNA and *amoA* gene (\*) are both shown in brackets.

		NaCl concentration (mM)							
Sample	Site	10	200	400	800	1400			
Н0а	Н0	+	+	+	-	+			
			(H0a-200: N.nitrosa, N.halophila)	(H0a-400: N.halophila)		(H0a-1400: N.halophila)			
H0b	Н0	-	-	-	-	-			
Hla	H1	+	+	+	+	+			
				(H1a-400: N.halophila)	(H1a-800: N.halophila*)	)			
H1b	H1	+	+	+	-	-			
		(H1b-10)	(H1b-200: <i>N.communis*</i> )	(H1b-400: <i>N.communis*</i> )					
H4	H4	-	-	+	+	+			
				(H4-400: <i>N.marina*</i> )					
Н6а	Н6	-	+	+	-	+			
			(H6a-200)	(H6a-400: N.halophila)					
H6b	Н6	+	-	+	+	+			
		(H6b-10: N.halophila)		(H6b-400: <i>N.halophila*</i> )	(H6b-800: N.halophila)	(H6b-1400)			
Н6с	Н6	-	+	+	+	+			
			(I	H6c-400: N.nitrosa, N.halophila	a*) (H6c-800: N.oligotropha	)			
H6d	Н6	-	+	+	+	+			
			(H6d-200: N. nitrosa)	(H6d-400: N.halophila)	(H6d-800)	(H6d-1400)			

Enrichment H1a from site H1 was found in a broad range of salinities. Enrichments from H0 and H1 showed a tendency to grow between 10 and 400 mM NaCl, enrichment of H4 grew between 400 and 1400 mM NaCl and most of the enrichments from H6 grew at higher concentrations than 200 mM NaCl.

#### **6.3.2** Bacterial communities in the enrichments

Bacterial community composition of the enrichment cultures was analyzed by 16S rDNA PCR-DGGE. Between 7 and 10 bands per sample were found (Data not shown). In total 171 bands were obtained and 48 of them were sequenced. The sequences were affiliated to *Cytophaga-Flavobacteria-Bacteroidetes* (CFB), *Gammaproteobacteria* and *Betaproteobacteria*. CFB and *Gammaproteobacteria* were the most frequent group found in all the enrichments and *Betaproteobacteria* were detected in 16 of the 21 enrichments (Fig. 6-1).



**Fig 6-1.** Phylogenetic affiliation and frequency of the DGGE sequenced bands from AOB enrichment cultures.

Despite the frequency of *Gammaproteobacteria*, no AOB related-*Gammaproteobacteria* were detected. Phylogenetic analysis of sequences from bands excised from the DGGE gels showed high diversity of the CFB group. Some sequences were related to *Nitrosomonas* sp. (Data not shown).

# 6.3.3 AOB composition inferred by 16S rDNA sequences

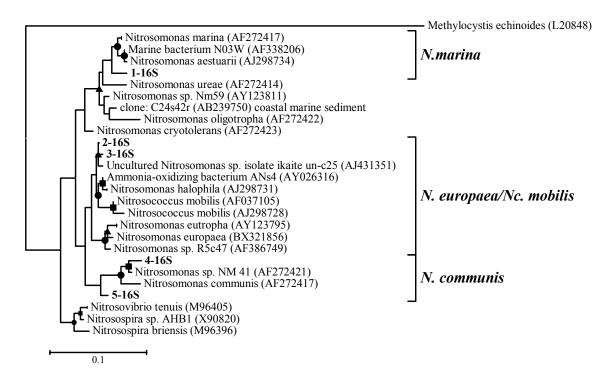
Clone libraries of beta-AOB 16S rDNA were made from cultures H1b-200, H4-400, H1b-400, H6b-400 and H1a-800. A single phylotype was found from each enrichment. The phylotype 1-16S (enrichment H4-400) was related to the *N. marina* cluster at 97% similarity with the first hit in BLAST (Table 6-3, Fig. 6-2).

**Table 6-3.** Sequence similarity of 16S rDNA from AOB phylotypes with GenBank entries (BLAST search).

		Closest relative in BLAST				
Phylotype	Enrichment culture	Name	Similarity (%)	Environment		
1-16S	H4-400	Uncultured <i>Nitrosomonas</i> sp. clone MZS-2 (DQ002465)	97	intertidal muddy sediments		
2-16S	H1a-800	Uncultured <i>Nitrosomonas</i> sp.ikaite unc25 (AJ431351)	99	ikaite tufa columns, Greenland		
3-16S	H6b-400	Uncultured <i>Nitrosomonas</i> sp. ikaite unc16 (AJ431350)	97	ikaite tufa columns, Greenland		
4-16S	H1b-200	Nitrosomonas sp. Nm 41 (AF272421)	98	soil		
5-16S	H1b-400	Nitrosomonas nitrosa isolate Nm90 (AJ298740)	97	activated sludge		

Phylotype 2-16S (enrichment H1a-800) and phylotype 3-16S (enrichment H6b-400) were 99% similar and were affiliated to the *N. europaea/Nitrosococcus mobilis* cluster. Both phylotypes had 99% similarity with an uncultured *Nitrosomonas* sp. retrieved in a clone library from ikaite tufa columns (columns formed with precipitated mineral ikaite) in Greenland (Stougaard et al., 2002). Phylotype 4-16S and phylotype 5-16S exhibited highest similarity with *Nitrosomonas* sp. NM41 (Purkhold et al., 2000) at 96 (enrichment H1b-200) and 98% (enrichment H1b-400), both affiliated to the *N*.

*communis* cluster (Table 6-3, Fig. 6-2). None of the enrichments were amplified with the NOC1 and NOC2 primers designed to amplify *Nitrosococcus oceanus*, a member of the gamma-AOB (Ward et al., 2000).



**Fig. 6-2.** Phylogenetic tree based on partial betaproteobacterial 16S rDNA sequences (≥800 bp) of AOB enrichment cultures inferred by maximum likelihood analysis. The scale bar represents 10% nucleotide sequence difference. Symbols on the branches indicate bootstrap confidence values as follows: ●, >80%; ■, >60-80%; ▲, 40-60%. *Methylocystis echinoides* (L20848) was used as outgroup. Abbreviation: Nc. mobilis, *Nitrosococcus mobilis*.

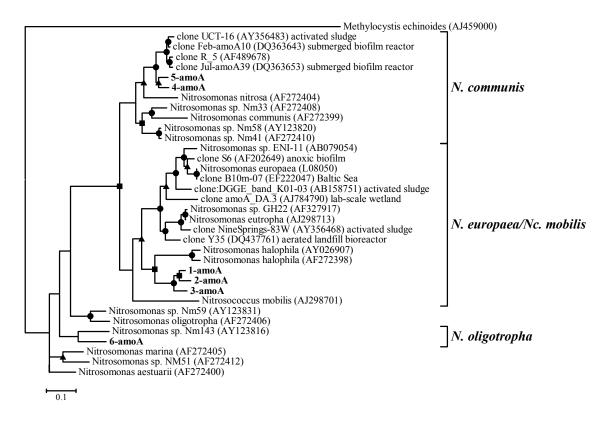
### 6.3.4 AOB composition inferred by *amoA* sequences

Clone libraries of bacterial *amo*A gene were made for eleven enrichments (H6b-10, H0a-200, H6d-200, H1a-400, H6c-400, H6a-400, H0a-400, H6d-400, H6c-800, H6b-800, H0a-1400). A total of six phylotypes were identified and were affiliated to three described clusters of *Nitrosomonas* (Fig. 6-3). Phylotype 1-amo, 2-amo and 3-amo had 83-84% similarity with *N. halophila* and >76% with *Nitrosococcus mobilis* and also low similarity (>84%) with the first hit in BLAST (Table 6-4).

 Table 6-4. Sequence similarity of amoA from AOB phylotypes with GenBank entries (BLAST search).

				Closest relative	tive in BLAST			
		Nucleotide		Protein				
Phylotype	Enrichment culture	Name	Similarity (%)	Environment	Name	Similarity (%)	Environment	
1-amoA	H6b-10	clone S6 (AF202649)	84	anoxic biofilm	clone S6 (AAF22967)	95	anoxic biofilm	
	H1a-400	idem	idem	idem	idem	idem	idem	
	H6c-400	idem	idem	idem	idem	idem	idem	
	H6a-400	idem	idem	idem	idem	idem	idem	
	H0a-400	idem	idem	idem	idem	idem	idem	
	H6d-400	idem	idem	idem	idem	idem	idem	
	H6c-800	idem	idem	idem	idem	idem	idem	
	H6b-800	idem	idem	idem	idem	idem	idem	
2-amoA	H1a-400	clone Y35 (DQ437761)	84	aerated landfill bioreactor	clone S6 (AAF22967)	93	anoxic biofilm	
3-amoA	H0a-200	clone Y35 (DQ437761)	84	aerated landfill bioreactor	clone S6 (AAF22967)	94	anoxic biofilm	
	H0a-400	idem	idem	idem	idem	idem	idem	
	H6c-400	idem	idem	idem	idem	idem	idem	
	H0a-1400	idem	idem	idem	idem	idem	idem	
4-amoA	H6d-200	clone Jul-amoA39 (DQ363653)	92	aerated submerged biofilm reactor	r clone RT-075_01 (ABF20600)	95	soil	
	H6c-400	idem	idem	idem	idem	idem	idem	
5-amoA	H0a-200	clone Feb-amoA10 (DQ363643)	92	aerated submerged biofilm reactor	r clone RT-075_01 (ABF20600)	94	soil	
6-amoA	H6c-800	clone Bsedi-01 (EF222068)	95	sea water-sediment interface	Bsedi-32 (ABN13010)	93	sea water-sediment interfac	

The protein sequence exhibits higher similarity (>93%) with available sequences and the three phylotypes were related with clone S6 retrieved from anoxic biofilm of a reactor with high anaerobic ammonia oxidation (Schmid et al., 2000). Phylotypes 4-amoA and 5-amoA were related with the *N. communis* cluster and they had >86% similarity with *N. nitrosa*. Both phylotypes had the same closest protein relative (clone RT\_075\_01) retrieved from soil (GenBank information). Phylotype 6-amoA was found in enrichment H6c-800 and had low similarity (83%) with *Nitrosomonas* sp. NM 143. No amplification was detected using archaeal *amoA* primers in the enrichment cultures.



**Fig. 6-3.** Phylogenetic tree based on *amo*A sequences (≥450 bp) of AOB enrichment cultures inferred by maximum likelihood analysis. Characteristics of the tree as in Fig 6-2. *Methylocystis echinoides* (AJ459000) was used as outgroup.

#### **6.4 DISCUSSION**

AOB, like all other bacteria, have distinctive ecophysiological preferences including salt concentrations, substrate affinity and habitat (e.g.: Geets et al., 2006; Webster et al., 2005; Koops and Pommerening-Röser, 2001). Using a range of salt concentrations, we examined the salt tolerance of AOB enrichment cultures collected from a saline water body that exhibits spatial and temporal variation in salt and nutrient concentrations. Considering the contrasting characteristics at the sites, we expected to find clear differences in AOB composition associated to site and media salinity. In some samples (e.g. H4) AOB grew only at higher salinities (above 400 mM NaCl), while sample H1b only grew at salt concentrations lower than 400 mM NaCl (Table 6-2). However, this association was not repeated in all samples and could reflect the high spatial variability of these sites (Chapter 4).

Salinity appears to be an important factor in determining the distribution of AOB in estuarine and river systems (Bernhard et al., 2005; Stehr et al., 1995) with low abundance and low diversity at high salt concentrations. In terms of AOB composition in the enrichments, heterotrophic members of CFB and Proteobacteria dominated the enrichments. These two groups have been described from altiplanic wetlands including the Salar de Huasco (Demergasso et al. 2004; Chapter 3), and there was no clear relationship between salinity and composition of the enrichments (Fig. 6-1). We used quite high concentrations of ammonia (10 mM) in our enrichments. At sites H0 and H1 ambient concentrations were lower (<1.3 mM) and possibly the lack of growth in samples from H0 may be explained by inhibition due to the ammonia concentration. Enrichments cultures have been used to detect AOB in several environments (e.g. seawater: McCaig et al., 1994; freshwater: Hiorns et al., 1995; calcareous grasslands: Kowalchuk et al., 2000)

at several initial ammonia concentrations, from 0.67 mM (Hastings et al., 1998) up to more than 100 mM (e.g. McCaig et al., 1994; Bruns et al., 1999) but usually enrichments result in the isolation of only a fraction of the total diversity of AOB present in a sample (Stephen et al., 1996). Molecular analyses have demonstrated that conclusions from cultivation approaches can misrepresent AOB populations in a sample. Nonetheless, enrichment cultures and pure cultures can provide important clues about their physiological properties and adaptation to conditions of their habitat (e.g. see Kowalchuck and Stephen, 2001).

Analysis of 16S rDNA and *amo*A gene shows that sequences from the enrichments belonged to the *N. marina*, *N. europaea/Nitrosococcus mobilis*, *N. communis* and *N. oligotropha* clusters having only low similarity with cultured representatives. Studies of enrichment cultures from sediment from the root zone of a macrophyte in lake Drontermeer in the Netherlands showed the dominance of the *Nitrosomonas oligotropha* cluster at low ammonia concentration (<10 mM) (Bollmann and Laanbroek, 2001) (also referred to as *Nitrosomonas* cluster 6a: Stephen et al. 1996). We found a single phylotype related to this cluster (6-amoA) obtained from the sample H6c-800 from site H6 which had higher ammonia concentration compared to the other sites (Table 6-1).

Ammonia oxidizers are phylogenetically and ecophysiologically diverse (Koops et al., 2006). *N. marina* and *N. europaea/Nitrosocuccus mobilis* have been described as obligately halophilic or halotolerant (e.g. optimal growth of *N. marina* is between 300 and 400 mM NaCl). On the other hand, *N. communis* and *N. oligotropha* do not have salt requirements for growth. We found these two groups at salinities between 200 and 800 mM NaCl demonstrating the high salt tolerance of these species of AOB from the Salar de Huasco. In addition, a reduced diversity of AOB was apparent. AOB in the

enrichments were dominated by a single phylotype using 16S rDNA analysis and by three phylotypes in the enrichment H6c-400 using *amoA* gene.

The most frequent phylotype recovered in *amoA* gene clone libraries from a wide range of salt concentrations (from 10 mM in H6b until 1400 mM in H0a) was clustered with *N. halophila* (Fig. 6-3). *N. halophila* has been isolated from the North Sea and have a maximum salt tolerance of 900 mM (Koops et al. 2006). In our study, the phylotype 3-16S sequence was 83% similar to *N. halophila* and was found at a NaCl concentration of 1400 mM, indicating that this species may have a higher tolerance to salt than previously supposed. Alternatively, this result may point to the existence of a new, more tolerant species closely related to *N. halophila*. Sequences related to *N. nitrosa* were found at salinities of 200 and 400 mM NaCl, but this species does not need salt to survive (Koops et al., 2006).

In a previous study we investigated AOB in water and sediment samples using clone libraries of betaproteobacteria and gammaproteobacteria 16S rDNA and the *amoA* gene. The 16S rDNA sequences obtained were not related with AOB and no amplification was detected using bacterial *amoA* gene (Data not shown). Additionally we detected archaeal *amoA* in water samples from site H0 (Chapter 4) and sequences related to annamox bacteria (Strous et al., 1999) (Data not shown).

We did not detect gamma-AOB or archaeal *amoA* in our enrichments despite the marked abundance of *Gammaproteobacteria* and the apparent presence of archaeal amoA identified after amplification from environmental samples (Chapter 4). The medium used to isolate *Nitrosopumilus maritimus*, (the only ammonia-oxidizing Crenarchaeota cultured to date: Könneke et al., 2005) differentiated from the culture media used for ammonia oxidizers (inorganic salt medium containing ammonia) with regard to the

presence of vitamins and trace elements. This might explain the previous failure to isolate ammonia-oxidizing Archaea (Nicol and Schleper, 2006).

Apparently, the Salar de Huasco supports both a reduced diversity and abundance of ammonia oxidizers. This might be related to nitrogen limitation in some sites, the low concentration of ammonia and nitrate (Table 6-1), the presence of anoxia at some sites (e.g. site H4: Chapter 2) and the salinity.

Recently, Valentine (2007) proposed that Archaea are better adapted than Bacteria under conditions of chronic energy stress. In case of nitrifiers, Archaea thrive at conditions of low energy availability (e.g. low ammonia concentration). The low diversity of AOB and the evidence of ammonia oxidizing Archaea (AOA) in the site H0 provide some clues regarding nitrification in the Salar de Huasco. However, future studies including the quantification and activity of AOB, AOA and anammox are required to further understand nitrification in high altitude wetlands.

