IX Reunión de la Red de Microorganismos Extremófilos

(IX Meeting of the Spanish Scientific Network on Extremophilic Microorganisms)

Scientific Program and Abstracts

IMEDEA — CSIC — Universitat de les Illes Balears

Alcúdia, 1 & 2 October

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Welcome

Dear participants,

Last year in Grazalema, during the VIII meeting of the Network we were engaged to lead the biannual project support RedEx, as well as organize the first meeting of this biannual period. We think that we can all be very satisfied because we have succeeded in maintaining the network, as well as we are having important attendance numbers and high scientific quality contributions.

The organization has chosen the Pollentia Resort because it integrates most of the needs to hold such a meeting. The resort offers the accommodation to the attendees, as well as the entire infrastructure to support scientific sessions. Besides, the resort is flanked by the Natural Park Albufereta (a small protected wetland yearly visited by migratory birds and with a remarkable niche diversity), and the sandy beach Can Cap de Bou just across the road. The bay of Pollença is one of the most attractive places in Mallorca due to its interesting landscape and relatively calm and clean waters. Besides, the Resort is very close to the City of Alcudia, the Capital of the Island during the Roman colonization.

It is very rewarding to see that all the efforts that we have joined to obtain economical support to the maintenance of the Spanish Extremophilic Organisms' Network have finally succeeded. The current meeting could not have taken place without the invaluable economic support of the three institutions Ministerio de Educación y Ciencia, Govern de les Illes Balears, and CSIC. With this financial support we can cover most of the expenses needed during the meeting as well the invitation of the four foreign specialists.

For this meeting we have overcome in 140% the initial expectations of participation. We expect the presence of nearly 70 Spanish experts in the field, as well four international scientists pioneer in their research fields. We count with 34 talks that range from the microbial diversity and ecology to genetics and biotechnology. The quality of the abstracts presented is of excellence. We have especially encouraged the participation of the junior scientists that will be the real protagonists of the meeting. Besides, we expect that the presence of a large list of senior scientists will promote active discussions. We anticipate that this get-together will promote new research knowledge exchange as well as tighten the scientific laces already existing within the network.

It is a pleasure of the organizers to welcome you in Mallorca, and hope that the environment and scientific quality of the meeting do cover your expectations.

On behalf of the organizing committee,

Ramon Rosselló-Móra

Acknowledgements

The meeting has been sponsored by three main funding institutions. In first instance, the **Ministerio de Educación y Ciencia** has granted the network with a two year Project **(BIO2008-04954-E)** that covers two national meetings (in years 2009 and 2010) and one practical seminar on extremophilic microorganisms that had taken place at the University of Alicante in June 2009. Besides, the IX national meeting of the network of extremophilic organisms has been sponsored by the **Conselleria d'Economia, Hisenda i Innovació of the Govern de les Illes Balears** with the project **AAEE006908-08**, and the **Consejo Superior de Investigaciones Científicas (CSIC)** with the project **MP-1776-EC**. All three institutions are acknowledged by the participants of the network. This economical support has permitted not only the participation nearly 70 Spanish scientists, but also the invitation of four international experts in the fields of Diversity, Ecology, Genetics and Biotechnology of extremophilic microorganisms.

The hosting institution, **Institut Mediterrani d'Estudis Avançats (IMEDEA)** is acknowledged as it has supported the meeting by means of bureaucratic and logistic infrastructure.

Finally, the organization wants to acknowledge **Empar Rosselló-Móra** for her altruistic support to the editing of the Abstracts book and the design of the RedEx 2009 Logo.

General information

Venue:

The meeting will take place at the Pollentia Club Resort (http://www.clubpollentia.com/), which is located at the north of the island in the Pollença Bay. Between the Can Cap de Bou beach and the Albufereta Natural Park. All participants will as well be accommodated in this resort, as well as all meals and social events will take place. The Resort is located about 2 Km from the city of Alcúdia. The contact information is the following: email: info@clubpollentia.com; Telephone: +34 971 54 69 96; Fax: +34 971 54 69 92. This is a complete tourist resort that accounts with three swimming pools (one indoors, one outdoor temperate for sports, and a large one for pleasure). Besides, the hotel accounts with additional services that can be checked in their website.

Language:

After some intense discussions within the RedEx participants we decided that the official languages will be both English and Spanish. The abstracts and the projected support will be written in English. The language of the oral presentations will be chosen by each of the speakers at their own convenience.

Registration:

The registration will take place between 15:00 and 16:00 at the Hotel Reception on October 1st. However, the desk will be opened through the meeting in case that some attendees had not been registered.

Scientific sessions:

The scientific program will take place in the "Salón Pollentia" of the same hotel. Situated on the first floor, with the access on the left side of the hotel reception.

The sessions are scheduled in two different lengths. Invited speakers will give a talk of about 25 minutes with 5 minutes discussion. On the other hand, the RedEx contributions (mostly given by junior scientists) are scheduled in about 12 minutes talk and 3 minutes discussion.

Talks will be presented as power point format or alternatively can be given as PDF. In all cases it is encouraged that the projected material is written in English. Please, give the presentation, at least 15 minutes before the session starts, to one of the organizers.

Meals:

The organization covers the dinners of the nights of the 1st and 2nd of October, as well as the lunch of the 2nd of October. These will take place in the restaurant of the resort. Additional meals should be arranged with the hotel. If any participant has special needs for their meals, please communicate it to the organization so that a special menu can be arranged.

Social program:

The meeting will have a Welcome Toast just after the last talk of 1st October, and before the dinner. Besides, we plan to have a get together happening on the night of the 2nd of October.

Accommodation Agency:
The Agency Diplomatic is supporting all side-needs of the meeting. They will arrange the accommodation and other non-scientific issues. The contact person is Esperanza Escandell (esperanza@diplomatic-services.com; Tel: +34 971 22 10 04; fax: +34 971 73 85 12), and she will be available during all the meeting.

Places of interest:
The resort is located at 2 Km of the city of Alcúdia (http://www.alcudia.net/). This was the first and most important roman settlement on the island. The ruins are just in the outskirts of the city and can be visited. The city is surrounded by a medieval fortification that still conserves most of its ancient shape. Worth to visit is the Pollentia Roman Museum located close to the main church. Besides, the city has numerous restaurants and cafes of different budget classes. Most likely the weekend of the meeting you will be able to enjoy the local annual agricultural fair.

The Alcúdia harbour is located about 3 Km from the resort, with a remarkable nightlife and lots of entertainment possibilities. The beach of Alcúdia is also the largest sand-shore on the island.

The natural park of the Albufera (http://www.mallorcaweb.net/salbufera/) is located about 5 Km from the resort, and is the largest wetland in the Balearic Islands. It can be visited and information can be obtained at the interpretation centre located in the middle of the park.

Other places of interest are: city of Palma de Mallorca (about 60 Km); village of Pollença (about 7 Km); Cape Formentor (about 15 Km); Closter Lluch in the heart of the Tramuntana mountains (30 Km); El Torrent de Pareis (about 45 Km); village of Sóller (about 60 Km); Drach Caves (about 50 Km), Artà Caves (about 40 Km). More information can be found at http://www.illesbalears.es/.

Public transport:
From Palma to Alcúdia one can just take a public bus. The timetable can be checked at:
http://www.caib.es/sacmicrofront/archivopub.do?ctrl=MCRST330ZI44993&id=44993

Despite the resort is close to Alcúdia, there is a bus driving between Alcudia – Pollença. The resort has a bus stop just in front of it. The timetable can be download at:
http://www.caib.es/sacmicrofront/archivopub.do?ctrl=MCRST330ZI44994&id=44994

Taxis in Palma Airport: can be taken just outside the main exit at the arrivals terminal. The transfer costs to Alcúdia are approximately 70€

Taxis in Palma: RADIO TAXI Tel. 971 755 440 - 971 764 545. Fax. 971 298 200; TAXI PALMA RADIO Tel. 971 401 414.Fax. 971 401 010; FONO TAXI Tel. 971 200 900 - 971 728 081. Fax. 971 728 288; TAXI TELEFONO Tel. 971 743 737 - 971 744 050

Taxis in Alcudia: Taxis Alcudia 971.54.90.04; 971.54.97.66; 971.54.98.70

Scientific Program:

Thursday 1 October

15:00 – 15:30 Registration

16:00 – 16:05 Opening Session

16:05 – 16:30 Wolfgang Ludwig **"Does the conserved core of prokaryotic genomes provide sufficient phylogenetic information for natural systematics?"**

16:30 – 17:30 Session I: Diversity and Ecology (Convener, Emilio O. Casamayor)

16:30 – 16:45 Angeles Aguilera, Elena González-Toril, Virginia Souza-Egipsy and Ricardo Amils. "Eukaryotic biodiversity and structure of phototrophic biofilms in extreme acidophilic environments. The Río Tinto case"

16:45 – 17:00 Irene Sánchez- Andrea , María Méndez, Nuria Fernández, Ricardo Amils, Jose Luis Sanz. "Prokaryotic Biodiversity in Anoxic Zones of an Extreme Acidic Environment: Río Tinto"

17:00 – 17:15 Elena González-Toril, Virginia Souza-Egipsy, Olaya Rendueles, Ricardo Amils and Angeles Aguilera. "Microbial diversity of phototrophic microbial mats in an Icelandic geothermal hot spring"

17:15 – 17:30 Tomàs Llorens-Mares, JC Auguet, N Nomokonova, M Vila-Costa and EO Casamayor. "Living in the Ice: Psychrophilic Bacteria of high-altitude lakes"

17:30 – 18:00 **Coffee Break**

18:00 – 19:00 Session II: Diversity and Ecology (Convener, Emilia Quesada)

18:00 – 18:15 Rafael R. de la Haba, M. Carmen Márquez and Antonio Ventosa. "Multilocus Sequence Analysis (MLSA) of the family *Halomonadaceae*"

18:15 – 18:30 Rocío Luque, Nahid Oueriaghli, Inmaculada llamas, Fernando Martínez-Checa, Emilia Quesada and V. Béjar. "Ubiquity and diversity of the genus *Halomonas*"

18:30 – 18:45 P. Corral, M. C. Gutiérrez, A. M. Castillo, H. Minegishi and A. Ventosa. "*Natronorubrum sediminis* sp. nov., an archaeon isolated from a saline lake"

18:45 – 19:00 A.B. Fernandez, C. Sanchez-Porro, M.A. Amoozegar, H. Babavalian Fard, M. Ramezani and A. Ventosa. "*Lentibacillus persicus* sp. nov., a moderately halophilic species isolated from a saline lake in Iran"

Scientific Program:

19:00 – 19:30 Mike Dyall-Smith. **"Haloviruses: a growing field of interest"**

21:00 **Dinner**

Friday 2 October

9:00 – 11:45. **Session III: Diversity and Ecology (Conveners, Pepa Antón & Ricardo Amils)**

9:00 – 9:15 M.C. Portillo and J.M. Gonzalez. "Thermophilic bacteria can mobilize sulfur from organic matter in temperate terrestrial environments"

9:15 – 9:30 Carlotta Vizioli, Ricardo Amils and Irma Marín. "Microbial community composition of Tirez lagoon, a athalassohaline environment"

9:30 – 9:45 Jocelyn Brito-Echeverría, Marianna Lucio, Arantxa López-López, Phillipe Schmitt-Kopplin and Ramón Rosselló-Móra "Changes in the culturability and metabolome composition during adverse environmental conditions in the extremely halophilic bacterium *Salinibacter ruber*"

9:45 – 10:00 Santos F., P. Yarza, C. Briones. V. Parro and J. Anton. "The metavirome of an hypersaline environment"

10:00 – 10:15 M. Gomariz, F. Santos, J. Antón, I. Meseguer. "Spatial and temporal changes on the prokaryotic community and retinal proteins bacteriorhodpsin and xanthorhodopsin along a salinity gradient in the course of one year"

10:15 – 10:30 Carmen Mª González-Domenech, Javier Tamames, Miguel Pignatelli, Victoria Béjar, Andrés Moya, Emilia Quesada. "An overview of the ecological distribution of the genus *Halomonas* studied using EnvDB, a database that correlates prokaryotic diversity and environmental information"