# 7. MOLECULAR ANALYSIS OF HALOPHILIC BACTERIA ISOLATES FROM SALAR DE HUASCO

#### 7.1 ABSTRACT

Several strains of halophilic Bacteria were isolated from water samples collected from Salar de Huasco, a high altitude (3800 m), saline, cold wetland located in the Chilean Altiplano. Maximum salt concentrations in Salar de Huasco (65 gL<sup>-1</sup>) were notably lower than those reported from environments where halophilic Archaea dominate. The isolates were able to grow in media with high salt concentration (3M-4M NaCl) designed for halophilic Archaea and all were affiliated to Gammaproteobacteria, including *Halomonas*, *Salinivibrio*, *Idiomarina* and *Marinobacter*. We also isolated strains in media for halophilic denitrifying Bacteria that were closely related to *Halomonas*, a genus capable of nitrate reduction. We propose that halophilic Bacteria are better adapted than Archaea to conditions in habitats such as salares.

#### 7.2 INTRODUCTION

Halophilic microorganisms (*i.e.* those that demonstrate the capability to live at high salt concentrations) are found in all three domains of life: Archaea, Bacteria and Eukarya (e.g. Oren, 2002; Imhoff, 1993). Most extreme-halophilic Archaea are aerobic chemoorganotrophs, that use a respiratory chain and molecular oxygen as the terminal electron acceptor, while utilizing organic material supplied by primary producers, such as the eukaryotic alga *Dunaliella* and halophilic cyanobacteria present in hypersaline environments (Chaban, 2006). Halophilic Archaea can be found in the Halobacteriales

order and also in some families of methanogens (Methanospirillaceae and Methanosarcinaceae) (Imhoff, 1993).

Halophilic Bacteria are moderately halophilic, metabolically versatile aerobes or facultative anaerobes that can be found in the Gammaproteobacteria (family Halomonadaceae, members of Chromatiaceae and Ectothiorhodospiraceae), Firmicutes (families Halanaerobiaceae and Halobacteroidaceae), Cyanobacteria or Cytophaga-Flavobacteria-Bacteroidetes group of Bacteria (e.g. Oren, 2005; Imhoff, 2001; Ventosa et al., 1998; Imhoff, 1993; Imhoff, 1988).

Suitable habitats for extreme halophiles include natural salt lakes, the Dead Sea, salt crystallization ponds, hypersaline soda lakes, saline soils, Antarctic salt lakes and salted foods like soy sauce (Imhoff, 1993). Also, halophilic Archaea have been recorded from low salinity estuarine environments (Purdy et al., 2004). Moderately halophilic Bacteria have been recorded from saline environments, including hypersaline Antarctic lakes, solar salterns and saline soils (Ventosa et al., 1998).

The Salar de Huasco is a high altitude (3800 m), cold, saline wetland located in northern Chile. This salar, like other "salares" located in the Chilean Altiplano are athaloassohaline water bodies subjected to extreme abiotic conditions including high UV radiation, low temperatures, negative water balance and variable salt concentrations. In northern Chile different saline deposits are recognized and classified according to their location. They are located at high altitude (Andean basins: salares and Andean Lakes) or lower altitude (Preandean basins: preandean salares) between latitudes 18° and 27° south (Chong, 1984). Microbiological surveys in these systems have been mostly conducted in salares located in the Atacama Desert, e.g. Salar de Atacama and Salar de Llamará

(Demergasso et al., 2003). Campos (1997) reported several isolates from different sites of the Salar de Atacama including moderate halophilic bacteria: *Marinomonas, Vibrio, Alteromonas, Marinococcus, Acinetobacter* and halotolerant bacteria: *Bacillus, Pseudomonas-Deleya, Micrococcus* and *Acinetobacter*. Studies in Lake Tebenquiche (Salar de Atacama) have led to the isolation of *Halorubrum tebenquichense*, an extremely halophilic archaeon (Lizama et al., 2002) and *Chromohalobacter nigrandesensis*, a moderately halophilic bacterium member of the Halomonadaceae (Prado et al., 2006). This bacterium is closely related to *Chromohalobacter sarecensis*, previously isolated from the high altitude (4 300 m) saline lake Laguna Verde located in south-west Bolivia (Quillaguamán et al., 2004).

In the present study we used three different media designed to cultivate halophilic Archaea and Bacteria from water samples of Salar de Huasco. We describe the existence of a putative new species and discuss the dominance of halophilic Bacteria over Archaea in the variable-salinity conditions of the Salar de Huasco.

#### 7.3 RESULTS AND DISCUSSION

# 7.3.1 Growth of halophilic microorganisms

The three different media used to cultivate halophilic microorganisms from water samples of Salar de Huasco all contained high NaCl concentrations (3M-4M) and yeast extract and casamino acids as nutrient sources (Table 2-3). Such conditions are frequently used for cultivation of halophilic Archaea (Oren, 2002) and halophilic Bacteria (Ventosa et al., 1998). Ambient salinities at the sample sites were considerably lower than those in the media (Table 7-1) and could explain the absence of growth in all sites using

Halobacterium medium. This medium has a total salt concentration of 272 gL<sup>-1</sup> (Table 2-3) an order of magnitude greater than concentrations recorded at the most saline site H4 (64.9 gL<sup>-1</sup>: see Table 2-2).

**Table 7-1.** Growth of halophilic microorganisms at the different sites and media.

	Sites					
Growth/conditions at the sites	Н0	H1	Н4	Н6		
HYM	-	=	+	+		
Halorubrum medium	+	+	+	+		
Halobacterium medium	-	-	-	-		

However, we detected growth of red-pink and white colonies using *Halorubrum* media at all sites, included those with low salt concentration (H0, H1). Growth in the HYM medium was detected only with samples from sites H4 and H6 as bubble formation (possibly N<sub>2</sub> production) and the growth of white colonies on agar plates. The ability to reduce nitrate is widespread in the members of Halobacteriales (Archaea): Halobacterium vallismorti, H. mediterranei, H. marismortui and H. denitrificans grow anaerobically only in the presence of nitrate (Mancinelli and Hochstein, 1986; Tomlinson et al., 1986). In case of Bacteria, Pseudomonas halophila, Halovibrio, Halomonas and Halospina (Gammaproteobacteria) have been described as halophilic denitrifiers (Soronkin et al., 2006). In hypersaline brines and sediments, ammonia is generally the predominant inorganic nitrogen compound and the concentrations of nitrate are low (Oren, 1994). The lost of nitrite have been related with denitrification in hypersaline environments as occurs in Lakes Wadi Natrun in Egypt (Imhoff, 1979). In Salar de Huasco nitrate concentrations range between 0.5 in site H6 and 1 µML<sup>-1</sup> in site H4 (data not shown). These values are low in comparison with other saline systems like Mono Lake, USA (>1µML<sup>-1</sup>: Jellison and Melack, 1988), seawater solar saltern in Alicante, Spain (3-6.2 uML<sup>-1</sup>: Joint et al., 2002) and Dead Sea (3.2-8 μML<sup>-1</sup>: Stiller and Nissenbaum, 1999). Hence, further studies are required to clarify the role and importance of denitrifying microorganisms at this site.

# 7.3.2 Phylogenetic analysis of the isolates

Colonies were selected for DNA extraction and sequencing according to morphology and color. In total 10 colonies were selected from the *Halorubrum* medium and 4 from HYM medium for sequencing. 16S rDNA sequence analysis of the isolates showed that all of them were affiliated to Gammaproteobacteria in the domain Bacteria. The 16S rDNA sequences were compared to the closest cultured relatives in GenBank and shared similarities between 99 and 88% with members of the genera *Halomonas*, *Salinivibrio*, *Idiomarina* and *Marinobacter*, all of which have been described as halophilic Bacteria (Table 7-2, Fig. 7-1).

**Table 7-2.** Closest relatives of the isolates recovered in Salar de Huasco.

Isolate	Site	Media	Closest relative	Accesion number	Similarity (%)	Reference
H6A/HYM	Н6	HYM	Halomonas ventosae	AY268080	97	Martínez-Cánovas et al., 2004a
H6B/HYM	Н6	HYM	idem	idem	98	idem
H6C/HYM	Н6	HYM	idem	idem	97	idem
H4/HYM	H4	HYM	Halomonas koreensis	AY382579	88	Lim et al., 2004
H0-2/Hr	H0	Halorubrum	Idiomarina ramblicola	AY526862	99	Martínez-Cánovas et al., 2004b
H4-2/Hr	H4	Halorubrum	idem	idem	98	idem
H0-42/Hr	H0	Halorubrum	idem	idem	98	idem
H1-2/Hr	H1	Halorubrum	Halomonas sulfidaeris	AF212204	97	Kaye et al., 2004
H0-4/Hr	H0	Halorubrum	idem	idem	98	idem
H6-1/Hr	Н6	Halorubrum	Halomonas hydrothermalis	AF212218	98	Kaye and Baross, 2000
H1-4/Hr	H1	Halorubrum	idem	idem	97	idem
H6-4/Hr	Н6	Halorubrum	idem	idem	97	idem
H4/Hr	H4	Halorubrum	Salinivibrio costicola	X95531	98	Mellado et al., 1996
H4-4/Hr	H4	Halorubrum	Marinobacter aquaeolei	AJ000726	96	Huu et al., 1999

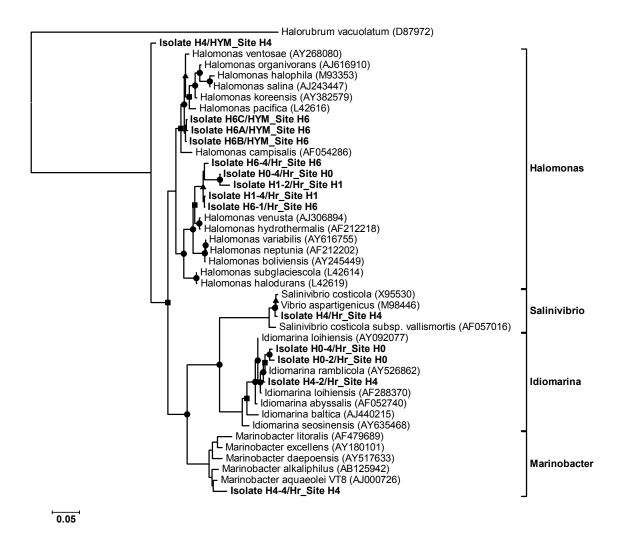
In the HYM medium, we recovered the isolates H6A/HYM, H6B/HYM and H6C/HYM. They were 97-98% similar to *Halomonas ventosae*, a moderately halophilic bacterium described by Martínez-Cánovas et al. (2004a) to grow optimally at NaCl

concentrations of 1.38 M and to denitrify. The isolate H4/HYM also grew in this medium, and had a low (93%) similarity with *Halomonas koreensis*, a moderately halophilic bacterium that reduces nitrate to nitrite, previously isolated from a solar saltern in the Dangjin area of the Yellow Sea in Korea (Lim et al., 2004). Due to the low similarity with the closest relative held in the database, we propose this strain possibly represents a putative new species.

Halomonas were grouped in two branches in the phylogenetic tree (Fig. 7-1) according with the polyphyletic character of Halomonas (Arahal et al., 2002). Isolates recovered from the HYM medium were affiliated with the Group I of Halomonas (Arahal et al., 2002). Five isolates from the Halorubrum medium clustered with the Group II of Halomonas (Arahal et al., 2002), and were 97-98% similar to Halomonas sulfidaeris and Halomonas hydrothermalis, both isolated from deep-sea hydrothermal vents (Kaye et al., 2004).

The isolate H4/Hr grew in the *Halorubrum* medium and was 98% similar with *Salinivibrio costicola*. This moderately halophilic bacterium was originally isolated from salted meats and brines, but has subsequently been shown to have a wide distribution in hypersaline environments (Mellado et al., 1996).

Three isolates (H0-4/Hr, H0-2/Hr, H4-2/Hr) had similarities ranging between 99-98% with *Idiomarina ramblicola*, previously isolated from hypersaline water in Murcia, Spain (Martínez-Cánovas et al., 2004b). This genus has been primarily described from deep-sea waters but in the last years have been found in other environments e.g. Korean seashore sands (Kwon et al., 2006) and hypersaline water collected from a solar saltern (Choi and Cho, 2005).



**Fig. 7-1.** Phylogenetic tree based on partial 16S rDNA sequences ( $\sim$ 700 bp) of halophilic isolated from water samples of Salar de Huasco inferred by maximum likelihood analysis. The scale bar represents 5% nucleotide sequence difference. Symbols on the branches indicate bootstrap values as follows:  $\bullet$  >80%;  $\blacksquare$  60-80%;  $\blacktriangle$  40-60%. *Halorubrum vacuolatum* (D87972) was used as outgroup.

The isolate H4-4/Hr was 96% similar to *Marinobacter aquaeoli*, isolated from an offshore oil-producing well off southern Vietnam, that grows optimally at an NaCl concentration of 0.85 M (Huu et al., 1999).

Using the *Halorubrum* medium, we isolated strains related to four different genera, revealing an elevated level of diversity of halophilic Bacteria in the Salar de Huasco.

# 7.3.3 Halophilic Archaea versus halophilic Bacteria

Typically, Archaea dominate over Bacteria under high salinity conditions and in systems with NaCl concentrations close to saturation, they are the only active aerobic heterotrophs (Oren, 2002). In saline athalassohaline water bodies, the ionic composition is different than in marine systems (thalassohaline), resulting in a particular microflora (Galinski and Trüper, 1994).

Halophilic Bacteria are adapted to life at lower salinities and have the possibility to adapt rapidly to external salinity changes. This contrasts with the halophilic Archaea, which depended on elevated concentrations of salt, and accordingly, these taxa occupy different niches (Ventosa et al., 1998). Modelling and laboratory (chemostat) studies of samples from Spanish solar salterns have revealed that competition between Archaea and Bacteria is largely dependent on salt concentration and temperature. At lower salt concentrations, moderate halophilic bacteria dominated, while at higher salt concentrations (35-40% salt) pigmented Archaea outperformed their bacterial competitors. At intermediate salt concentrations (20-30% salt), temperature proved to be the decisive competitive factor, and bacteria were favored under low temperatures (<25°C) (Rodriguez-Valera et al., 1980; del Moral et al., 1987).

Salar de Huasco could be considered as moderately saline wetland (Table 2-2) and the presence of extremely halophilic Archaea would be predicted only in sites with salt concentrations higher than 150 gL<sup>-1</sup>. In a parallel study at the same sites, using clone libraries of 16S rDNA of water and sediment samples (Chapter 4), we found a single clone with high similarity to halophilic Archaea, supporting the results of the present study.

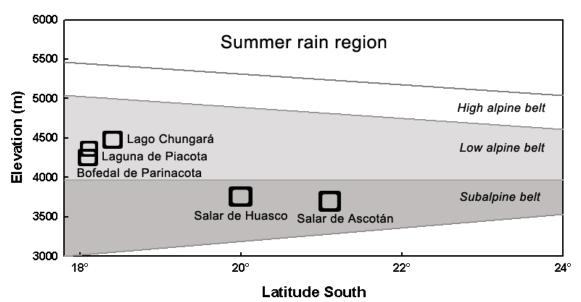
Sodium is toxic at high intracellular levels due to electrochemical and osmotic interactions with nucleic acids and proteins. Halophilic microorganisms living in high salt and low water activity environments have to maintain the intracellular solute concentrations at a level osmotically equivalent with the salt concentration of the environment. Archaea of the order Halobacteriales (also some Bacteria e.g. Halanaerobiales, Salinibacter) accumulate inorganic ions (mainly K<sup>+</sup> and Cl<sup>-</sup>) at high concentrations in the cytoplasm giving a limited adaptability to changing conditions (Imhoff, 1986; Imhoff, 1993). However, halophilic Bacteria and halophilic Eukarya utilize low-molecular-weight organic osmotic solutes to maintain intracellular ionic concentrations at low levels, permitting greater flexibility to fluctuations in salt concentrations (Oren, 2002). The Salar de Huasco is subject to considerable spatial and temporal variability including water level fluctuations (Garreaud et al., 2003), which could affect the composition of the microbial communities (Chapter 4). Although Archaea would dominate any competitive interactions with Bacteria at high salt concentrations (Valentine, 2007), we suggest that in this wetland, halophilic Bacteria would be better competitors under the conditions in these specialized and fluctuating environments (salares) than halophilic Archaea.

#### 8. DISCUSSION

# 8.1 Contrasting water bodies

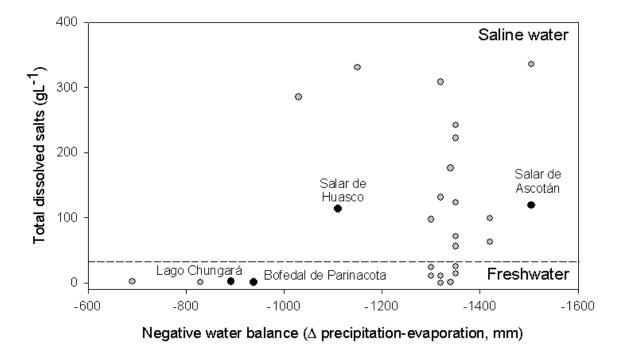
This thesis examined several different aspects of microbial communities from high altitude wetlands of the Altiplano. The specific climate of the Altiplano, combined with its temporal variability at different scales, the volcanic origin of the basins and the geographic isolation of the area, all combine to unique aquatic systems that support a biota adapted to these particular conditions.

At a more local scale, unique microbial diversity patterns are apparent within the various high altitude wetlands located in the Altiplano, with clear differentiation between high altitude lakes (Lago Chungará), freshwater wetlands (Bofedal de Parinacota and Laguna de Piacota) and saline wetlands (Salar de Huasco and Salar de Ascotán) (Chapter I). These contrasting aquatic systems are distributed between latitudes 18 and 21° south and between altitudes of 3700 and 4500 m.



**Fig. 8-1.** Location of the studied wetlands in relation with the vegetational belts (see: Squeo et al., 2006)

Distinct ecozones have been defined for the Altiplano according to the type and abundance of vegetation (e.g. Squeo et al., 2006). Salar de Huasco and Salar de Ascotán are both located within the subalpine belt (dominated by shrubs), Lago Chungará and the freshwater wetlands Bofedal de Parinacota and Laguna de Piacota are located in the low alpine belt (characterized by the presence of shrubs, grasses and cushion plants) (Fig. 8-1). The differences in salt concentration between the aquatic systems examined in this thesis are significant and also reflect the negative water balance at these sites (Fig. 8-2).



**Fig. 8-2.** Influence of the negative water balance and salt concentration on the type of altiplanic wetland. Lago Chungará and Bofedal de Parinacota are freshwater systems while Salar de Huasco and Salar de Ascotán are saline water bodies.

The ecological differentiation highlighted by the different vegetation belts reflects variation in the environmental conditions that also shape distinct aquatic systems in these areas. As might be expected, the biota and in this particular case, the microbial communities, in the different water bodies of the Altiplano varied according to their location (Chapter 3). In contrast to the vegetation belts, the composition of microbial

communities in aquatic systems is strongly related to the chemical and physical conditions of the water as well as other environmental conditions.

# 8.2 Specific microbial diversity pattern

#### 8.2.1 Freshwater water bodies

Lago Chungará and associated freshwater wetlands are located above an altitude of 4400 m. Mean annual temperatures in this area (Lauca National Park) are 5.1°C (maximum) and -2°C (minimum) (Rundell and Palma, 2000). Many of the sequences retrieved from water samples from these sites were highly related to described psychrophilic bacteria (e.g. Gammaproteobacteria: Psychrobacter sp., Pseudomonas congelans, CFB: Flavobacterium psychrolimnae). The abundance of Cytophaga-Flavobacteria-Bacteroidetes in water samples of Lago Chungará indicates that these taxa are likely to play an important role in the degradation of organic matter in this lake, which is considered as oligo-mesotrophic (Mühlhauser et al., 1995). In sediment samples, sequences from these sites were highly similar with sulfate-reducing bacteria (e.g. Deltaproteobacteria: Desulfobacterium phototrophic sp.), bacteria (e.g. Betaproteobacteria: Rhodoferax antarcticus; Alphaproteobacteria: Rhodobacter sp.), Actinobacteria and aceticlastic methanogens (e.g. Methanobacteria: Methanosarcina sp., *Methanosaeta* sp.).

#### **8.2.2** Saline water bodies

In the salares, most sequences were highly similar with aerobic planktonic bacteria with characteristic extreme halotolerance (e.g. Gammaproteobacteria: *Marinobacter* sp., *Halomonas* sp.) and anaerobic fermentative halophilic bacteria (Firmicutes: *Halanaerobium* sp.). Psychrotolerant halophilic Archaea (Halobacteria:

Halobacterium lacusprofundis) and alkaliphilic Archaea (Natronobacterium sp.) were also reported, reflecting the low temperature and saline conditions of Salar de Huasco and Salar de Ascotán. High sulfide concentrations in the sediments are indicative of reductive conditions. The presence in water and sediment samples of sequences related with sulfate-reducing bacteria (Deltaproteobacteria: Desulfotignum sp., Desulfobacterium sp.) and methanogens in Salar de Huasco appears to support this. Sequences related to the families Methanosarcinae were found in both water and sediment samples but sequences related to the Methanomicrobiaceae were only recorded from sediment samples. Members of the Methanosarcinae are able to utilize acetate and other organic acids to produce methane, while members of the Methanomicrobiaceae grow by reducing CO<sub>2</sub>, using H<sub>2</sub> and formate as electron donors. Competition between sulfate-reducing bacteria and methanogens for available energy sources when sulfate is not limiting has been widely described (Ollivier et al., 1994). This would be the case in the salares because they exhibit high contents of sulfate of volcanic origin (Risacher et al., 2003).

# 8.3 Salar de Huasco as a model of altiplanic wetlands

Comparisons between the different altiplanic wetlands investigated here is difficult, as they are heterogeneous water bodies with distinct chemical, physical and biological characteristics. The Salar de Huasco was selected as a model of altiplanic wetlands because it is subject to low anthropogenic perturbations and exhibits significant spatial variability: freshwater streams (e.g. site H0), bofedales and lagoons with different salt concentration (e.g. sites H1, H4, H6) are all found in an area of 51 km<sup>2</sup>. This contrasts with the Salar de Ascotán, where boron is mined (Chong, 1984; Chong et al., 2000). Different microbial communities were identified in this wetland. Most sequences

of Archaea (Chapter 4) and Cyanobacteria (Chapter 5) had low similarities with their closest relatives held in GenBank and in case of the Archaea, most had no similarity with cultured relatives.

#### 8.3.1 Salt tolerance

The microorganisms that inhabit Salar de Huasco appear to display a considerable tolerance to salt, which was examined using several media with different salt concentrations (Chapters 5, 6, 7). We studied four distinct sites in Salar de Huasco that differed particularly with regard to salt concentration. Generally, sites H4 and H6 had lower water level than sites H0 and H1 and consequently displayed increased salt concentrations. Hence, we can expect to find more salt-tolerant species in sites H4 and H6 than H0 and H1. In case of AOB we found growth at all salinities (<1400 mM NaCl) and sites (Chapter IV) and most sequences (identified by 16S rDNA and amoA sequences) were highly similar with *Nitrosomonas halophila*, for which salt tolerance of 900 mM have been reported (Koops et al. 2006). We attempted to isolate halophilic Archaea from samples collected in the Salar de Huasco (Chapter V). The media used were designed for the recovery of Halobacteria and Halorubrum (200-270 gL<sup>-1</sup> total dissolved salts) but interestingly, we isolated only halophilic bacteria of the genera Halomonas, Salinivibrio and Marinobacter, all salt tolerant Gammaproteobacteria. This result is in accordance with the almost complete lack of *Halobacteria* as reported in Chapter 4 (both studies were made with samples taken in January 2005, rainy season). Phototrophic bacteria, were able to grow in media with 15% of total salts (Chapter VI), which is not too surprising, as many members of the Chromatiaceae have particularly high tolerance to salt, e.g. Halorhodospira halophila has a salt optimum of 32% salt (Imhoff, 2001). Therefore, it is likely that species inhabiting the Salar de Huasco would

have elevated salt tolerances. The variability in the availability of water in the lagoons of Salar de Huasco could affect the composition of the microbial communities. Further studies including sampling over shorter time scales (monthly, weekly, daily) would help to understand how microbial community dynamics are related to the availability of water.

### 8.4 Approximation of biogeochemical cycles

As mentioned above, the analysis of 16S rDNA sequences does not give information on the metabolic activities of the different species (Stackebrandt and Göbel, 1994). However, sequences with high similarity to known metabolic groups, permited the identification of the following groups and their predicted importance for key biogeochemical cycles.

- i) Nitrogen cycle: Some sequences were closely related to *Nostoc* sp. and *Nodularia spumigena*, two described halophilic nitrogen fixers (Chapter 5). Also nitrification can be inferred from 16S rDNA and bacterial *amoA* sequences that were affiliated to the genera *Nitrosomonas* (Chapter 6) and to Archaeal *amoA* (Chapter 4). The reduced diversity of AOB and the evidence of ammonia-oxidizing Archaea (AOA) at site H0 provide some clues regarding nitrification in the Salar de Huasco. Nitrification by AOA has been shown to be predominant in soil (Leininger et al., 2006) and in open waters (e.g. Francis et al., 2005). Nitrifying Archaea thrive under conditions of low energy availability compared to their bacterial counterparts (Valentine, 2007). Isolates of halophilic denitrifying bacteria were identified and related to several species of *Halomonas* (Chapter 7).
- ii) Carbon cycle: in sediment samples, a diverse and abundant community of methanogenic Archaea was found. They were related to methylotrophic (e.g.

Methanomethylovorans) and aceticlastic organisms (e.g. Methanosarcina, Methanosaeta). Phototrophic microorganisms were also found in samples of Salar de Huasco, anoxygenic and oxygenic phototrophs and Cyanobacteria (Chapter 5) were retrieved from water and sediment samples.

iii) Sulfur cycle: sulfate concentrations in water samples were greater than 4.3 gL<sup>-1</sup> at site H6 (data not shown). Native sulfur is abundant in the Western Cordillera, and its oxidation produces SO<sub>4</sub><sup>2-</sup> that subsequently acidifies inflowing waters. This drastically reduces the carbonate content of these systems, resulting in non-alkaline waters (pH~7-8). Carbonate is also abundant in this region, resulting from atmospheric deposition of gypsum (CaSO<sub>4</sub>×2H<sub>2</sub>O) from the Central Valley or Atacama Desert (Risacher et al., 1999; 2003). Generally, the sediments have a white-cream surface (S°) with red-pink or green patches (from phototrophic bacteria and Cyanobacteria), but only a few millimeters below the surface, sediments are completely black and H<sub>2</sub>S can be detected by odor. Bacteria related to sulfate reduction were found in sediment samples (Chapter 3) but sequences related to sulfur-oxidizing bacteria not.

# 8.5 "Everything is everywhere, but, the environment selects"

This quote from the Dutch microbiologist Baas Becking (1934) is frequently used as the starting point of many studies on prokaryotic and protist biodiversity and biogeography (de Wit and Bouvier, 2006). "Everything is everywhere" reflects the concept that all microorganisms are cosmopolitan and "the environment selects" that specific microorganisms are observed in their characteristic environments. The literature includes much evidence supporting the cosmopolitan character of prokaryotes, however estimates of the scope for their distribution are affected by the level of taxonomic

resolution applied and the technique used to identify them. For example, it is well accepted that Bacteria and Archaea are globally distributed (using 16S rDNA sequences) (DeLong and Pace, 2001) but at lower taxonomic levels (e.g. genus level) the prokaryotes have a cosmopolitan distribution in their respective habitats (Rammete and Tiedje, 2006). Following this concept, the 16S rDNA sequences retrieved in the present thesis had high similarity with other clone libraries made from extremely dissimilar environments including Antarctic microbial mats and lakes (Chapter 3, 5), hydrothermal vents (Chapter 4), hypersaline lakes (Chapter 3, 7) and seawater (e.g. Roseobacter-like sequences, Chapter 5). If we consider that the distinctive environmental characteristics of the Salar de Huasco include (amongst others) cold temperatures and the variable salinity, we might expect a microbial community adapted to such conditions and also similar with other comparative environments. The high similarity between Cyanobacteria from the Salar de Huasco and Antarctica might be explained by temperature (e.g. low) similarities in the region, and by dispersion factors. However, there is no clear explanation for the presence of other groups, for example those found in hydrothermal vents (e.g. Marine Benthic Groups of Archaea: Chapter 4). Importantly, comparisons of microbial diversity of five separate altiplanic wetlands (Chapter 3) resulted in the conclusion that the contrasting physical and chemical conditions in these different systems was reflected in the presence or absence of certain groups of Bacteria or Archaea, even though they were located in the same geographic region under similar environmental conditions. Therefore, the influence of habitat plays an important role in defining some of these biogeographic distributions.

The biogeography of Bacteria and Archaea has increasingly become topical, especially following the development of culture-independent methods. However, the debate regarding whether microorganisms exhibit biogeographic patterns still remains

(Martiny et al., 2006). The lack of a general ecological theory related to microorganisms (e.g. Prosser et al., 2007) also restricts the scope of any generalizations or predictions regarding many aspects of microbial diversity.

# 8.6 Future developments in the study of microbial communities in altiplanic wetlands

This thesis covered a broad range of aspects of microbial ecology in altiplanic wetlands. Phylogeny: the evolutionary relationships between species (or in this case, phylotypes), diversity: presence and distribution of distinct phylotypes, and function: the role of microorganisms in biogeochemical cycles. The Altiplanic wetlands are fragile aquatic systems that can undergo significant community change through environmental variability. Studies of microbial community dynamic at small temporal scales (e.g. 24 h cycle) will aid our understanding of the effect of environmental variability and how microorganisms adapt under changing conditions. Biogeochemical cycles in the altiplanic wetlands can still be largely considered a black box in terms of rates and components. However, the results detailed here have revealed an elevated level of microbial diversity that needs to be considered to delineate future studies of specific physiological groups.

#### CONCLUSION

Altiplanic wetlands exhibited unique microbial community structures, that were adapted to environmental conditions of the Altiplano, and that cannot be easily compared with any other environments on Earth. Members of Bacteria were more abundant than Archaea in all sites and diversity in sediment samples was higher than in water. Generally, 16S rDNA sequences had reduced similarity with cultured relatives and most of them were related with uncultured Bacteria or Archaea. Further studies focused on particular biogeochemical cycles are necessary to understand the role of the microorganisms in these systems. Also, studies examining the adaptations of the various microorganisms that inhabit these habitats are interesting to study, as they thrive under particularly extreme conditions, that to date, have not been well described. Because of the almost unexplored character of the altiplanic wetlands, the microbiology of the Altiplano would be a fascinating area of future research at the same level of others extreme environments such as hydrothermal vents or Antarctic habitats, and could help to provide information regarding the unique characteristics of these systems to promote their conservation.

# INDIVIDUAL SCIENTIFIC CONTRIBUTIONS TO MULTIPLE-AUTHOR PUBLICATIONS

Results of this thesis have been submitted for publication with the following manuscripts:

Cristina Dorador, Irma Vila, Karl-Paul Witzel and Johannes F. Imhoff. Unique microbial communities in contrasting aquatic environments of the high altitude Andean Altiplano (northern Chile). Submitted as research paper to Applied and Environmental Microbiology (Status: in review)

Sampling was conducted by Cristina Dorador and Irma Vila. Irma Vila contributed with chemical analysis and supervision of the limnological aspects. The manuscript was prepared by Cristina Dorador under the supervision of Karl-Paul Witzel and Johannes F. Imhoff. Experimental work, evaluation of data, phylogenetic analysis and preparation of the manuscript was done by Cristina Dorador.

Cristina Dorador, Irma Vila, Johannes F. Imhoff and Karl-Paul Witzel. Archaeal diversity in Salar de Huasco, a high altitude saline wetland in Northern Chile including evidence for ammonia oxidizing Crenarchaeota. Submitted as research paper to Environmental Microbiology (Status: in review).

Sampling was conducted by Cristina Dorador, Irma Vila and Karl-Paul Witzel.

The manuscript was prepared by Cristina Dorador under the supervision of Karl-Paul

Witzel and Johannes F. Imhoff. Experimental work, evaluation of data, phylogenetic analysis and preparation of the manuscript was done by Cristina Dorador.

Cristina Dorador, Irma Vila, Johannes F. Imhoff and Karl-Paul Witzel. Cyanobacteria from the Salar de Huasco, a high altitude saline wetland in Northern Chile, are extremely similar to Antarctic cyanobacteria. Submitted as research paper to FEMS Microbiology Ecology (Status: in review).

Sampling was conducted by Cristina Dorador, Irma Vila and Karl-Paul Witzel. The manuscript was prepared by Cristina Dorador under the supervision of Karl-Paul Witzel and Johannes F. Imhoff. Experimental work, evaluation of data, phylogenetic analysis and preparation of the manuscript was conducted by Cristina Dorador.

Cristina Dorador, Annika Busekow, Irma Vila, Johannes F. Imhoff and Karl-Paul Witzel.