10:30 – 11:00 **Coffee Break**

11:00 – 11:15 Pedrós-Alió, Carlos, Escudero L., Chong-Díaz G., Demergasso C. "Microbial life in the high-altitude, saline, water bodies in the Andes of northern Chile"

11:15 – 11:30 B.Cámara, A. De los Ríos, C. Ascaso, J.Wierzchos. "Microbial and lichen colonization of gypsum crust in the Atacama Desert"

11:30 – 11:45 Abraham Esteve-Núñez. "Breathing the unbreathable: Redox potential as a new form of extremophilia"

Scientific Program:

11:45 – 13:30 **Session IV: Genetics and Biotechnology (Convener, José Berenguer)**

11:45 – 12:00 Eloy R. Ferreras, Aurelio Hidalgo and José Berenguer. "A putative "penicillin acylase" from *Thermus thermophilus* HB27"

12:00 – 12:15 Federico Acosta y José Berenguer. "Implication of Omp85 on the secretion and insertion of the S-layer protein from *Thermus thermophilus*"

12:15 – 12:30 Laura Álvarez, Carlos Bricio, Zahra Chahlafi, y José Berenguer. "Comparative genomics of partial and complete denitrificant strains of *Thermus thermophilus*"

12:30 – 13:00. Milton Da Costa **"Compatible solutes in thermophiles and hyperthermophiles. From the esoteric to the applied"**

14:00 **Lunch**

16:00 – 17:45 **Session V: Genetics and Biotechnology (Convener José Nieto)**

16:00 – 16:15 García-Yoldi, A., Argandoña, M., Nieto, J.J., Vargas, C. "Curated annotation and in silico reconstruction of the central metabolism of *Chromohalobacter salexigens* DSM 3043"

16:15 – 16:30 Salvador-De Lara, M., Argandoña, M., Iglesias-Guerra, F., Nieto, J.J. and Vargas, C. "Involvement of the global regulators *rpoS* and *rpoD* in the synthesis of the bioestabilizers ectoine and hydroxyectoine in *Chromohalobacter salexigens*"

16:300 – 16:45 Rodríguez-Moya, J., Argandoña, M., Iglesias-Guerra, F., Vargas, C. and Nieto, J.J. "Effect of knocking out the uptake of the ectoine and hydroxyectoine and co-expression of the synthesis genes to enhance hydroxyectoine production by *C. salexigens*"

16:45 – 17:00 M. Reina-Bueno, M. Argandoña, F. Iglesias-Guerra, J. J. Nieto, C. Vargas. "Role of trehalose in the response to hypersaline, dehydration and heat stress in halophilic and drought-tolerant bacteria"

17:00 – 17:15 Laia Pedro Roig, Mónica Camacho Carrasco, Vanesa Bautista Saiz, Julia Esclapez y Mª José Bonete Pérez. "Gene analysis and heterologous expression of PII proteins from the haloarchaea *Haloferax mediterranei*"

17:15 – 17:45 **Coffee Break**

17:45 – 19:00 **Sesión VI: Genetics and Biotechnology (Convener, Maria José Bonete)**

17:45 – 18:00 M.L Moreno, M.T. García, A. Ventosa y E. Mellado. "Characterization and cloning of the protease Salipro produced by the extremely halophilic bacterium *Salicola marasensis* IC10"

18:00 – 18:15 Domencech, J. Baker, P. Rice, D.W. and Ferrer, J. "Determination and analysis of the three-dimensional structure of a new D-2-hydroxyacid dehydrogenase NAD(P) H-dependent from *Haloferax mediterranei*"

18:15 – 18:30 Inmaculada llamas, Diana Carranza, M. Carmen Ruiz-Ruiz, Ignacio J. Molina y Emilia Quesada. "B100S, a highly sulphated exopolysaccharide produced in its native state by the bacterium *Halomonas maura*, causes the death of hematopoietic tumour cells by apoptosis via the mitochondrial pathway"

18:30 – 18:45 Basilio Zafrilla, María José Bonete, Rosa María Martínez-Espinosa, Gloria Bravo-Barrales. "Homologous expression: A new and simple alternative for halophilic enzymes production"

18:45 – 19:00 D. Pérez1, E. Mellado1, A. Ventosa1, G. Fernández-Lorente2, C. Mateo2, M. Filice2 y J. M. Guisán. "Purification and characterization of LipBL, a lipase with high homology to the class C beta-lactamases"

19:00 – 19:15 C. Pire, L. Mellini, L. Pedro-Roig, F. Pérez -Pomares and M.J. Bonete. "Transcript levels of glutamine synthetase and glutamate synthase genes in response to the nitrogen source in *Haloferax mediterranei*"

19:15 – 19:45 Gerard Muyzer and Dimitry Yu. Sorokin **"Ecology and biotechnology of halo-alkaliphilic sulfur bacteria "**

19:45 – 20:00 **Closing session**

21:00 **Farewell dinner**

ABSTRACTS
(Invited Speakers)

Does the conserved core of prokaryotic genomes provide sufficient phylogenetic information for natural systematics?

Wolfgang Ludwig

Dept. of Microbiology, Technische Universität München, Am Hochanger 4, D-85354 Freising, Germany

Nowadays, comparative rRNA sequence analysis certainly represents the most commonly applied method for elucidating phylogenetic relationships supporting microbial taxonomy and identification. The majority of higher taxa - above the species level - have been created based on rRNA derived data. However, there is a continuous debate concerning the justification and power of a single marker molecule for elucidating and establishing the phylogeny and taxonomy of organisms, respectively. The progress in genome sequencing helps evaluating rRNA based phylogenetic conclusions to some extent. The available genome databases allowed defining a small set of genes representing the conserved core of prokaryotic genomes. Genes encoding rRNA polymerases, translation initiation, elongation and release factors, proton translocating ATPases, heat shock proteins, recA, and few others are used as non-rRNA phylogentic markers. Even in the age of genomics, the datasets for these alternative markers are poor in comparison to approximately 1 million rRNA primary structures available in general and special databases. Comparative phylogenetic analyses are complicated by the occurrence of duplicates (paralogs) in many cases of non-rRNA markers. Careful data analyses showed global support of the small subunit rRNA derived view of prokaryotic evolution. Although the tree topologies reconstructed from alternative markers differ in detail, the major groups and – in many cases - taxa are verified or at least not disproved.