Molecular analysis of enrichment cultures of ammonia oxidizers from the Salar de

Huasco, a high altitude saline wetland in northern Chile. Submitted as research paper
to Extremophiles (Status: in review).

Sampling was done by Cristina Dorador, Irma Vila and Karl-Paul Witzel. Manuscript was prepared by Cristina Dorador under the supervision of Karl-Paul Witzel and Johannes F. Imhoff. Experimental work was done by Annika Busekow and Cristina Dorador. Evaluation of data, phylogenetic analysis and preparation of the manuscript was done by Cristina Dorador.

#### **REFERENCES**

- Abed, R.M.M., Garcia-Pichel, F. and Hernández-Mariné, M. (2002) Polyphasic characterization of benthic, moderately halophilic, moderately thermophilic cyanobacteria with very thin trichomes and the proposal of *Halomicronema* excentricum gen. nov., sp. nov. *Arch Microbiol* 177, 361-370.
- Abell, G.C.J. and Bowman, J.P. (2005) Ecological and biogeographic relationships of class *Flavobacteria* in the Southern Ocean. *FEMS Microbiol Ecol* **51**, 265-277.
- Abulencia, C.B., D.L. Wyborski, J.A. Garcia, M. Podar, W. Chen, S. H. Chang, H. W. Chang, D. Watson, E. L. Brodie, T. C. Hazen, and M. Keller (2006) Environmental whole-genome amplification to access microbial populations in contaminated sediments. *Appl Environ Microbiol* **72**, 3291-3301.
- Aceituno, P. (1997) Aspectos generales del clima en el Altiplano Sudamericano. In *El Altiplano: Ciencia y Conciencia de los Andes* ed. González, C. pp.63-69. Santiago, Chile: Editorial Artegrama.
- Achenbach, L.A. and Coates, J.D. (2000) Disparity between bacterial phylogeny and physiology. *ASM News* **66**, 714-715.
- Akasaka, H., Izawa, T., Ueki, K. and Ueki, A. (2003) Phylogeny of numerically abundant culturable anaerobic bacteria associated with degradation of rice plant residue in Japanese paddy field soil. *FEMS Microbiol Ecol* **43**, 149-161.
- Albuquerque, L., Simoes, C., Nobre, M.F., Pino, N.M., Battista, J.R., Silva, M.T., Rainey, F.A. and da Costa, M.S. (2005) *Truepera radiovictrix* gen. nov., sp. nov., a new radiation resistant species and the proposal of Trueperaceae fam. nov. *FEMS Microbiol Lett* **247**, 161-169.
- Allgaier, M., Uphoff, H., Felske, A. and Wagner-Döbler, I. (2003) Aerobic anoxygenic photosynthesis in *Roseobacter* clade bacteria from diverse marine habitats. *Appl Environ Microbiol* **69**, 5051-5059.
- Amachi, S., Muramatsu, Y., Akiyama, Y., Miyazaki, K., Yoshiki, S., Hanada, S., Kamagata, Y., Ban-nai, T., Shinoyama, H. and Fujii, T. (2005) Isolation of iodide-oxidizing bacteria from iodide-rich natural gas brines and seawaters. *Microb Ecol* 49, 547-557.

- APHA (1999) Standard Methods for the Examination of Water and Wastewater.

  Baltimore, Maryland: American Public Health Association.
- Arahal, D.R., Ludwig, W., Schleifer, K.H. and Ventosa, A. (2002) Phylogeny of the family Halomonadaceae based on 23S and 16S rDNA sequence analyses. *Int J Syst Evol Microbiol* **52**, 241–249.
- Arroyo, M.K., Squeo, F.A., Veit, H., Cavieres, L., León, P. and Belmonte, E. (1997) Flora and vegetation of northern Chilean Andes. In *El Altiplano: Ciencia y Conciencia de los Andes* ed. González, C. pp.167-178. Santiago, Chile: Editorial Artegrama.
- Baas Becking, L.G.M. (1934) *Geobiologie of inleiding tot de milieukunde*. The Hage, the Netherlands: W. P. Van Stockum & Zoon.
- Barns, S.M., Fundyga, R.E., Jeffries, M.W. and Pace, N.R. (1994) Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment. *Proc Natl Acad Sci USA* **91**, 1609-1613.
- Barns, S.M., Delwiche, C.F., Palmer, J.D. and Pace, N.R. (1996) Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. *Proc Natl Acad Sci USA* **20**, 9188-9193.
- Benoit, I. (1989) *Libro Rojo de la Flora Terrestre de Chile*. Santiago, Chile: CONAF, Ministerio de Agricultura.
- Bernhard, A.E. and Peele, E.R. (1997) Nitrogen limitation of phytoplankton in a shallow embayment in northern Puget Sound. *Estuaries* **20**, 759-769.
- Bernhard, A.E., Donn, T., Giblin, A.E. and Stahl, D.A. (2005) Loss of diversity of ammonia-oxidizing bacteria correlates with increasing salinity in an estuary system. *Environ Microbiol* **7**, 1289–1297.
- Berrini, C.C., De Appolonia, F., Dalla Valle, L., Komarek, J. and Andreoli, C. (2004) Morphological and molecular characterization of a thermophilic cyanobacterium (Oscillatoriales) from Euganean Thermal Springs (Padua, Italy). *Arch Hydrobiol Suppl Algol Stud* **113**, 73-85.
- Betancourt, J.L., Latorre, C., Rech, J.A., Quade, J. and Rylander, K.A. (2000) A 22,000-year record of monsoonal precipitation from Northern Chile's Atacama Desert. *Science* **289**, 1542-1546.

- Béjà, O., Suzuki, M.T., Heidelberg, J.F., Nelson, W.C., Preston, C.M., Hamada, T., Elsen, J.A., Fraser, C.M. and DeLong, E.F. (2002) Unsuspected diversity among marine aerobic anoxygenic phototrophs. *Nature* **415**, 630-633.
- Biddle, J.F., Lipp, J.S., Lever, M.A., Lloyd, K.G., Sørensen, K.B., Anderson, R., Fredricks, H.F., Elvert, M., Kelly, T.J., Schrag, D.P., Sogin, M.L., Brenchley, J.E., Teske, A., House, C.H. and Hinrichs, K.-U. (2006) Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proc Natl Acad Sci USA* 103, 3846-3851.
- Bidle, K., Amadio, W., Oliveira, P., Paulish, T., Hicks, S. and Earnest, C. (2005) A phylogenetic analysis of haloarchaea found in a solar saltern. *Bios* **76**, 89-96.
- Blazejak, A., Erseus, C., Amann, R. and Dubilier, N. (2005) Coexistence of bacterial sulfide oxidizers, sulfate reducers, and spirochetes in a gutless worm (Oligochaeta) from the Peru margin. *Appl Environ Microbiol* **71**, 1553-1561.
- Bollmann, A. and Laanbroek, H.J. (2002) Influence of oxygen partial pressure and salinity on the community composition of ammonia-oxidizing bacteria in the Schelde estuary. *Aquat Microb Ecol* **28**, 239-247.
- Bothe, H., Jost, G., Schloter, M., Ward, B.B. and Witzel, K.-P. (2000) Molecular analysis of ammonia oxidation and denitrification in natural environments. *FEMS Microbiol Rev* **24**, 673-690.
- Bowman, J.P., McCammon, S.A., Lewis, T., Skerratt, J.H., Brown, J.L., Nichols, D.S. and McMeekin, T.A. (1998) *Psychroflexus torquis* gen. nov., sp. nov., a psychrophilic species from antarctic sea ice, and reclassification of *Flavobacterium gondwanense* (Dobson et al. 1993) as *Psychroflexus gondwanense* gen. nov., comb. nov. *Microbiology* **144**, 1601-1609.
- Bowman, J., McCammon, S.A., Rea, S.M. and McMeekin, T.A. (2000) The microbial composition of three limnologically disparate hypersaline Antarctic lakes. *FEMS Microbiol Lett* **183**, 81-88.
- Bowman, J.P. and Nichols, D.S. (2002) *Aequorivita* gen. nov., a member of the family Flavobacteriaceae isolated from terrestrial and marine Antarctic habitats. *Int J Syst Evol Microbiol* **52**, 1533-1541.
- Bruns, M.A., Stephen, J.R., Kowalchuk, G.A., Prosser, J.I. and Paul, E.A. (1999)

- Comparative diversity of ammonia oxidizer 16S rRNA gene sequences in native, tilled and successional soils. *Appl Environ Microbiol* **65**, 2994-3000.
- Cabrera, S., Bozzo, S. and Fuenzalida, H. (1994) Variation in UV radiation in Chile. *J Photochem Photobiol B: Biol* **28**, 137-142.
- Cameron, R.E., Gensel, D.R. and Blank, G.B. (1966) Abundance of microflora in soil samples from the Chilean Atacama Desert. *JPL Space Programs Summary* IV, 37-38.
- Campos, V. (1997) Microorganismos de ambientes extremos: Salar de Atacama, Chile. In *El Altiplano: Ciencia y Conciencia de los Andes* ed. González, C. pp.143-147. Santiago, Chile: Editorial Artegrama.
- Casamatta, D.A., Johansen, J.R., Vis, M.L. and Broadwater, S.T. (2005) Molecular and morphological characterization of ten polar and near-polar strains within the oscillatoriales (cyanobacteria). *J Phycol* **41**, 421-438.
- Castiglioni, B., Rizzi, E., Frosini, A., Sivonen, K., Rajaniemi, P., Rantala, A., Mugnai, M.A., Ventura, S., Wilmotte, A., Boutte, C., Grubisic, S., Balthasart, P., Consolandi, C., Bordoni, R., Mezzelani, A., Battaglia, C. and De Bellis, G. (2004) Development of a universal microarray based on the ligation detection reaction and 16S rRNA gene polymorphism to target diversity of cyanobacteria. *Appl Environ Microbiol* 70, 7161-7172.
- Caumette, P., Baulaigue, R. and Matheron, R. (1988) Characterization of *Chromatium salexigens* sp. nov., a halophilic *Chromatiaceae* isolated from mediterranean salinas. *System Appl Microbiol* **10**, 284-292.
- Chaban, B., Ng, S.Y.M. and Jarrell, K.F. (2006) Archaeal habitats from the extreme to the ordinary. *Can J Microbiol* **52**, 73-116.
- Chan, O.C., Claus, P., Casper, P., Ulrich, A., Lueders, T. and Conrad, R. (2005) Vertical distribution of structure and function of the methanogenic archaeal community in Lake Dagow sediment. *Environ Microbiol* 7, 1139-1149.
- Chao, A. (1984) Non-parametric estimation of the classes in a population. *Scand J Statistics* **11**, 265-270.
- Chao, A. (1987) Estimating the population size for capture-recapture data with unequal catchability. *Biometrics* **43**, 783-791.

- Charrier, R. and Muñoz, N. (1997) Geología y tectónica del Altiplano Chileno. In *El Altiplano: Ciencia y Conciencia de los Andes* ed. González, C. pp.23-31. Santiago, Chile: Editorial Artegrama.
- Choi, D.H. and Cho, B.C. (2005) *Idiomarina seosinensis* sp. nov., isolated from hypersaline water of a solar saltern in Korea. *Int J Syst Evol Microbiol* **55**, 379–383.
- Chong, G. (1984) Die Salare in Nordchile: Geologie, Struktur und Geochemie. *Geotekt Forsch* **67**, 1-146.
- Chong, G., Pueyo, J.J. and Demergasso, C. (2000) Los yacimientos de boratos de Chile. *Rev Geol Chile* **27**, 99-119.
- Cohen, Y. and Gurevitz, M. (2006) The Cyanobacteria ecology, physiology and molecular genetics. In *The Prokaryotes* eds. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. and Stackebrandt, E. pp.1074-1098. New York, USA: Springer.
- Comte, K., Sabacká, M., Carré-Mlouka, A., Elster, J. and Komárek, J. (2007) Relationships between the Arctic and theAntarctic cyanobacteria; three *Phormidium*-like strains evaluated by a polyphasic approach. *FEMS Microbiol Ecol* **59**, 366-376.
- CONAMA (2006) Estudio de la variabilidad climática en Chile para el siglo XXI. Santiago, Chile: Departamento de Geofísica, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile.
- Curtis, T.P., Head, I.M., Lunn, M., Woodcock, S., Schloss, P.D. and Sloan, W.T. (2006) What is the extent of prokaryotic diversity? *Philos Trans R Soc Lond B Biol Sci* **361**, 2023-2037.
- de Souza, M.P., Amini, A., Dojka, M.A., Pickering, I.J., Dawson, S.C., Pace, N.R. and Terry, N. (2001) Identification and characterization of bacteria in a selenium-contaminated hypersaline evaporation pond. *Appl Environ Microbiol* **67**, 3785-3794.
- de Wit, R. and Bouvier, T. (2006) "Everything is everywhere, but, the environment selects"; what did Baas Becking and Beijerinck really say? Environ Microbiol 8, 755-758.
- del Moral, A., Quesada, E., Bejar, V. and Ramos-Cormenzana, A. (1987) Evolution of bacterial flora from a subterranean saline well by gradual salinity changes in enrichment media. *J Appl Bacteriol* **62**, 465-471.
- DeLong, E.F. (1992) Archaea in coastal marine environments. Proc Natl Acad Sci USA

- **89**, 5685-5689.
- DeLong, E.F. (1998) Everything in moderation: Archaea as "non-extremophiles". *Curr Opin Gen Dev* **8**, 649-654.
- DeLong, E.F. and Pace, N.R. (2001) Environmental diversity of Bacteria and Archaea. *Syst Biol* **50**, 470-478.
- Demergasso, C., Chong, G., Galleguillos, P., Escudero, L., Martínez-Alonso, M. and Esteve, I. (2003) Microbial mats from the Llamará salt flat, northern Chile. *Rev Chil Hist Nat* **76**, 485-499.
- Demergasso, C., Casamayor, E., Chong, G., Galleguillos, P., Escudero, L. and Pedrós-Alió, C. (2004) Distribution of prokaryotic genetic diversity in athalassohaline lakes of the Atacama Desert, Northern Chile. *FEMS Microbiol Ecol* **48**, 57-69.
- Dojka, M.A., Hugenholtz, P., Haack, S.K. and Pace, N.R. (1998) Microbial diversity in a hydrocarbon- and chlorinated-solvent-contaminated aquifer undergoing intrinsic bioremediation. *Appl Environ Microbiol* **64**, 3869-3877.
- Dong, H., Zhang, G., Jiang, H., Yu, B., Chapman, L.R., Lucas, C.R. and Fields, M.W. (2006) Microbial diversity in sediments of saline Qinghai Lake, China: linking geochemical controls to microbial ecology. *Microbiol Ecol* **51**, 65-82.
- Dorador, C., Pardo, R. and Vila, I. (2003) Variaciones temporales de parámetros físicos, químicos y biológicos: el caso del lago Chungará. *Rev Chil Hist Nat* **76**, 15-22.
- Dorador, C., Castillo, G., Carú, M. and Vila, I. (2005) Microbial communites structure in freshwater systems of different trophic state using T-RFLP. In *Tercer Taller Internacional de Eutrofización de Lagos y Embalses* eds. Vila, I. and Pizarro, J. Santiago, Chile: CYTED XVI B.
- Dorador, C., Castillo, G., Witzel, K.-P. and Vila, I. (2007) Bacterial diversity in the sediments of a temperate artificial lake, Rapel reservoir. *Rev Chil Hist Nat* **80**, 213-224.
- Drees, K.P., Neilson, J., Betancourt, J.L., Quade, J., Henderson, D., Pryor, B.M. and Maier, R.M. (2006) Bacterial community structure in the hyper-arid core of the Atacama Desert, Chile. *Appl Environ Microbiol* **72**, 7902-7908.
- Earle, L.R., Warner, B.G. and Aravena, R. (2003) Rapid development of an unusual peat-accumulating ecosystem in the Chilean Altiplano. *Quaternary Res* **59**, 2-11.