Haloviruses: a growing field of interest

Mike Dyall-Smith
Department of Membrane Biochemistry, Max Planck Institute of Biochemistry, Martinsried 82152, München, Germany.

Hypersaline waters, like the oceans, contain high numbers of viruses but very few have been isolated or studied. Of those that infect haloarchaea, there are three major morphological groups: spindle-shaped, round, and head-tail. My laboratory has been studying representatives of all three morphotypes, and I will summarise our work, including genome structure, relationships between the known haloviruses, virus promoters and transcription programs. The representatives haloviruses are outlined below:

1) His1 and His2 are representatives of the spindle-shaped morphological group (Salterprovirus), and have linear dsDNA genomes that are around 15 kb in length. They have been sequenced, and except for their DNA polymerase genes, show no significant homology in their other genes. Both use a protein-primed replicase. Very recently, the Bamford group (Finland) have isolated a ssDNA halovirus that shows a close relationship to His2.

2) SH1 and PH1 are representatives of the round virus morphology, and are closely related. Their genomes are linear dsDNA and around 30 kb in length and, like the salterproviruses, use protein-primed replication. Strangely, the DNA polymerase is not encoded by the virus.

3) HF1 and HF2 are very closely related haloviruses that have a head-tail morphology, similar to bacteriophages. They have relatively large genomes, around 77kb of linear dsDNA. Over 100 ORFs are annotated but very few have identifiable functions. Replication is via concatemer formation and precise cutting, similar to the mechanism seen in phage T3.

The transcription programs of members of all three groups have been investigated, and all seem to produce very long transcripts, often on both strands (ie. Transcripts and counter-transcripts). It seems likely that these would interact to produce dsRNA molecules.

We have also begun to develop genetic systems to enable the analysis of gene function in haloviruses. Using transfection and transposon mutagenesis we have shown that infectious virus can be recovered that contain single transposon insertions, offering the possibility to knock-out specific genes and test their effects.

Compatible solutes in thermophiles and hyperthermophiles. From the esoteric to the applied.

Milton S. da Costa

Departamento de Bioquímica and Centro de Neurociências e Biologia Celular, Universidade de Coimbra, 3001-401 Coimbra, Portugal. Email: milton@ci.uc.pt, numenius@cnc.uc.pt

Many organisms that live at very high temperatures have been isolated from shallow marine and abyssal thermal environments, where the geothermal water may reach the salinity of the surrounding seawater. These organisms, like all other microorganisms must adjust, within intrinsic limits, to alterations in the water activity of the environments. The majority of microorganisms adjust to osmotic stress by the selective accumulation of small molecular weight organic compounds. Thermophiles and hyperthermophiles accumulate a few compatible solutes that are also common in mesophilic bacteria and archaea, namely trehalose and glutamate and even the very rare glucosylglycerate (GG). However, the majority of the compatible solutes encountered in hyper/thermophiles are unique to these organisms. These compatible solutes include mannosylglycerate, di-myo-inositol-phosphate and the very rare compatible solutes di-glycerol-phosphate, di-mannosyl-di-myo-inositol-phosphate and mannosylglyceramide.

In recent years we have studied the synthesis of mannosylglycerate (MG) and trehalose in *Thermus thermophilus* (Phylum *Deinococcus*/*Thermus*), *Rubrobacter xylanophilus* (Phylum *Actinobacteria*) and *Persephonella marina* (Order *Aquificales*). The species of the genus *Thermus* have optimum growth temperatures that range between 70 and 75ºC and, with the exception of *Thermus thermophilus*, a maximum growth temperature below 80ºC. The species *Thermus thermophilus* has a maximum growth temperature of about 82 to 83ºC. This species is also capable of growing in media containing 3 to 5% NaCl. The strains of *T. thermophilus* accumulate primarily trehalose and lower levels of mannosylglycerate (MG) during osmotic adjustment. Recombinant mutants lacking the genes for the synthesis of trehalose, MG or both, result in a profound effect on the ability of organisms to grow in media containing NaCl. The synthesis of MG by *T. thermophilus* proceeds via a two step pathway catalyzed by mannosyl-phosphoglycerate synthase (MpgS) and mannosyl-phosphoglycerate phosphatase (MpgP) from GDP-mannose and 3-phosphoglycerate. These enzymes are very similar to those found in other hyper/thermophilic organisms, however the MpgS and the MpgP from *R. xylanophilus* have little or no identity to the previous enzymes. The homologous enzymes of *R. xylanophilus* lead, depending of the substrate, to the synthesis of MG or GG. The thermophilic bacterium *P. marina*, on the other hand, is the only known thermophile to accumulate GG in response to salt stress and possesses genes that are also very different from the ones mentioned above. Moreover, this organism possesses two pathways for the synthesis of GG. The physiological relevance of MG and GG accumulation in these thermophilic bacteria and the evolution of MG and GG biosynthesis in prokaryotes are discussed. These studies led us to encounter a gene in the species of *Mycobacterium* that leads to the synthesis of GG that is bound to a polysaccharide and does not serve as a compatible solute. We are now continuing these studies in mycobacteria and have initiated several other studies in an attempt to understand the regulation of the synthesis of MG in T. thermophilus and the characterization of the synthesis and degradation of mannosyl-glucosylglycerate (MGG) in members of the Planctomyces.

Ecology and biotechnology of halo-alkaliphilic sulfur bacteria

Gerard Muyzer[1] and Dimitry Yu. Sorokin[1,2]

[1]Dept. of Biotechnology, Delft University of Technology, Delft, The Netherlands, [2]Winogradsky Institute of Microbiology RAS, Moscow, Russia

Soda lakes are the only habitats on Earth with extreme soluble carbonate alkalinity maintaining a stable pH between 9.5 and 11. Most of these lakes are located in arid areas of Central Asia, Eastern Africa, and the Northwestern part of the USA. In general, soda lakes harbor an enormous diversity of microorganisms, which have attracted considerable attention, because of the possible biotechnological use of their enzymes, such as extracellular hydrolases in laundry detergents. Another group of bacteria from soda lakes that draw industrial attention are the chemolithoautotrophic sulfide-oxidizing bacteria (SOB) of the genus *Thioalkalivibrio*, which are excellently suited for the removal of noxious sulfur compounds from waste streams and energy carriers. Today more than 100 different *Thioalkalivibrio* strains have been isolated, mostly from sediments of hypersaline soda lakes. Recently, the genomes of two *Thioalkalivibrio* strains were sequenced by the Joint Genome Institute (JGI) of the Department of Energy (DOE) in the USA. *Thioalkalivibrio* sp. HL-EbGR7 is a moderately salt-tolerant alkaliphile, which was isolated from a full-scale sulfide-removing bioreactor. The other strain, *Thioalkalivibrio* sp. K90mix, is an extreme Na/K carbonate-tolerant alkaliphile isolated from a mixture of soda lake sediments. Here I will discuss the ecology of halo-alkaliphilic sulfur bacteria, and their application in the removal of noxious sulfur compounds from waste streams and energy carriers. In addition, the first results of comparative genomics will be presented.

ABSTRACTS
(RedEx Contributions)

Eukaryotic biodiversity and structure of phototrophic biofilms in extreme acidophilic environments. The Río Tinto case.

Angeles Aguilera[A], Elena González-Toril[A], Virginia Souza-Egipsy[A] and Ricardo Amils[A,B]

(a) Centro de Astrobiología (INTA-CSIC). Carretera de Ajalvir Km 4, Torrejón de Ardoz. 28850 Madrid. Spain.

(b) Centro de Biología Molecular (UAM-CSIC). Universidad Autónoma de Madrid. Canto Blanco. 28049 Madrid.

Río Tinto (SW, Spain) is one of the largest and most extreme acidic environments studied to date. The river has a constant low pH (mean 2.3), buffered by ferric iron and with high concentrations of dissolved heavy metals, reaching 20 g l^{-1} of Fe. However, what makes Río Tinto a unique extreme environment is that eukaryotic organisms are the principal contributors of biomass, over 65% of the total biomass is due to a remarkable degree of eukaryotic diversity. Most of these communities are distributed in extensive biofilms along the riverbed. The macroscopic shape and species composition of the biofilms vary greatly throughout the river. Some of them adopt filamentous morphologies in flowing water while others form thick colourful patches firmly attached to the mineral substrates. We present here a description of the eukaryotic biodiversity, biofilm development and structural organization of several phototrophic biofilms monitored monthly over a year. Fourteen taxa were recognized as constituents of the eukaryotic assemblages. The lowest diversity was found at the most extreme locations, in terms of pH and heavy metal concentrations. The biofilms were mainly formed by species from the genera *Dunaliella* and *Cyanidium*. Two genera of filamentous algae, *Zygnemopsis* and *Klebsormidium,* were principally responsible for the variability in the cell number throughout the year. The microcolonization sequence showed an initial accumulation of amorphous particles composed of bacteria and inorganic grains of minerals. By the end of the second month the organic matrix was also populated by fungi, bacteria and few eukaryotic heterotrophs such amoebae and small flagellates. Diatoms only showed significant colonization in those regions where mycelial matrices were first established. Flagellated green algae like *Dunaliella* or *Chlamydomonas* as well as *Euglena* were also present at the very beginning of the biofilm development. After the flagellated cells, sessile species of algae such *Chlorella* or *Cyanidium* appeared. Filamentous algae were the last species to colonize the biofilms. Most of the biofilms were structures composed of different species organized in different layers separated by extracellular polymeric substances. The possible implications of the biofilm structure in the adaptation to this extreme habitat will be discussed.

Prokaryotic Biodiversity in Anoxic Zones of an Extreme Acidic Environment: Río Tinto

Irene Sánchez- Andrea , María Méndez, Nuria Fernández, Ricardo Amils, Jose Luis Sanz.

Molecular Microbiology Department, Autonomous University of Madrid, Spain

Río Tinto is an extreme unusual ecosystem due to its size (100-km long), constant acidic pH (mean pH 2.3), high concentration of heavy metals (Fe, Cu, Zn, As, Mn and Cr) and microbial diversity. The combined use of conventional and molecular microbial ecology methods has led to the identification of the most representative microorganisms of the Río Tinto basin. Eighty percent of the diversity of the water column corresponds to only three bacterial genera: *Leptospirillum*, *Acidithiobacillus*, and *Acidiphilium;* although other iron-oxidizing (*Ferroplasma* spp. and *Thermoplasma acidophilum)* or iron–reducing ("*Ferromicrobium*" spp.) bacteria have been also detected (González-Toril et al., *Appl Environ Microbiol* 69:4853-4865, 2003). An unexpected degree of eukaryotic diversity has been found in its waters (Aguilera et al., *Appl Environ Microbiol* 72: 5325-5330, 2006).

In contrast with the microbiota inhabited the water column, there is limited knowledge on the microbial ecology of the sediments and the anaerobic zones of the river. The goal of this study is to go deep in it. Two sampling sites, where deep sediment accumulation takes place, were selected. Cores of 7ID cm and 45 cm long were taken with a liner sampler. Protocols, based on the amplification and molecular cloning of metagenomic DNA extracted from the powdered samples, have been developed for microbial analyzing of the sediments. Results show that amplifiable and clonable DNA could be extracted from the sediments making some modifications to the standard protocols of extraction, leading to the detection of iron-oxidizing and iron–reducing, sulfur-oxidizing, sulphate-reducing, nitrate-reducing microorganisms, and others bacteria implicated in the anaerobic degradation of the organic matter, like H_2 producers. A geomicrobiological model of the different cycles operating in the system has been developed.