- Eggen, R., Harmsen, H., Geerling, A. and de Vos, W.M. (1989) Nucleotide sequence of a 16S rRNA encoding gene from the archaebacterium *Methanothrix soehngenii*. *Nucleic Acids Res* **17**, 9469.
- Fahy, A., McGenity, T.J., Timmis, K.N. and Ball, A.S. (2006) Heterogeneous aerobic benzene-degrading communities in oxygen-depleted groundwaters. *FEMS Microbiol Ecol* **58**, 260-270.
- Fernández Zenoff, V., Siñeriz, F. and Farías, M.E. (2006) Diverse responses to UV-B radiation and repair mechanisms of Bacteria isolated from high altitude aquatic environments. *Appl Environ Microbiol* **72**, 7857-7863.
- Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E. and Oakley, B.B. (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc Natl Acad Sci USA* **102**, 14683-14688.
- Fräre, M., Rijks, J.Q. and Rea, J. (1975) *Estudio agroclimatológico de la zona andina*. Roma: FAO.
- Freitag, T.E. and Prosser, J.I. (2003) Community structure of ammonia-oxidizing bacteria within anoxic marine sediments. *Appl Environ Microbiol* **69**, 1359-1371.
- Freitag, T.E., Chang, L. and Prosser, J.I. (2006) Changes in the community structure and activity of betaproteobacterial ammonia-oxidizing sediment bacteria along a freshwater-marine gradient. *Environ Microbiol* **8**, 684–696.
- Galinski, E.A. and Trüper, H. (1994) Microbial behaviour in salt-stressed ecosystems. *FEMS Microbiol Rev* **15**, 95-108.
- Garreaud, R., Mathias, V. and Clement, A.C. (2003) The climate of the Altiplano: observed current conditions and mechanisms of past changes. *Palaeogeogr Palaeocl Palaeoecol* **194**, 5-22.
- Geets, J., Boon, N. and Verstraete, W. (2006) Strategies of aerobic ammonia-oxidizing bacteria for coping with nutrient and oxygen fluctuations. *FEMS Microbiol Ecol* **58**, 1-13.
- Glade, A. (1993) *Libro Rojo de los Vertebrados Terrestres de Chile*. Santiago, Chile: CONAF, Ministerio de Agricultura.
- Glatz, R.E., Lepp, P.W., Ward, B.B. and Francis, C.A. (2006) Planktonic microbial community composition across steep physical/chemical gradients in permanently

- ice-covered Lake Bonney, Antarctica. Geobiology 4, 53-67.
- Glöckner, F.O., Zaichikov, E., Belkova, N., Denissova, L., Pernthaler, J., Pernthaler, A. and Amann, R. (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of Actinobacteria. *Appl Environ Microbiol* **66**, 5053-5065.
- Go, Y.S., Han, S.K., Lee, I.G. and Ahn, T.Y. (2000) Diversity of the domain Archaea as determined by 16S rRNA gene analysis in the sediments of Lake Soyang. *Arch Hydrobiol* **149**, 459-466.
- Gómez-Consarnau, L., González, J.M., Coll-Lladó, M., Gourdon, P., Pascher, T., Neutze, R., Pedrós-Alió, C. and Pinhassi, J. (2007) Light stimulates growth of proteorhodopsin-containing marine Flavobacteria. *Nature* 445, 210-213.
- Grosjean, M. (1994) Paleohydrology of the Laguna Lejía (north Chilean Altiplano) and climatic implications for the late-glacial times. *Palaeogeogr Palaeocl Palaeoecol* **109**, 89-100.
- Grosjean, M. and Veit, H. (2005) Water resources in the arid mountains of the Atacama Desert (N Chile): past climate changes and modern conflicts. In *Global Change and Mountain Regions: A State of Knowledge Overview* eds. Huber, U.M., Bugmann, H.K.M. and Reasoner, M.A. pp.96-104. Dordrecht: Springer.
- Guindon, S., Lethiec, F., Duroux, P. and Gascuel, O. (2005) PHYML Online, a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Res* **33**, W557-W559.
- Gundlapally, S.R. and Garcia-Pichel, F. (2006) The community and phylogenetic diversity of biological soil crusts in the colorado plateau studied by molecular fingerprinting and intensive cultivation. *Microb Ecol* **52**, 345-357.
- Hallam, S., Mincer, T., Schleper, C., Preston, C., Roberts, K., Richardson, P. and DeLong, E.F. (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine *Crenarchaeota*. *PLoS Biol* 4, e95.
- Hardy, D.R., Vuille, M., Braun, C., Keimig, F. and Bradley, R.S. (1998) Annual and daily meteorological cycles at high altitude on a tropical mountain. *Bull Am Meteorol Soc* **79**, 1899-1913.

- Hartley, A.J., Chong, G., Houston, J. and Mather, A.E. (2005) 150 million years of climatic stability: evidence from the Atacama Desert, northern Chile. *J Geol Soc London* **162**, 421-424.
- Hastings, R.C., Saunders, J.R., Hall, G.H., Pickup, R.W. and McCarthy, A.J. (1998) Application of molecular biological techniques to a seasonal study of ammonia oxidation in a eutrophic freshwater lake. *Appl Environ Microbiol* **64**, 3674-3682.
- Hecky, R.E., Campbell, P. and Hendzel, L.L. (1993) The stoichiometry of carbon, nitrogen and phosphorus in particulate matter of lakes and oceans. *Limnol Oceanogr* **38**, 709-724.
- Hiorns, W.D., Hastings, R.C., McCarthy, A.J., Saunders, J.R., Pickup, R.W. and Hall, H. (1995) Amplification of 16S rDNA genes of autotrophic ammonia-oxidizing bacteria demostrates the ubiquity of nitrosospiras in the environment. *Microbiology* **141**, 2793-2800.
- Holmes, M.L., Olsen, G.J. and Dyall-Smith, M.L. (1990) The halophilic archaebacteria *Hb. lacusprofundi* and *Hb. saccharovorum* are closely related: 16S rRNA sequence comparison. *Nucleic Acids Res* **18**, 4607.
- Holmes, D.E., Nevin, K.P. and Lovley, D.R. (2004) Comparison of 16S rRNA, nifD, recA, gyrB, rpoB and fusA genes within the family *Geobacteraceae* fam. nov. *Int J Syst Evol Microbiol* **54**, 1591-1599.
- Hongoh, Y., Deevong, P., Hattori, S., Inoue, T., Noda, S., Noparatnaraporn, N., Kudo, T. and Ohkuma, M. (2006) Phylogenetic diversity, localization, and cell morphologies of members of the Candidate Phylum TG3 and a Subphylum in the Phylum Fibrobacteres, recently discovered bacterial groups dominant in termite guts. Appl Environ Microbiol 72, 6780-6788.
- Huber, H., Hohn, M.J., Rachel, R., Fuchs, T., Wimmer, V.C. and Stetter, K.O. (2002) A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature* **417**, 63-67.
- Hugenholtz, P., Pitulle, C., Hershberger, K. and Pace, N.R. (1998) Novel division level bacterial diversity in a Yellowstone hot spring. *J Bacteriol* **180**, 366-376.
- Hughes, J.B., Hellmann, J.J., Taylor, R.H. and Bohannan, J.M. (2001) Counting the uncountable: statistical approaches to estimating microbial diversity. *Appl Environ*

- Microbiol 67, 4399-4406.
- Humayoun, S.B., Bano, N. and Hollibaugh, J.T. (2003) Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. *Appl Environ Microbiol* **69**, 1030-1042.
- Hunter, E.M., Mills, H.J. and Kostka, J.E. (2006) Microbial community diversity associated with carbon and nitrogen cycling in permeable shelf sediments. *Appl Environ Microbiol* **72**, 5689-5701.
- Huu, N.B., Denner, E.B.M., Ha, D.T.C., Wanner, G. and Stan-Lotter, H. (1999) *Marinobacter aquaeolei* sp. nov., a halophilic bacterium isolated from a Vietnamese oil-producing well. *Int J Syst Bacteriol* **49**, 367–375.
- Imhoff, J.F. (1979) The Wadi Natrun: chemical composition and microbial mass developments in alkaline brines of eutrophic desert lakes. *Geomicrobiol J* 1, 219-234.
- Imhoff, J.F. (1986) Osmoregulation and compatible solutes in eubacteria. *FEMS Microbiol Rev* **39**, 57-66.
- Imhoff, J.F. (1988) Halophilic phototrophic bacteria. In *Halophilic Bacteria* ed. Rodriguez-Valera, F. pp.85-108. Boca Raton, Florida: CRC Press.
- Imhoff, J.F. (1988) Anoxygenic phototrophic bacteria. In *Methods in Aquatic Bacteriology* ed. Austin, B. pp.207-240: John Wiley & Sons Ltd.
- Imhoff, J.F. (1993) Osmotic adaptation in halophilic and halotolerant microorganisms. In *The Biology of Halophilic Bacteria* eds. Vreeland, R.H. and Hochstein, L.J. pp.211-253. Boca Raton, Florida: The CRC Press.
- Imhoff, J.F. and Süling, J. (1996) The phylogenetic relationship among Ectothiorhodospiraceae: A reevaluation of their taxonomy on the basis of rDNA analyses. *Arch Microbiol* **165**, 106-113.
- Imhoff, J.F. (2001) True marine and halophilic anoxygenic phototrophic bacteria. *Arch Microbiol* **176**, 243-254.
- Imhoff, J.F. (2006a) The phototrophic Alpha-proteobacteria. In *The Prokaryotes* eds. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. and Stackebrandt, E. pp.41-64. New York, USA: Springer.
- Imhoff, J.F. (2006b) The Chromatiaceae. In *The Prokaryotes* eds. Dworkin, M., Falkow,

- S., Rosenberg, E., Schleifer, K.-H. and Stackebrandt, E. pp.846-873. New York, USA: Springer.
- Imhoff, J.F. (2006c) The family Ectothiorhodospiraceae. In *The Prokaryotes* eds. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. and Stackebrandt, E. pp.874-886. New York, USA: Springer.
- Inagaki, F., Suzuki, M., Takai, K., Oida, H., Sakamoto, T., Aoki, K., Nealson, K.H. and Horikoshi, K. (2003) Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. *Appl Environ Microbiol* **69**, 7224-7235.
- Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F.S., Nealson, K.H., Horikoshi, K., D'Hondt, S. and Jørgensen, B. (2006) Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc Natl Acad Sci USA* 103, 2815–2820.
- Jellison, R. and Melack, J.M. (1988) Photosynthetic activity of phytoplankton and its relation to environmental factors in hypersaline Mono Lake, California. *Hydrobiologia* **158**, 69-88.
- Jiang, H., Dong, H., Zhang, G., Yu, B., Chapman, L. and Fields, M. (2006) Microbial diversity in water and sediment of Lake Chaka an athalassohaline lake in northwestern China. *Appl Environ Microbiol* 72, 3832-3845.
- Joint, I., Henriksen, P., Garde, K. and Riemann, B. (2002) Primary production, nutrient assimilation and microzooplankton grazing along a hypersaline gradient. *FEMS Microbiol Ecol* 39, 245-257.
- Jonkers, H.M., De Bruin, S. and Van Gemerden, H. (1998) Turnover of dimethylsulfoniopropionate (DMSP) by the purple sulfur bacterium *Thiocapsa roseopersicina* M11: ecological implications. *FEMS Microbiol Ecol* **27**, 281-290.
- Jonkers, H.M., Jansen, M., Van der Maarel, M.J.E.C. and Van Gemerden, H. (1999) Aerobic turnover of dimethyl sulfide by the anoxygenic phototrophic bacterium *Thiocapsa roseopersicina*. *Arch Microbiol* **172**, 150-156.
- Jung, D.O., Achenbach, L.A., Karr, E.A., Takaichi, S. and Madigan, M.T. (2004) A gas vesiculate planktonic strain of the purple non-sulfur bacterium *Rhodoferax*

- antarcticus isolated from Lake Fryxell, Dry Valleys, Antarctica. Arch Microbiol **182**, 236-243.
- Jungblut, A.-D., Hawes, I., Mountfort, D., Hitzfeld, B., Dietrich, D.R., Burns, B.P. and Neilan, B.A. (2005) Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. *Environ Microbiol* 7, 519-529.
- Jurgens, G., Glöckner, F., Amann, R., Saano, A., Montonen, L., Likolammi, M. and Münster, U. (2000) Identification of novel Archaea in bacterioplankton of a boreal forest lake by phylogenetic analysis and fluorescent in situ hybridization. *FEMS Microbiol Ecol* 34, 45-56.
- Kaye, J.Z., Márquez, M.C., Ventosa, A. and Baross, J.A. (2004) *Halomonas neptunia* sp. nov., *Halomonas sulfidaeris* sp. nov., *Halomonas axialensis* sp. nov. and *Halomonas hydrothermalis* sp. nov.: halophilic bacteria isolated from deep-sea hydrothermal-vent environments. *Int J Syst Evol Microbiol* **54**, 499–511.
- Kemp, P. and Aller, J. (2003) Bacterial diversity in aquatic and other environments: what 16S rDNA libraries can tell us. *FEMS Microbiol Ecol* **47**, 161-177.
- Kemp, P. and Aller, J. (2004) Estimating prokaryotic diversity: When are 16S rDNA libraries large enough? *Limnol Oceanogr: Methods* **2**, 114-125.
- Kendall, M.M., Wardlaw, G.D., Tang, C.F., Bonin, A.S., Liu, Y. and Valentine, D.L. (2007) Diversity of Archaea in marine sediments from Skan Bay, Alaska, including cultivated methanogens, and description of *Methanogenium boonei* sp. nov. *Appl Environ Microbiol* 73, 407-414.
- Kirchman, D. (2002) The ecology of *Cytophaga-Flavobacteria* in aquatic environments. *FEMS Microbiol Ecol* **39**, 91-100.
- Klohn, W. (1972) Hidrografía de la zona desértica de Chile. In *Investigaciones de los Recursos Hidráulicos en el Norte Grande* ed. Burz, J. p.185. Santiago de Chile: Programa de las Naciones Unidas para el Desarrollo.
- Koch, C., Schumann, P. and Stackebrandt, E. (1995) Reclassification of *Micrococcus agilis* (Ali-Cohen 1889) to the genus *Arthrobacter* as *Arthrobacter agilis* comb. nov. and emendation of the genus *Arthrobacter*. *Int J Syst Bacteriol* **45**, 837-839.
- Koops, H.-P., Böttcher, B., Möller, U.C., Pommerening-Röser, A. and Stehr, G. (1991)

- Classification of eight new species of ammonia-oxidizing bacteria: *Nitrosomonas communis* sp. nov., *Nitrosomonas ureae* sp. nov., *Nitrosomonas aestuarii* sp. nov., *Nitrosomonas marina* sp. nov., *Nitrosomonas nitrosa* sp. nov., *Nitrosomonas eutropha* sp. nov., *Nitrosomonas oligotropha* sp. nov. and *Nitrosomonas halophila* sp. nov. *J Gen Microbiol* **137**, 1689-1699.
- Koops, H.-P. and Pommerening-Röser, A. (2001) Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species. *FEMS Microbiol Ecol* **37**, 1–9.
- Koops, H.-P., Purkhold, U., Pommerening-Röser, A., Timmermann, G. and Wagner, M. (2006) The lithotrophic ammonia-oxidizing bacteria. In *The Prokaryotes*. pp.778-811. New York: Springer.
- Kowalchuk, G.A., Stienstra, A.W., Heilig, G.H.J. and Stephen, J.R. (2000) Changes in the community structure of ammonia-oxidizing bacteria during secondary succession of calcareous grasslands. *Environ Microbiol* **2**, 99-110.
- Kowalchuk, G.A. and Stephen, J.R. (2001) Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Ann Rev Microbiol* **55**, 485-529.
- Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B. and Stahl, D.A. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**, 543-546.
- Krebs, C.J. (1998) Ecological Methodology. New York: Benjamin Cummings.
- Kumar, S., Tamura, K. and Nei, M. (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150-163.
- Kwon, S.-W., Kim, B.-Y., Weon, H.-Y., Baek, Y.-K., Koo, B.-S. and Go, S.-J. (2006) *Idiomarina homiensis* sp. nov., isolated from seashore sand in Korea. *Int J Syst Evol Microbiol* **56**.
- La Nación (2007) La guerra por el agua, 23 April 2007.
- Labrenz, M. and Banfield, J.F. (2004) Sulfate-reducing bacteria-dominated biofilms that precipitate ZnS in a subsurface circumneutral-pH mine drainage system. *Microb Ecol* **47**, 205-217.
- Lamb, S. and Davis, P. (2003) Cenozoic climate change as a possible cause for the rise of the Andes. *Nature* **425**, 792-797.
- Latorre, C., Betancourt, J.L., Rylander, K.A. and Quade, J. (2002) Vegetation invasion

- into absolute desert: A 45 k.y. rodent midden record from the Calama-Salar de Atacama basins, northern Chile (lat 22-24°S). *GSA Bulletin* **114**, 349-366.
- Leininger, S., Urich, T., Scloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C. and Schleper, C. (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442**, 806-809.
- Li, L., Kato, C. and Horikoshi, K. (1999) Bacterial diversity in deep-sea sediments from different depths. *Biodivers Conserv* **8**, 659-677.
- Lim, J.-M., Yoon, J.-H., Lee, J.-C., Jeon, C.O., Park, D.-J., Sung, C. and Kim, C.-J. (2004) *Halomonas koreensis* sp. nov., a novel moderately halophilic bacterium isolated from a solar saltern in Korea. *Int J Syst Evol Microbiol* **54**, 2037–2042.
- Liu, Y., Boone, D.R. and Choy, C. (1990) *Methanohalophilus oregonense* sp. nov., a methylotrophic methanogen from an alkaline, saline aquifer. *Int J Syst Bacteriol* **40**, 111-116.
- Liu, W.T., Marsh, T.L., Cheng, H. and Forney, L.J. (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environ Microbiol* 63, 4516-4522.
- Liu, Y., Y. Tandong, J. Nianzhi, S. Kang, Y. Zeng, and S. Huang (2006) Microbial community structure in moraine lakes and glacial meltwaters, Mount Everest. *FEMS Microbiol Lett* 265, 98-105.
- Lizama, C., M. Monteoliva-Sanchez, Suárez-García, A., Roselló-Mora, R., Aguilera, M., Campos, V. and Ramos-Cormenzana, A. (2002) *Halorubrum tebenquichense* sp. nov., a novel halophilic archaeon isolated from the Atacama Saltern, Chile. *Int J Syst Evol Microbiol* **52**, 149-155.
- Lomans, B.P., Maas, R., Luderer, R., Camp, H.O.d., Pol, A., Drift, C.v.d. and Vogels, G.D. (1999) Isolation and characterization of *Methanomethylovorans hollandica* gen. nov., sp. nov., isolated from freshwater sediment, a methylotrophic methanogen able to grow on dimethyl sulfide and methanethiol. *Appl Environ Microbiol* **65**, 3641-3650.
- Loy, A., Beisker, W. and Meier, H. (2005) Diversity of bacteria growing in natural mineral water after bottling. *Appl Environ Microbiol* **71**, 3624-3632.
- Löffler, F.E., Sun, O., Li, J. and Tiedje, J. (2000) 16S rRNA gene-based detection of

- tetrachloroethene-dechlorinating *Desulfuromonas* and *Dehalococcoides* species. *Appl Environ Microbiol* **66**, 1369-1374.
- Ludwig, W., Strunk, O., Klugbauer, S., Klugbauer, N., Weizenegger, M., Neumaier, J., Bachleitner, M. and Schleifer, K.H. (1998) Bacterial phylogeny based on comparative sequence analysis. *Electrophoresis* **19**, 554-568.
- Luebert, F. and Gajardo, R. (2005) Vegetación alto andina de Parinacota (norte de Chile) y una sinopsis de la vegetación de la Puna meridional. *Phytocoenologia* **35**, 79-128.
- Lyra, C., Laamanen, M., Lehtimaki, J.M., Surakka, A. and Sivonen, K. (2005) Benthic cyanobacteria of the genus *Nodularia* are non-toxic, without gas vacuoles, able to glide and genetically more diverse than planktonic *Nodularia*. *Int J Syst Evol Microbiol* **55**, 555-568.
- Madigan, M.T., Jung, D.O., Woese, C.R. and Achenbach, L.A. (2000) *Rhodoferax* antarcticus, sp. nov. a moderately psychrophilic purple nonsulfur bacterium from an Antarctic microbial mat. *Arch Microbiol* **173**, 269-277.
- Mancinelli, R.L. and Hochstein, L.I. (1986) The occurrence of denitrification in extremely halophilic bacteria. *FEMS Microbiol Lett* **35**, 55-58.
- Marquardt, J. and Palinska, K.A. (2007) Genotypic and phenotypic diversity of cyanobacteria assigned to the genus *Phormidium* (Oscillatoriales) from different habitats and geographical sites. *Arch Microbiol* **187**, 397-413.
- Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumis, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Øvreås, L., Reysenbach, A.-L., Smith, V.H. and Staley, J.T. (2006) Microbial biogeography: putting microorganisms on the map. *Nat Rev Microbiol* 4, 102-112.
- Martínez-Cánovas, M.J., Quesada, E., Llamas, I. and Béjar, V. (2004a) *Halomonas ventosae* sp. nov., a moderately halophilic, denitrifying, exopolysaccharideproducing bacterium. *Int J Syst Evol Microbiol* **54**, 733–737.
- Martínez-Cánovas, M.J., Béjar, V., Martínez-Checa, F., Páez, R. and Quesada, E. (2004b) *Idiomarina fontislapidosi* sp. nov. and *Idiomarina ramblicola* sp. nov., isolated from inland hypersaline habitats in Spain. *Int J Syst Evol Microbiol* **54**, 1793–1797.

- Maturrano, L., Santos, F., Roselló-Mora, R. and Antón, J. (2006) Microbial diversity in maras Salterns, a hypersaline environment in the Peruvian Andes. *Appl Environ Microbiol* **72**, 3887-3895.
- Márquez, M.C., Quesada, E., Bejar, V. and Ventosa, A. (1993) A chemotaxonomic study of some moderately halophilic Gram-positive isolates. *J Appl Bacteriol* **75**, 604-607.
- McCaig, A.E., Embley, T.M. and Prosser, J.I. (1994) Molecular analysis of enrichment cultures of marine ammonia oxidisers. *FEMS Microbiol Lett* **120**, 363-368.
- Mellado, E., Moore, E.R.B., Nieto, J.J. and Ventosa, A. (1996) Analysis of 16S rRNA gene sequences of *Vibrio costicola* strains: description of *Salinivibrio costicola* gen. nov., comb. nov. *Int J Syst Bacteriol* **46**, 817–821.
- Moffitt, M.C., Blackburn, S.I. and Neilan, B.A. (2001) rRNA sequences reflect the ecophysiology and define the toxic cyanobacteria of the genus *Nodularia*. *Int J Syst Evol Microbiol* **51**, 505-512.
- Moreno, A., Giralt, S., Valero-Garcés, B., Sáez, A., Bao, R., Prego, R., Pueyo, J.J., González-Sampériz, P. and Taberner, C. (2007) A 14 kyr record of the tropical Andes: The Lago Chungará sequence (18°S, northern Chilean Altiplano). *Quatern Int* **161**, 4-21.
- Muñoz, S., Núñez, M.H. and Yáñez, J. (1996) Libro Rojo de los Sitios Prioritarios para la Conservación de la Diversidad Biológica. Santiago, Chile: CONAF, Ministerio de Agricultura.
- Muyzer, G., de Waal, E.C. and Uitterlinden, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59**, 695-700.
- Muyzer, G. (1999) DGGE/TGGE a method for identifying genes from natural ecosystems. *Curr Opin Microbiol* **2**, 317-322.
- Mühlhauser, H.A., Hrepic, N., Mladinic, P., Montecino, V. and Cabrera, S. (1995) Water quality and limnological features of a high altitude andean lake, Chungará, in northern Chile. *Rev Chil Hist Nat* **68**, 341-349.
- Nadeau, T.-L., Milbrandt, E.C. and Castenholz, R.W. (2001) Evolutionary relationships of cultivated Antarctic oscillatoriaceans (cyanobacteria). *J Phycol* **37**, 650-654.

- Nicol, G.W. and Schleper, C. (2006) Ammonia-oxidizing Crenarchaeota: important players in the nitrogen cycle? *Trends Microbiol* **14**, 207-212.
- Norris, T.B. and Castenholz, R.W. (2006) Endolithic photosynthetic communities within ancient and recent travertine deposits in Yellowstone National Park. *FEMS Microbiol Ecol* **57**, 470-483.
- Nübel, U., Garcia-Pichel, F. and Muyzer, G. (1997) PCR primers to amplify 16S rRNA gene from Cyanobacteria. *Appl Environ Microbiol* **63**, 3327-3332.
- Núñez, L. (1992) *Cultura y Conflicto en los Oásis de San Pedro de Atacama*. Santiago, Chile: Editorial Universitaria.
- Núñez, L., Grosjean, M. and Cartajena, I. (2002) Human occupations and climate change in the Puna de Atacama, Chile. *Science* **298**, 821-824.
- O'Sullivan, L.A., Weightman, A.J. and Fry, J.C. (2002) New degenerate *Cytophaga-Flexibacter-Bacteroides*-specific 16S ribosomal DNA-targeted oligonucleotide probes reveal high bacterial diversity in River Taff epilithon. *Appl Environ Microbiol* **68**, 201-210.
- Okubo, Y., Futamata, H. and Hiraishi, A. (2005) Distribution and capacity for utilization of lower fatty acids of phototrophic purple nonsulfur bacteria in wastewater environments. *Microbes Environ* **20**, 135-143.
- Ollivier, B., Caumette, P., Garcia, J.-L. and Mah, R.A. (1994) Anaerobic bacteria from hypersaline environments. *Microb Rev* **58**, 27-38.
- Opfell, J.B. and Zebal, G.P. (1967) Ecological patterns of micro-organisms in desert soils. *Life Sci Space Res* **5**, 187-203.
- Oren, A. (1994) The ecology of the extremely halophilic archaea. *FEMS Microbiol Rev* **13**, 415-440.
- Oren, A. (2002) Molecular ecology of extremely halophilic Archaea and Bacteria. *FEMS Microbiol Ecol* **39**, 1-7.
- Oren, A. (2005) Halophilic Microorganisms: Physiology and Phylogeny. In *Origins* ed. Seckbach, J.: Springer, Netherlands.
- Oren, A. (2006) The Order Halobacteriales. In *The Prokaryotes*. pp.113-164. New York: Springer.
- Overmann, J., Fischer, U. and Pfennig, N. (1992) A new purple sulfur bacterium from

- saline littoral sediments, *Thiorhodovibrio winogradskyi* gen. nov. and sp. nov. *Arch Microbiol* **157**, 329-335.
- Overmann, J. and Garcia-Pichel, F. (2006) The phototrophic way of life. In *The Prokaryotes* eds. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. and Stackebrandt, E. pp.32-85. New York, USA: Springer.
- Oz, A., Sabehi, G., Koblizek, M., Massana, R. and Béjà, O. (2005) *Roseobacter*-like bacteria in Red and Mediterranean Sea aerobic anoxygenic photosynthetic populations. *App Environ Microbiol* **71**, 344-353.
- Palinska, K.A., Thomasius, C.F., Marquardt, J. and Golubic, S. (2006) Phylogenetic evaluation of cyanobacteria preserved as historic herbarium exsiccata. *Int J Syst Evol Microbiol* **56**, 2253–2263.
- Patel, G.B. and Sprott, G.D. (1990) *Methanosaeta concilii* gen. nov., sp. nov. ('Methanothrix concilii') and Methanosaeta thermoacetophila nom. rev., comb. nov. *Int J Syst Bacteriol* **40**, 79-82.
- Pedrós-Alió, C. (2005) Diversity of microbial communities: the case of solar salterns. In *Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya* eds. Gunde-Cimerman, N., Oren, A. and Plemenitaš, A. p.577. Dordrecht, The Netherlands: Springer.
- Petsch, S.T., Eglinton, T.I. and Edwards, K.J. (2001) 14C-Dead living biomass: Evidence for microbial assimilation of ancient organic carbon during shale weathering. *Science* **292**, 1127-1131.
- Prado, B., Lizama, C., Aguilera, M., Ramos-Cormenzana, A., Fuentes, S., Campos, V. and Monteoliva-Sánchez, M. (2006) *Chromohalobacter nigrandesensis* sp. nov., a moderately halophilic, Gram-negative bacterium isolated from Lake Tebenquiche on the Atacama Saltern, Chile. *Int J Syst Evol Microbiol* **56**, 647-651.
- Preston, C.M., Wu, K.Y., Molinski, T.F. and DeLong, E.F. (1996) A psychrophilic crenarchaeon inhabits a marine sponge: *Cenarchaeum symbiosum* gen. nov., sp. nov. *Proc Natl Acad Sci USA* **93**, 6241-6246.
- Prosser, J.I., Bohannan, B.J.M., Curtis, T.P., Ellis, R.J., Firestone, M.K., Freckleton, R.P., Green, J.L., Green, L.E., Killham, K., Lennon, J.J., Osborn, M.A., Solan, M., van der Gast, J. and Young, P.W. (2007) The role of ecological theory in microbial

- ecology. Nat Rev Microbiol 5, 384-392.
- Purdy, K.J., Cresswell-Maynard, T.D., Nedwell, D.B., McGenity, T.J., Grant, W.D., Timmis, K.N. and Embley, T.M. (2004) Isolation of haloarchaea that grow at low salinities. *Environ Microbiol* **6**, 591–595.
- Purkhold, U., Pommerening-Roser, A., Juretschko, S., Schmid, M.C., Koops, H.-P. and Wagner, M. (2000) Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoA sequence analysis: implications for molecular diversity surveys. *Appl Environ Microbiol* **66**, 5368-5382.
- Purkhold, U., Wagner, M., Timmermann, G., Pommerening-Roser, A. and Koops, H.-P. (2003) 16S rRNA and amoA-based phylogeny of 12 novel betaproteobacterial ammonia-oxidizing isolates: extension of the dataset and proposal of a new lineage within the nitrosomonads. *Int J Syst Evol Microbiol* **53**, 1485-1494.
- Quillaguamán, J., Delgado, O., Mattiasson, B. and Hatti-Kaul, R. (2004) *Chromohalobacter sarecensis* sp. nov., a psychrotolerant moderate halophile isolated from the saline Andean region of Bolivia. *Int J Syst Evol Microbiol* **54**, 1921–1926.
- Raggi, L.A. (1997) La fauna altiplánica. In *El Altiplano: Ciencia y Conciencia de los Andes* ed. González, C. pp.199-202. Santiago, Chile: Editorial Artegrama.
- Rainey, F.A., Zhilina, T.N., Boulygina, E.S., Stackebrandt, E., Tourova, T.P. and Zavarzin, G.A. (1995) The taxonomic status of the fermentative halophilic anaerobic bacteria: description of *Haloanaerobiales* ord. nov., *Halobacteroidaceae* fam. nov., *Orenia* gen. nov. and further taxonomic rearrangements at the genus and species level. *Anaerobe* 1, 185-199.
- Ramette, A. and Tiedje, J. (2006) Biogeography: an emerging cornerstone for understanding prokaryotic diversity, ecology, and evolution. *Microb Ecol* **53**, 197-207.
- Rech, J.A., Currie, B.S., Michalski, G. and Cowan, A.M. (2006) Neogene climate change and uplift in the Atacama Desert, Chile. *Geology* **34**, 761-764.
- Reddy, G.S., Raghavan, P.U., Sarita, N.B., Prakash, J.S., Nagesh, N., Delille, D. and Shivaji, S. (2003) *Halomonas glaciei* sp. nov. isolated from fast ice of Adelie Land, Antarctica. *Extremophiles* **7**, 55-61.