Microbial diversity of phototrophic microbial mats in an Icelandic geothermal hot spring

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The microbial community composition and the three-dimensional structure of diverse phototrophic microbial mats from a hot spring in Iceland (Seltun geothermal areas) were explored by 16S and 18S rRNA gene cloning and sequencing and comparing microbiotic assemblages of mats. Sample mats were collected in July 2007 from 15 sampling stations across thermal, chemical and pH gradients following this hot spring. Physicochemical data revealed high variability in terms of pH (ranged from 2.8 to 7) with high concentrations of heavy metals including up to 20g l^{-1} Fe, 80 mg l^{-1} Zn,117 mg l^{-1} Cu, and 39 mg l^{-1} Ni in the most acidic sampling points. Phylogenetic analysis of 16S and 18S rDNA genes revealed a diversity of sequences related to several taxa including typical members of Bacteria and Archaea domain typical in hot spring microbial ecosystems (*Chloroflexi*, *Thermus*, *Synechococcus*) as well as acidophilic bacteria (*Ferrimicrobium* spp., *Acidiphilium*, *Acidobacteria*) or sulphur cycle bacteria (*Acidithiobacillus* and *Thiomonas*). 18S rDNA genes revealed a diversity of sequences related to several taxa including members of the *Bacillariophyta*, *Chlorophyta*, *Rhodophyta* and *Euglenophyta* phyla as well as ciliates, amoebae or stramenopiles. The closest relative to some of the eukaryotic sequences detected came from acidophilic organisms, even when the samples were collected at circumneutral water locations. This diversity distribution is perfectly correlated with the physicochemical gradient present along the spring. The electron microscopy analysis showed that most of the microecosystems analyzed were organized as phototrophic microbial mats in which filamentous cyanobacteria usually appear as a major component. Deposition of amorphous minerals rich in silica, iron and aluminium around the filaments was frequently found. Taking into consideration the characteristics of this extreme environment, the physiological gradient and spatial distribution of the identified microorganisms, a model for its geomicrobiological evolution is advanced.

Living in the Ice: Psychrophilic Bacteria of high-altitude lakes

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High altitude lakes are covered by ice and snow for several months of the year, and a rich and very active community of psychrophilic microorganisms naturally develops in the slush layers. These slush layers contain a mixture of snow and water at temperatures near 0 Celsius degrees. Late in fall an ice sheet forms on the top of mountain lakes and the first snows accumulated. Next in winter and spring, surface lake water floods the cover as a result of the hydrostatic adjustment induced by the progressive accumulation of snow on top of the ice sheet. The mixing of snow and water gives rise to slush layers in successive episodes converting the cover into a complex vertical stratigraphical structure. In spite of the low temperature and the seasonal occurrence of the habitat, microbial biomass and activity are usually greater in the slush layers than in the lake water. Here, we present a preliminary study, carried out in the high mountain Lake Redon (2,240 m altitude in the Pyrenees) which describes the presence and diversity of abundant and active bacterial and archaeal communities in the slush layers of Lake Redon as compared with the assemblages inhabiting the water column, and an ice-melt pond by cloning and sequencing the 16S rRNA gene and FISH analysis. Up to 20% of total "bacterial" cells in the slush hybridized with the Archaea probe ARC915 and were mostly Crenarchaeota. Most of the Bacteria in the slush were Beta-Proteobacteria and Sphingobacteria (Bacteroidetes) and a few Actinobacteria, closely related to other clones found worldwide. These cold-adapted microorganisms showed permanent growth and offer exciting biotechnological perspectives as a valuable source of enzymes for processes operated at low temperatures, and to study how biomolecules remain active near 0°C. For the microbial ecologist, the origin and the fate of these populations, and the active carbon cycle developed in these layers are questions of great interest.

Multilocus Sequence Analysis (MLSA) of the family *Halomonadaceae*

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The family *Halomonadaceae*, belonging to the subclass *Gammaproteobacteria*, was described in 1988 by Franzmann *et al.* Nowadays, this family includes nine validly published genera names: *Halomonas* (57 species), *Chromohalobacter* (9 species), *Kushneria* (4 species), *Carnimonas*, *Cobetia*, *Halotalea*, *Modicisalibacter*, *Salinicola* and *Zymobacter* (each containing one single species). The heterogenicity of this family is well-known, grouping halophilic, halotolerant and non-halophilic bacteria.

The currently taxonomic technique based on 16S rRNA gene sequence analysis does not permit to distinguish between closely related species because of the high level of sequence conservation. Therefore, in order to overcome these disadvantages and to deeply know the evolutionary history of this family, alternative phylogenetic markers (housekeeping genes) are been implemented.

In this study, some low quality 16S rRNA gene sequences of members of this family were resequenced as recommended by the minimal standards for *Halomonadaceae* (Arahal *et al.*, 2007). Besides, the complete 23S rRNA gene sequence and partial *gyrB* (β-subunit of DNA gyrase) and *rpoD* (σ^{70} factor of RNA polymerase) gene sequences were obtained. Based on these individual and concatenated data, phylogenies were inferred and trees were constructed by using different algorithm methods.

Results showed that housekeeping genes sequence phylogenies are not always in agreement with 16S rRNA gene sequence phylogeny. Trees based on 23S and 16S rRNA gene sequences are quite similar, but 23S rRNA gene showed more clearly the phylogenetic relationships than 16S rRNA gene. However, similarly to 16S rRNA, 23S rRNA does not always let us distinguish between closely related species of the family *Halomonadaceae*. Evolutionary rate of *gyrB* and *rpoD* genes is faster than 16S/23S rRNA genes and, therefore, their resolution power is higher. On the other hand, phylogenetic history of all these genes reveals an important number of horizontal gene transfers between species sharing the same habitat.

At present, the sequence analysis of other housekeeping genes is under investigation.

References:

Franzmann, P. D., Wehmeyer, U., and Stackebrandt, E. (1988). *Halomonadaceae* fam. nov., a new family of the class *Proteobacteria* to accommodate the genera *Halomonas* and *Deleya*. *Syst Appl Microbiol* 11, 16–19.

Arahal, D. R., Vreeland, R. H., Litchfield, C. D., Mormile, M. R., Tindall, B. J., Oren, A., Bejar, V., Quesada, E. y Ventosa, A. (2007). *Recommended minimal standards for describing new taxa of the family* Halomonadaceae. Int J Syst Evol Microbiol *57, 2436–2446.*

Ubiquity and diversity of the genus *Halomonas*

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The genus *Halomonas* belongs to the class *Gamma-Proteobacteria* and includes more than 60 species of halophilic and halotolerant bacteria, some of which have proved to be of great interest for biotechnological purposes. *Halomomas* species have been isolated from a wide range of environments, both saline and non-saline, but despite our observations *in vitro* we are still not sure about the way they behave and relate to other organisms in their natural environment.