- Reysenbach, A.L., Longnecker, K. and Kirshtein, J. (2000) Novel bacterial and archaeal lineages from an in situ growth chamber deployed at a Mid-Atlantic Ridge hydrothermal vent. *Appl Environ Microbiol* **66**, 3798-3806.
- Risacher, F. and Fritz, B. (1991) Geochemistry of Bolivian salars, Lipez, southern Altiplano: Origin of solutes and brine evolution. *Geochim Cosmochim Ac* **55**, 687-705.
- Risacher, F., Alonso, H. and Salazar, C. (1999) *Geoquímica de aguas en cuencas cerradas: I, II y III regiones Chile*. Santiago, Chile: Ministerio de Obras Públicas, Dirección General de Aguas.
- Risacher, F., Alonso, H. and Salazar, C. (2003) The origin of brines and salts in Chilean salars: a hydrochemical review. *Earth-Sci Rev* **63**, 249-293.
- Risgaard-Petersen, N., Nicolaisen, M.H., Revsbech, N.P. and Lomstein, B.A. (2004) Competition between ammonia-oxidizing bacteria and benthic microalgae. *Appl Environ Microbiol* **70**, 5528-5537.
- Rivadeneyra, M.-A., Delgado, G., Soriano, M., Ramos-Cormenzana, A. and Delgado, R. (1999) Biomineralization of carbonates by *Marinococcus albus* and *Marinococcus halophilus* isolated from the Salar de Atacama (Chile). *Curr Microbiol* **39**, 53-57.
- Rodriguez-Valera, F., Ruiz-Berraquero, F. and Ramos-Cormenzana, A. (1980) Behaviour of mixed populations of halophilic bacteria in continuous cultures. *Can J Microbiol* **26**, 1259-1263.
- Rodriguez-Valera, F., Juez, G. and Kushner, D.J. (1983) *Halobacterium mediterranei* spec. nov., a new carbohydrate-utilizing extreme halophile. *Syst Appl Microbiol* **4**, 369–381.
- Roeselers, G., Norris, T.B., Castenholz, R.W., Rysgaard, S., Glud, R.N., Kühl, M. and Muyzer, G. (2007) Diversity of phototrophic bacteria in microbial mats from Artic hot springs (Greenland). *Environ Microbiol* **9**, 26-38.
- Romero, H., Rivera, A. and Fernández, P. (1997) Climatología de la puna de Atacama y su relación con los recursos hídricos. In *El Altiplano: Ciencia y Conciencia de los Andes* ed. González, C. pp.87-93. Santiago, Chile: Editorial Artegrama.
- Rotthauwe, J.H., Witzel, K.-P. and Liesack, W. (1997) The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural

- ammonia-oxidizing populations. Appl Environ Microbiol 63, 4704-4712.
- Rudi, K., Skulberg, O.M., Larsen, F. and Jakobsen, K.S. (1997) Strain characterization and classification of oxyphotobacteria in clone cultures on the basis of 16S rRNA sequences from the variable regions V6, V7, and V8. *Appl Environ Microbiol* **63**, 2593-2599.
- Rundell, P.W. and Palma, B. (2000) Preserving the unique Puna ecosystems of the Andean Altiplano: A descriptive account of Lauca National Park, Chile. *Mt Res Dev* **20**, 262-271.
- Sabbe, K., Hodgson, D.A., Verleyen, E., Taton, A., Wilmotte, A., Vanhoutte, K. and Vyverman, W. (2004) Salinity, depth and the structure and composition of microbial mats in continental Antarctic lakes. *Freshwater Biol* **49**, 296–319.
- Salazar, C. (1997) Hidrología del sector altiplánico chileno. In *El Altiplano: Ciencia y Conciencia de los Andes* ed. González, C. pp.71-77. Santiago, Chile: Editorial Artegrama.
- Sanguinetti, C.J., Dias-Neto, E. and Simpson, A.J.G. (1994) Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* **17**, 915-919.
- Sangwan, P., Kovac, S., Davis, K.E., Sait, M. and Janssen, P.H. (2005) Detection and cultivation of soil Verrucomicrobia. *Appl Environ Microbiol* **71**, 8402-8410.
- Saul, D.J., J. A. Aislabie, C. E. Brown, L. Harris, and J. M. Foght (2005) Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica. *FEMS Microbiol Ecol* **53**, 141-155.
- Schink, B., V. Thiemann, H. Laue, and M. W. Friedrich (2002) *Desulfotignum phosphitoxidans* sp. nov., a new marine sulfate reducer that oxidizes phosphite to phosphate. *Arch Microbiol* **177**, 381-391.
- Schleper, C., Jurgens, G. and Jonuscheit, M. (2005) Genomic studies of uncultivated Archaea. *Nat Rev Microbiol* **3**, 479-488.
- Schmid, M., Twachtmann, U., Klein, M., Strous, M., Juretschko, S., Jetten, M., Metzger, J.W., Schleifer, K.H. and Wagner, M. (2000) Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation. *Syst Appl Microbiol* 23, 93-106.

- Schmidt, M., Prieme, A. and Stougaard, P. (2006) Bacterial diversity in permanently cold and alkaline ikaite columns from Greenland. *Extremophiles* **10**, 551-562.
- Schwarz, J.I.K., Lueders, T., Eckert, W. and Conrad, R. (2007) Identification of acetate-utilizing Bacteria and Archaea in methanogenic profundal sediments of Lake Kinneret (Israel) by stable isotope probing of rRNA. *Environ Microbiol* **9**, 223-237.
- Selje, N., Simon, M. and Brinkhoff, T. (2004) A newly discovered *Roseobacter* cluster in temperate and polar oceans. *Nature* **427**, 445-448.
- Servant, M. and Fontes, J.C. (1978) Les lacs quaternaires des hauts plateaux des Andes boliviennes. Premières interprétations paléoclimatiques. In *Cahiers ORSTOM*, *série Géologie*. pp.79-97.
- Shao, P., Chen, Y.Q., Zhou, H., Qu, L.H., Jiao, N.Z., Ma, Y., Li, Z.Y. and Jiao, N. (2004) Phylogenetic diversity of Archaea in aquafarm sediment. *Mar Biol* **146**, 133-142.
- Simankova, M.V., Parshina, S.N., Tourova, T.P., Kolganova, T.V., Zehnder, A.J. and Nozhevnikova, A.N. (2001) *Methanosarcina lacustris* sp. nov., a new psychrotolerant methanogenic archaeon from anoxic lake sediments. *Syst Appl Microbiol* **24**, 362-367.
- Simberloff, D.S. (1972) Properties of the rarefaction diversity measurement. *Am Natur* **106**, 414-418.
- Sokolova, T.G., Kostrikina, N.A., Chernyh, N.A., Kolganova, T.V., Tourova, T.P. and Bonch-Osmolovskaya, E.A. (2005) *Thermincola carboxydiphila* gen. nov., sp. nov., a novel anaerobic, carboxydotrophic, hydrogenogenic bacterium from a hot spring of the Lake Baikal area. *Int J Syst Evol Microbiol* **55**, 2069-2073.
- Sorokin, D.Y., Tourova, T.P. and Muyzer, G. (2005) Oxidation of thiosulfate to tetrathionate by an haloarchaeon isolated from hypersaline habitat. *Extremophiles* **9**, 501-504.
- Sorokin, D.Y., Tourova, T.P., Galinski, E.A., Belloch, C. and Tindall, B.J. (2006) Extremely halophilic denitrifying bacteria from hypersaline inland lakes, *Halovibrio denitrificans* sp. nov. and *Halospina denitrificans* gen. nov., sp. nov., and evidence that the genus name *Halovibrio* Fendrich 1989 with the type species *Halovibrio variabilis* should be associated with DSM 3050. *Int J Syst Evol Microbiol* **56**, 379-388.

- Sørensen, K.B., Canfield, D.E., Teske, A.P. and Oren, A. (2005) Community composition of a hypersaline endoevaporitic microbial mat. *Appl Environ Microbiol* **71**, 7352-7365.
- Sørensen, K.B. and Teske, A. (2006) Stratified communities of active Archaea in deep marine subsurface sediments. *Appl Environ Microbiol* **72**, 4596-4603.
- Squeo, F., Warner, B.R., Aravena, D. and Espinoza, D. (2006) Bofedales: high altitude peatlands of the central Andes. *Rev Chil Hist Nat* **79**, 245-255.
- Squeo, F.A., Tracol, Y., López, D., Gutiérrez, J.R., Córdova, A.M. and Ehleringer, J.R. (2006) ENSO effects on primary productivity in Southern Atacama desert. Adv Geosci 6, 273-277.
- Stackebrandt, E. and Woese, C.R. (1981) The evolution in prokaryotes. In *Molecular and Cellular Aspects of Microbial Evolution* eds. Carlise, M.J., Collins, J.R. and Moseley, B.E.B. pp.1-31. Cambridge: Cambridge University Press.
- Stackebrandt, E. and Liesack, W. (1993) Nucleic acids and classification. In *Handbook of New Bacterial Systematics* ed. Goodfellow, M., and A. G. O'Donnell. pp.152-189. London: Academic Press.
- Stackebrandt, E. and Göbel, B.M. (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846-849.
- Stal, L.J. (1995) Physiological ecology of cyanobacteria in microbial mats and the other communities. *New Phytol* **131**, 1-32.
- Stehr, G., Böttcher, B., Dittberner, P., Rath, G. and Koops, H.-P. (1995) The ammonia-oxidizing nitrifying population of the River Elbe estuary. *FEMS Microbiol Ecol* **17**, 177-186.
- Stephen, J.R., McCaig, A.E., Smith, Z., Prosser, J.I. and Embley, T.M. (1996) Molecular diversity of soil and marine 16S rRNA gene sequences related to beta-subgroup ammonia-oxidizing bacteria. *Appl Environ Microbiol* **62**, 4147-4154.
- Stiller, M. and Nissenbaum, A. (1999) Geochemical investigation of phosphorus and nitrogen in the hypersaline Dead Sea -1979-1982. *Geochim Cosmochim Ac* **63**, 3467-3475.
- Stougaard, P., Jørgensen, F., Johnsen, M.G. and Hansen, O.C. (2002) Microbial diversity

- in ikaite tufa columns: an alkaline, cold ecological niche in Greenland. *Environ Microbiol* **4**, 487–493.
- Strous, M., Kuenen, J.G. and Jetten, M.S.M. (1999) Key physiology of anaerobic ammonium oxidation. *Appl Environ Microbiol* **65**, 3248-3250.
- Svenning, M.M., Eriksson, T. and Rasmussen, U. (2005) Phylogeny of symbiotic cyanobacteria within the genus *Nostoc* based on 16S rDNA sequence analyses. *Arch Microbiol* **183**, 19-26.
- Takai, K. and Horikoshi, K. (1999) Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics* **152**, 1285-1297.
- Takai, K., Moser, D.P., DeFlaun, M., Onstott, T.C. and Fredrickson, J.K. (2001) Archaeal diversity in waters from deep South African gold mines. *Appl Environ Microbiol* **67**, 5750-5760.
- Tamura, T., Hayakawa, M. and Hatano, K. (1999) *Sporichthya brevicatena* sp. nov. *Int J Syst Bacteriol* **49**, 1779-1784.
- Tarlera, S. and Denner, E.B. (2003) *Sterolibacterium denitrificans* gen. nov., sp. nov., a novel cholesterol-oxidizing, denitrifying member of the beta-Proteobacteria. *Int J Syst Evol Microbiol* **53**, 1085-1091.
- Taton, A., Grubisic, S., Brambilla, E., de Wit, R. and Wilmotte, A. (2003) Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo dry valleys, Antarctica): a morphological and molecular approach. *Appl Environ Microbiol* **69**, 5157-5169.
- Taton, A., Grubisic, S., Balthasart, P., Hodgson, D.A., Laybourn-Parry, J. and Wilmotte,
   A. (2006a) Biogeographical distribution and ecological ranges of benthic
   cyanobacteria in East Antarctic lakes. FEMS Microbiol Ecol 57, 272-289.
- Taton, A., Grubisic, S., Ertz, D., Hodgson, D.A., Piccardi, R., Biondi, N., Tredici, M.R., Mainini, M., Losi, D., Marinelli, F. and Wilmotte, A. (2006b) Polyphasic study of antarctic cyanobacterial strains. *J Phycol* 42, 1257-1270.
- Teske, A., Hinrichs, K.U., Edgcomb, V., de Vera Gomez, A., Kysela, D., Sylva, S.P., Sogin, M.L. and Jannasch, H.W. (2002) Microbial diversity of hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities. *Appl Environ Microbiol* **68**, 1994-2007.

- Tomlinson, G.A., Jahnke, L.L. and Hochstein, L.I. (1986) *Halobacterium denitrificans* sp. nov., an extremely halophilic denitrifying bacterium. *J Syst Bacteriol* **36**, 66–70.
- Tourova, T.P., Kolganova, T.V., Kuznetsov, B.B. and Pimenov, N.V. (2002) Phylogenetic diversity of the archaeal component in microbial mats on coral-like structures associated with methane seeps in the Black Sea. *Microbiology* **71**, 196-201.
- Ulrich, A.C. and Edwards, E.A. (2003) Physiological and molecular characterization of anaerobic benzene-degrading mixed cultures. *Environ Microbiol* **5**, 92-102.
- Valderrama, M.J., Prado, B., Del Moral, A., Rios, R., Ramos-Cormenzana, A. and Campos, V. (1991) Numerical taxonomy of moderately halophilic gram-positive cocci isolated from the Salar de Atacama (Chile). *Microbiología (Madrid, Spain)* 7, 35-41.
- Valentine, D.L. (2007) Adaptations to energy stress dictate the ecology and evolution of the Archaea. *Nat Rev Microbiol* **5**, 316-323.
- Vargas, C. (2002) Interacciones tróficas del lago Chungará, mediante análisis bibliográfico. In *Departamento de Ciencias Ecológicas*. p.45. Santiago, Chile: Universidad de Chile.
- Ventosa, A., Nieto, J.J. and Oren, A. (1998) Biology of moderately halophilic aerobic Bacteria. *Microbiol Mol Biol Rev* **62**, 504–544.
- Vetriani, C., Jannasch, H.W., MacGregor, B.J., Stahl, D.A. and Reysenbach, A.L. (1999) Population structure and phylogenetic characterization of marine benthic Archaea in deep-sea sediments. *Appl Environ Microbiol* **65**, 4375-4384.
- Vila, I. and Mühlhauser, H.A. (1987) Dinámica de lagos de altura, perspectivas de investigación. *Arch Biol Med Exp* **20**, 95-103.
- Vila, I. (2002) Sistemas intertropicales de altura: humedales altiplánicos. In *El agua en Iberoamérica; de la escasez a la desertificación* eds. Fernández Cirelli, A. and Abraham, E. pp.63-72. Buenos Aires: CYTED XVII, CETA, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires.
- Vila, I. (2006) A new species of Killifish in the genus *Orestias* (Teleostei: Cyprinodontidae) from the Southern High Andes, Chile. *Copeia* **3**, 472-477.
- Villagrán, C. and Castro, V. (1997) Etnobotánica y manejo ganadero de las vegas,

- bofedales y quebradas en el Loa superior, Andes de Antofagasta, Segunda Región, Chile. *Chungará (Chile)* **29**, 275-304.
- Vincent, W., Wurtsbaugh, W., Vincent, C. and Richerson, P.J. (1984) Seasonal dynamics of nutrients limitation in a tropical high-altitude lake (Lake Titicaca, Perú-Bolivia): Application of physiological bioassays. *Limnol Oceanogr* **29**, 540-552.
- Vincent, W., Vincent, C.L., Downes, M.T. and Richerson, P.J. (1985) Nitrate cycling in Lake Titicaca (Perú-Bolivia): the effects of high-altitude and tropicality. *Freshwater Biol* **15**, 31-42.
- Vincent, W.F. (2000) Evolutionary origins of antarctic microbiota: invasion, selection and endemism. *Antarct Sci* **12**, 374-385.
- Voytek, M.A. and Ward, B.B. (1995) Detection of ammonium-oxidizing bacteria of the beta-subclass of the class Proteobacteria in aquatic samples with the PCR. *Appl Environ Microbiol* **61**, 1444-1450.
- Wagner-Döbler, I. and Biebl, H. (2006) Environmental biology of the marine *Roseobacter* lineage. *Annu Rev Microbiol* **60**, 255-280.
- Ward, B.B., Martino, D.P., Diaz, M.C. and Joye, S.B. (2000) Analysis of ammonia-oxidizing bacteria from hypersaline Mono Lake, California, on the basis of 16S rRNA sequences. *Appl Environ Microbiol* **66**, 2873-2881.
- Waterbury, J.B. (2006) The Cyanobacteria isolation, purification and indentification. In *The Prokaryotes* eds. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. and Stackebrandt, E. pp.1053-1073. New York, USA: Springer.
- Webster, G., Embley, T.M., Freitag, T.E., Smith, Z. and Prosser, J.I. (2005) Links between ammonia oxidizer species composition, functional diversity and nitrification kinetics in grassland soils. *Environ Microbiol* 7, 676-684.
- Weidler, G.W., Dornmayr-Pfyffenhuemer, M., Gerbl, F.W., Heinen, W. and Stan-Lotter, H. (2007) Communities of Archaea and Bacteria in a subsurface radioactive thermal spring in the Austrian Central Alps, and evidence of ammonia-oxidizing *Crenarchaeota*. *Appl Environ Microbiol* 73, 259-270.
- Williams, W.D. (2002) Environmental threats to salt lakes and the likely status of inland saline ecosystems in 2025. *Environ Conserv* **29**, 154-167.
- Woese, C.R. (1987) Bacterial evolution. *Microb Rev* **51**, 221-271.