At the moment we are analysing some twenty saline and non-saline environments in southern and eastern Spain, northern Morocco and the "el Salar de Atacama" in Chile. Our results so far indicate that *Halomonas* species represent a high percentage of those species isolated at random from the culture media that we normally use in our laboratory to isolate mesophilic, aerobic, halophilic bacteria with heterotrophic metabolisms. Furthermore, species of this genus seem to be ubiquitous and highly diverse.

Despite this we do not believe that *Halomonas* is one of the most abundant bacteria in nature. Using DGGE and primers specific to the genus *Halomonas* we detected it in most of the environments studies, but when using primers universal to the *Bacteria* domain we found few *Halomonas* species in any of our samples.

Natronorubrum sediminis sp. nov., an archaeon isolated from a saline lake

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The genus *Natronorubrum* includes a group of haloalkaliphilic archaea, which stain as Gram-negative, non-motile, showing pleomorphic, triangular, discs, square and polygonal shapes when grow under favorable conditions. This genus comprises four validly published species names: *Natronorubrum bangense* (type species), *Natronorubrum aibiense, Natronorubrum sulfidifaciens* and *Natronorubrum tibetense*.

In this study we have isolated two novel haloalkaliphilic archaeal strains, designated CG-6 and CG-4, from the sediment of the salt lake Chagannor in Inner Mongolia, China. These strains were pleomorphic, strictly aerobic and non-motile. They required at least 2.5 M NaCl for growth, with an optimal concentration of 3.4 M NaCl, grew in a pH range of 8.0 to 11.0 with an optimum pH of 9.0. Hypotonic conditions with less than 1.5 M NaCl produced lysis of the cells.

The analysis of lipids indicates that both strains have a similar composition of polar lipids, $C_{20}C_{20}$ and $C_{20}C_{25}$ derivatives of phosphatidylglycerol phosphate and phosphatidylglycerol phosphate methyl ester. No glycolipids were detected. Comparison of 16S rRNA sequences and phenotypic features placed them in the genus *Natronorubrum*.The percentages of similarity between the sequences of 16S rRNA and species of the genus *Natronorubrum* were 96.2 to 93.8%. Studies of DNA-DNA hybridization between the two strains revealed that they represent a new species of the genus *Natronorubrum* for which it is proposed the name *Natronorubrum sediminis* sp. nov.

Lentibacillus persicus sp. nov., a moderately halophilic species isolated from a saline lake in Iran

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The landscape of Iran is dominated by rugged mountains, dense rain forests, deserts as well as some salt lakes. Up to date, the microbial diversity of these salt lakes has not been studied in detail. In 2008 extensive screenings were carried out in several lakes of Iran. The lakes Urmia, Aran-Bidgol, Howz-Soltan, Bakhtegan and Maharloo were selected for these studies. Water and sediment samples were collected and inoculated on different culture media. Many isolates were obtained and were subjected to a polyphasic taxonomic study. Some selected isolates were studied in more detail. In fact, those with a 16S rRNA similarity lower than 97% with the type strain of species validly published, were selected and subjected to an exhaustive phenotypic, genotypic, phylogenetic and chemotaxonomic analysis.

A Gram-positive, moderately halophilic bacterium, designated strain Amb31, isolated from water of the hypersaline lake Aran-Bidgol was taxonomically characterized using a polyphasic approach. Cells were rod-shaped, motile and able to produce ellipsoidal endospores at a central position in swollen sporangia. It was facultatively anaerobic, catalase and oxidase positive. Amb31 grew in a complex medium supplemented with 3-25% (w/v) NaCl, with the optimum at 7.5-10% (w/v) NaCl. The optimal growth was at 30-35ºC and pH 7.5. Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed that strain Amb31 belonged to the genus *Lentibacillus*; it exhibited 16S rRNA gene sequence similarity values of 96.8, and 96.4% to the type strain of *Lentibacillus salicampi* and *Lentibacillus salinarum*, respectively and values of 95.9-94.7% to the type strains of other recognized species of *Lentibacillus*. Strain Amb31 had cell wall peptidoglycan based on *meso*-diaminopimelic acid and MK-7 as the respiratory isoprenoid quinone. The major fatty acids were anteiso-$C_{15:0}$ (44.7 %), iso-$C_{16:0}$ (21.4 %) and anteiso-$C_{17:0}$ (15.9 %) and its polar lipid pattern consisted of phosphatidylglycerol, diphosphatidylglycerol, five phospholipids and a glycolipid. The DNA G+C content was 44.1 mol%. All those features confirmed the placement of isolate Amb31 within the genus *Lentibacillus*; it could be clearly differentiated from other species of *Lentibacillus* on the basis of several phenotypic, genotypic and chemotaxonomic features. The DNA-DNA hybridization with the most closely related species, *Lentibacillus salicampi* DSM 16425 was 28%. Therefore, strain Amb31 represents a novel species of the genus *Lentibacillus*, for which the name *Lentibacilus persicus* sp. nov. is proposed.

Thermophilic bacteria can mobilize sulfur from organic matter in temperate terrestrial environments

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Microbial diversity is represented by a huge number of different microorganisms in most natural environments although the ecological function of each of them remains to be understood (Curtis et al. 2002). The presence of thermophilic bacteria in temperate environments has been previously reported (Ghani et al. 1993). Herein, we report on a potential ecological role of a group of thermophilic bacteria commonly present in temperate terrestrial environments. These bacteria belong mainly to the Firmicutes and grow above 45ºC up to 75ºC, and are able to produce sulfate during heterotrophic growth on S-containing organic compounds (i.e., proteins). These bacteria are shown to be active during high temperature periods as judged by their detection through RNA-based molecular techniques in natural samples. Sulfur in soils is mostly found as organic compounds and the mobilization of organic sulfur as sulfate at low temperature has been reported to be poor (Ghani et al. 1993). Thus, thermophilic bacteria could represent an important link within the sulfur cycle suggesting the seasonality of this process which can be increased during current climate warming. This is an example of thermophilic bacterial processes occurring in terrestrial temperate environments.