- Woese, C.R., Kandler, O. and Wheelis, M.L. (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci USA* **87**, 4576-4579.
- Wu, X.L., M. W. Friedrich, and Conrad, R. (2006) Diversity and ubiquity of thermophilic methanogenic archaea in temperate anoxic soils. *Environ Microbiol* **8**, 394-404.
- Yuhana, M. (2005) Microbial diversity and community changes in high mountain habitats. p.125. Zürich: University of Zürich.
- Yurkov, V.V. and Beatty, T.J. (1998) Aerobic anoxygenic phototrophic bacteria. *Microbiol Mol Biol Rev* **62**, 695-724.
- Yurkov, V.V. (2006) Aerobic phototrophic proteobacteria. In *The Prokaryotes* eds. Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. and Stackebrandt, E. pp.562-584. New York, USA: Springer.
- Zabel, H.P., König, H. and Winter, J. (1984) Isolation and characterization of a new coccoid methanogen, *Methanogenium tatii* spec. nov. from a solfataric field on Mount Tatio. *Arch Microbiol* **137**, 308-315.
- Zellner, G., Boone, D.R., Keswani, J., Whitman, W.B., Woese, C.R., Hagelstein, A., Tindall, B.J. and Stackebrandt, E. (1999) Reclassification of *Methanogenium tationis* and *Methanogenium liminatans* as *Methanofollis tationis* gen. nov., comb. nov. and *Methanofollis liminatans* comb. nov. and description of a new strain of *Methanofollis liminatans*. *Int J Syst Bacteriol* 49, 247-255.
- Zengler, K., Toledo, G., Rappe, M., Elkins, J., Mathur, E.J., Short, J.M. and Keller, M. (2002) Cultivating the uncultured. *Proc Natl Acad Sci USA* **99**, 15681-15686.
- Zúñiga, L.R., Campos, V., Pinochet, H. and Prado, B. (1991) A limnological reconnaissance of Lake Tebenquiche, Salar de Atacama, Chile. *Hydrobiologia* **210**, 19-24.

### **ACKNOWLEDGEMENTS**

Al desierto y mar de Antofagasta.

A mi familia por su constante, incondicional y eterno apoyo. Gracias Papá por tu fuerza, mamá por tu cariño inmenso, Claudio, Iván y Leo (y las descendencias) por sus alegrías y esperanzas compartidas.

I would like to thank my supervisor Dr. K-P. Witzel for the opportunity to conduct this thesis in his laboratory, for his constant support, affection and understanding, for showing me the fascinating world of the microbial ecology and for giving me another perspective on doing science. Furthermore, I wish to thank Prof. Dr. Johannes F. Imhoff for giving me the opportunity to work with phototrophic bacteria and for his important academic and personal support during these three years. I also thank Prof. Dr. Winfried Lampert and Prof. Dr. Diethard Tautz for allowing me to work at the Max Planck Institute for Limnology (MPIL, now Max Planck Institute for Evolutionary Biology).

I could not be here without the help and support of my Chilean supervisor Prof. Dr. Irma Vila who pushed me to follow the scientific career and supported me to work in the wetlands of the Altiplano, also special thanks to Prof. Gabriela Castillo for giving me the opportunity to learn microbiology.

Special thanks to the Deutscher Akademischer Austausch Dienst (DAAD) for giving me the financial support to live in Germany and for allowing me to learn German.

Thanks to Ms. Maria Hartmann from the DAAD for the help and support.

Sampling in the Altiplano is not easy. Many people helped me directly or indirectly. I would like to thank: Carolina Vargas, Vilma Barrera, Rodrigo Pardo, Patricio Acuña, Gabriela Castillo, Margarita Carú, Marta Cariceo, Cristóbal Espinoza, Manon Kayser and my family.

I would like to express my gratitude for the excellent technical assistance of Annika Busekow and Conny Burghardt at the MPIL and Frank Lappe at the IFM-GEOMAR. During these three years I met very nice people in the Schlößchen, many thanks to Sunny, Ora, Verónica, Pilar, In-Seon, Nathaly, Karin Olsen, Karin Eckert, Sara Beier. Also, I would like to thank colleagues at the IFM-GEOMAR for their help: Andrea Gärtner, Jörg Süling, Jutta Wiese, Vera Thiel, Mirjam Perner and Bettina Reuter.

This thesis would not be possible without the help of Chris Harrod who gave me a very important support during this time and also corrected the English of the whole work. Thanks to all my friends and mates for the good moments, support and affection that I received during this period in Germany: Andrea, Juan, Manon, Xavier, Pedro, Cristóbal, Chica, Edu, Viviana, Pato, Edmundo, Magdalena, Mery, Julen, Cristian Salineros, Oli, Alethya, Noel, Paulinha, Naoya, Martin, Walter, Ricardo, Sarah Yeates, Vanessa, Christophe, Tobias, Kamilla, Loly, Celeste, Pamela, Ivonne, Cristian Agurto, Eduardo, Greys, Felipe, Angélica, Rodrigo, Carolina and Annika.

At last I would like to thank the Symphonisches Orchester Plön, especially Peter Schmidt and Dr. Werner Bodendorff, for giving me the opportunity to play double bass again and for making me feel like home.

## **CURRICULUM VITAE**

## **Cristina Inés Dorador Ortiz**

geboren am 28.02.1980 in Antofagasta, Chile

## Ausbildung

seit 04.2004	Promotion am Max Planck Institut für Limnologie in Plön and im
	Leibniz-Institut für Meereswissenschaften (IFM-GEOMAR) in
	Kiel, Germany.
06.2003	Abschluss des Biologie Studiums. Fakultät für
	Naturwissenschaften. Universidad de Chile, Santiago, Chile.
03.1998/12.2001	Hochschulbildung: Licenciatura für Naturwissenschaften mit
	Schwerpunkt Biologie. Fakultät für Naturwissenschaften.
	Universidad de Chile, Santiago, Chile.
03.1994/12.1997	Oberschule: Liceo Experimental Artístico (Musische Oberschule)
	Antofagasta, Chile.
03.1986/12.1993	Grundschule: Escuela D-75 "Darío Salas Díaz", Antofagasta,
	Chile.

### Publikationen

- Dorador, C., Pardo, R., and Vila, I. (2003) Temporal variations of physical, chemical and biological parameters of a high altitude lake: the case of Chungará lake. *Rev. Chil. Hist. Nat.* **76**: 15-22.
- Dorador, C., Castillo, G., Carú, M., and Vila, I. (2005) Microbial communites structure in freshwater systems of different trophic state using T-RFLP. In *Tercer Taller Internacional de Eutrofización de Lagos y Embalses*. Vila, I., and Pizarro, J. (eds). Santiago, Chile: CYTED XVI B.
- Dorador, C., Castillo, G., Witzel, K.-P., and Vila, I. (2007) Bacterial diversity in the sediments of a temperate artificial lake, Rapel reservoir. *Rev. Chil. Hist. Nat.* **80**: 213-224.

# **ERKLÄRUNG**

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbstständig und ohne unerlaubte Hilfe angefertigt habe und dass sie nach Form und Inhalt meine eigene Arbeit ist. Sie wurde keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Dies ist mein einziges und bisher erstes Promotionsverfahren. Die Promotion soll im Fach Mikrobiologie erfolgen. Des Weiteren erkläre ich, dass ich Zuhörer bei der Disputation zulasse.

\_\_\_\_

Cristina Dorador

## **APPENDIX**

Posters related to this thesis were presented at the following symposia:

Cristina Dorador, Karl-Paul Witzel, Carolina Vargas, Irma Vila and Johannes F. Imhoff. Bacterial communities in high Andean wetlands: the case of Huasco Salar, Chile. ASLO 2005 Summer Meeting, June 19-24, 2005, Santiago de Compostela, Spain.

Cristina Dorador, Karl-Paul Witzel, Irma Vila and Johannes F. Imhoff. High archaeal diversity in high altitude saline wetlands. 11<sup>th</sup> International Symposium on Microbial Ecology ISME-11, August 20-25, 2006, Vienna, Austria.

Cristina Dorador, Karl-Paul Witzel, Irma Vila and Johannes F. Imhoff. Archaeal diversity in contrasting high altitude wetlands in the Chilean Altiplano. Annual Conference of the Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM), April 1-4, 2007, Osnabrück, Germany.

# The case of Salar de Huasco, Chile <sup>1</sup>Dorador C., <sup>1</sup>Witzel K. P., <sup>2</sup>Vargas C., <sup>2</sup>Vila I. & <sup>3</sup>Imhoff J. F <sup>1</sup>Max Planck Institute for Limnology, Plön, Germany <sup>2</sup>Faculty of Sciences, University of Chile, Santiago, Chile 3Leibniz-Institute of Marine Sciences, Kiel, Germany Abstract Conclusions The results of the physical and chemical analyses geological and hydrological features. From one of these salt flats, Salar de Huasco demonstrate a high temporal variability (periods) and spatial variability (stations) of the water in Salar de Huasco. The DGGE analyses show differences in the number and we analyzed bacteria and archaea assemblages in water and sediment, using PCRwe analyzed bacteria and archied assertinates in the rest and sequencing of 165 rRNA gene fragments. The clonal diversity included organisms related to alpha, beta, gamma and delta Proteobacteria, Cytophaga-Flavobacteria and halophilic archaea. We conclude that the spatial and pattern of bands. The clone library confirms the variability of the system and the high diversity in the sediment and water temporal variations of the environment determine the diversity of bacteria and archaea with different metabolic pathways, which play an important role in the trophic interactions within this high andean ecosystem. \* Due to the high evaporation that results in significant salinization, the bacterial communities play a more important role in the biogeochemical cycles than higher organisms. Results Introduction The environmental conditions of the andean altiplano are very harsh. Salars are a testimony of a more wet past climate and there are more than one hundred of them in the Chiean Andes. High radiation, high altitude, diyness and rain periods with large amplitude of temperatures configure a unique environment. The hydrographic basins are hydrologically closed, with evaporation as the origidischarge mechanism (Monti & Herrilquez 1972). The vetlands located in this area present high salinity and in some cases, high nutrient concentrations. Water of the high andean wettands is the central and most important factor that allows the development of the andean blota in this highland desert. The chemical characteristics of these waters vary from those of melting snow to brine water (Alonso & Vargas 1988). The geographic isolation, the high diversity of habitats and the impact of human activities (i.e. mining) make these ecosystems an important study object (Vila & Muhlhauser 1967). Temperature, pH, dissolved oxygen (D. O.) and conductivity varies largely between the different stations. D. O. and conductivity have also differences between periods (ANOVA two ways). Oxygen values are low because of the high attitude, pressure and salimity. The environment tends to be alikaline with pH in the range of 7-9. The nutrient content indicates a meso-eutrophic system. Total nitrogen reached values up to 23,700 µg L-1 at station H4 in March 2003 (minimum 300 µg L-1 at station H0, March 2003). Total phosphorus values reached a maximum of 11,000 µg L-1 in H4 in September 2003, and a minimum of 39 µg L-1 in H1 in September 2003. DGGE, clone libraries The DGGE analyses show differences in the band pattern of the 4 Salar stations in sediment and water (Fig. 2). The Ilbrary, composition is show in Fig. 3. In water samples the Cytophaga Flavobacteria Bacteroidetes group is the most representative group followed by Proteobacteria (alpha, gamma, beta and debal.) For sediments, the major group is Proteobacteria (beta, alpha, gamma and detal) and others like Verrumicrobia and Planctomycetales. The Archaea clone library is dominated by Halobacteriales. For phylogenetic tree construction we used sediment samples from stations H1 and H4 (Fig. 4A) and water samples from H1 and H2 (Fig. 4B). ISS (SIMA clone libraries from station H1 and a comparative system, Salar de Ascotán were used for archaea (Fig. 4C). Salar de Huasco is a high altitude basin located at 3,800 mosl in the Chilean salari un rhaeco à a rigin attitude durain rocces di 3,000 millosi in la crisi della di altiplano (20 05 S, 6815 W). Until now, this system was maintained under natural conditions with a high biodiversity, and was declared as a Ramsar site. Like other ecosystems of the altiplano, Huasco is subjected to high annual and seasonal variability of natural environmental factors such as dryness and rain periods. The absence of anthropogenic perturbations and the presence of different microenvironments ("bofedal", river and salt flat) allow to use this wetland as a reference for other altiplanic basins. ASLO 2005 Session: SS44 Session: SS44 Poster Number: 24 Interpretación hidrogeólógica de la génesis de los salares y lagunes del elliptiano chières. Actas II Congreso Gest. Chile 3:49-81 Hidrogeológimica de legares del Altysien, "Sepunda Región. V. Cang. Geológica Chileno, V. 2, pp. 036-043. Disalenca de lagor de altrus, preprietros de investigación. Anol. Ball. Mad. Elsc. 2015-07.

Bacterial communities in high andean wetlands:

**Appendix A-1**. Poster presented at the ASLO Summer Meeting June 19-24, 2005, Santiago de Compostela, Spain.

Acknowledgments

Gärtner and Conny Burghardt for her kindly assistance.

#### HIGH ARCHAEAL DIVERSITY IN A HIGH ALTITUDE SALINE WETLAND

Dorador, C.1,3, Witzel, K.-P.1, Vila, I.2 and Imhoff, J. F.3

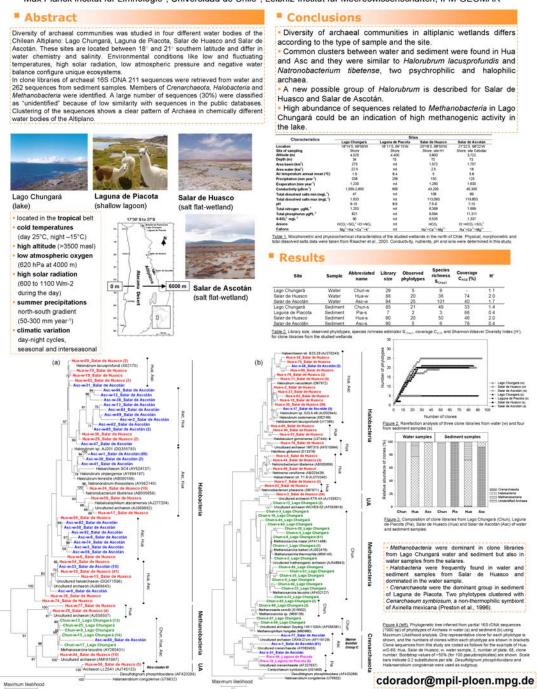
1 Max Planck Institute for Limnology, Plön, Germany. 2 Universidad de Chile, Santiago, Chile. 3 Leibniz-Institute of Marine Sciences, Kiel, Germany Microbial diversity in high altitude saline wetlands has found little attention so far, in spite of unique extreme environme Archaea are a diverse and widespread group involved in different biogeochemical cycles, e.g. those of carbon, nitrogen and suffur. Their diversity and ecological role in these systems is almost unknown. In this work the archaeal diversity in <u>sediments</u> was investigated using 165 rONA clone libraries from four sites of <u>Salar de Hussoo</u> located in <u>porthern Chile</u> at 3800 meters altitude. The studied sites have different salinity and nutrient concentrations. Methodology annual and seasonal variability of natural environmental fa dryness and rain periods. Inside the Salar different pol distinguished with different chemical composition (Table 2). to determinate actionated orientary in the sequences sour coorse sorianse were made. In total, 227 closes with the right insert were obtained and 197 sequences were retrieved. Rarefaction curves showed saturation in all libraries (Figure 2). The majority of the clones (64%) belonged to Euryarchaeota, 33% were clustered in Methanomicrobiales and 21% in sarcinales orders. 30% of the clones were not affiliated with an cultured representative of Euryarchaeota, they were "Unclassified Euryarchaeota". The phylum Cronarch represented by 10% of the clones. 6% of the clones were no memaranecionames. Ciones incin riv., n I and n 4 were cuisareca imi group. Five chiestes with only Salar de Huasco representatives (Hi. Group) were defined: ) HG-II (H0 and H1 clones), ii) HG-III (H0 clones) ii) HG-IV (H4 clones), iv) HG-V (H1 clones) and V) HG-VI (H1 clones Methanosarcinales. Clones from H0 and H1 were related to the gi Conclusions Salar de Huasco is a high altitude "salt flat" having high salt concentration, for this reason the absence of Halobacteria representatives in the libraries is remarkable.

The four studied sites showed environmental heterogeneity and this is reflected in the archaeal composition. The lower diversity index was found in H4 (anoxic and predominance of Methanomicrobiales) and the higher in H6 (76% of the clones were unaffiliated). \*12 Salar de Huasco subclusters were defined. Thei validity was probed by bootstrap analysis (Data not show). \*Most of the clones were not related with cultur representatives in the databases (GenBank, RD Sequences were affiliated only until the Order level but the najority had not clear phylogenetic affiliation.

**Appendix A-2**. Poster presented at the 11<sup>th</sup> International Symposium on Microbial Ecology ISME-11, August 20-25, 2006, Vienna, Austria.

# Archaeal diversity in contrasting high altitude wetlands in the Chilean Altiplano

<u>Dorador C</u><sup>1</sup>., K.-P. Witzel<sup>1</sup>, I. Vila<sup>2</sup> and J. F. Imhoff<sup>3</sup>
Max Planck Institut für Limnologie<sup>1</sup>, Universidad de Chile<sup>2</sup>, Leibniz-Institut für Meereswissenschaften, IFM-GEOMAR<sup>3</sup>



**Appendix** A-3. Poster presented at the Annual Conference of the Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM), April 1-4, 2007, Osnabrück, Germany.