References.

Curtis et al. 2002. Proc Nat Acad Sci USA 99: 10494-10499.
Ghani et al. 1993. Soil Biol Biochem 25: 1739-1744.

Marchant et al. 2002. Environ Microbiol 4: 595-602.

Microbial community composition of Tirez lagoon, a athalassohaline environment

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The hypersaline lagoon of Tirez is located in the southern subplateau of central Spain's La Mancha region and is one of several endorheic hypersaline lakes originated under semiarid conditions in the Iberian Peninsula. Hydrogeochemical and mineralogical analyses show that Tírez waters corresponded to Mg-Na-SO_4-Cl brines with epsomite, hexahydrite, and halite as end members. Tirez is indeed proposed as a terrestrial analogue of Europa's ocean. The proposal is based on the comparison of the hydrogeochemistry of Tírez lagoon with the geochemical features of the alteration mineralogy of meteoritic precursors and with Galileo's Near Infrared Mapping Spectrometer data on Europa's surface. The study of biodiversity in halophilic environments is restricted by the lack of adequate methodologies. Molecular ecology techniques have produced a revolution in microbial ecology and in this study it has been combined culture-dependent and indipendent techniques such as microscopy, cloning, Denaturing Gradient Gel Electrophoresis (DGGE) to study the microbial ecology of this sulfated athalasohaline environment. Algae (Dunaliella) and Cyanobacteria (*Nodularia, Microcoleus, Anabaena, Nostoc, Leptolyngbya* and *Pseudoanabaena*) were the main photosynthetic primary producers detected in the ecosystem all of them are frequently found in hypersaline systems. These microorganisms play an important role in the first steps of mineral global cycles. In some of the low ionic samples the rotifer *Hexartra* sp. and the artropod *Artemia* sp. were identified Also dinoflagelates and filamentous fungi have been identified in the water column. The highest phylotype abundance corresponded to members of the bacterial phylum *Proteobacteria*, mainly to *Gamma-* and *Alphaproteobacteria* classes. Gram positive *Firmicutes* and *Actinobacteria* have been isolated and identified in Tirez samples. The comparison with other athalassohaline environments allowed us to identify ubiquitous microorganisms (*Haloarchaea*, *Bacteroidetes*, *Delta-proteobacteria*, *Firmicutes* and *Actinobacteria*).

Changes in the culturability and metabolome composition during adverse environmental conditions in the extremely halophilic bacterium *Salinibacter ruber*.

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A combination of conventional culture methods and a metabolomic approach has been applied to the study the main changes in the viability, culturability and metabolome composition during the transition from exponential growth to stationary phase, and the response to different stress conditions of two strains of the extremely halophilic bacterium *Salinibacter ruber* (M8 and M31) isolated in Majorca.

We have determined how the transition from exponential to stationary growth, and to different stress treatments, influence the metabolome composition. We studied the viability and culturability of the cells by colony forming units (CFU) counts in SW 25% agar plates, and by fluorescence microscopy. The metabolomic composition was analyzed by high-field ion cyclotron Fourier transform mass spectrometry (ICR-FT/MS).

During the maximum exponential growth, only around 40-50% of total DAPI-stained cells could be cultured in agar plates. However, about 98% of the DAPI-stained cells could be regarded as potentially active as revealed by FISH. During stationary phase the capacity of forming colonies decreased to 9% indicating a strong loss of culturability. A similar trend occurred already after 2 hours under all stress conditions assayed. However, we could always find a significant fraction of non-cultivable but FISH-detectable cells under stationary growth and stress conditions meaning that those cells might remain potentially active, and thus retain their viability. In the same way, the metabolome composition of M8 and M31 strains during stationary phase and stress conditions revealed changes that could explain the metabolism modifications. Both strains showed common mechanisms in adapting to adverse environmental conditions. However, despite their strong similarity, some strain-specific differences could also be detected.

The metavirome of an hypersaline environment

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Hypersaline environments show one of the highest concentrations of viruses reported for aquatic systems. All the halophages characterized so far are isolates obtained by cultivation from described haloarchaeal species that have low ecological relevance in the environment. In our work, we applied a multiphasic culture-independent approach to characterize the viral community in a crystallizer pond. The study included TEM, SYBR-green staining, PFGE and metagenomic analyses. TEM analysis revealed several morphologies of viruses in the hypersaline water while the analysis of viral assemblages with PFGE showed a dominant band of viral DNA around 37 kb. The extraction and cloning of this viral DNA in fosmids allowed the characterization of the nearly complete genomic sequence of the environmental halophage 1 (EHP-1), and, lately, the construction of new metaviromic libraries, both in fosmids and plasmids (shot-gun library).

In the shot-gun library, 325 kb of DNA (650 clones) were sequenced. Five hundred and forty one open reading frames were predicted in 272 contigs, with an average of 1.67 ORFs/kb. The mean value for the G+C content was 52.5%. Bioinformatic analyses revealed a great amount of hypothetical proteins (75.6%). Ten ORFs were related to regulatory proteins and 20 predicted peptides were related to DNA replication. Nucleases, terminases and methyltransferases were also found. Interestingly, only 5 ORFs encoded integrases. The rest of the ORFs were included in groups of structural phage proteins, methyltransferases, terminases, and hydrolytic or metabolic enzymes. In many cases, the closest relatives from some predicted proteins were proteins belonging to halophilic prokaryotes or viruses. The mean of every predicted isoelectric point was 5.21, an acidic value characteristic of halophilic proteins. Single nucleotide polimorphisms (SNPs) were also analyzed, showing that mutation frequency in the community of halophages could be even higher than that for RNA viruses. ORFs found in the shot-gun library were also observed in the fosmid library, in which 23 clones were end-sequenced (25 kb of DNA) and compared by restriction pattern analysis. The analyses of this halophilic metavirome show a high genetic diversity that seems mainly composed by lytic phages.

All the contigs were also analyzed according to their dinucleotide frequency and sorted in groups of different GC content, showing a good correlation between both parameters. Clustering analysis indicates the presence of nine different groups of viruses in the saltern and their distribution showes groups that would be infecting the two most abundant putative hosts in the crystallizer: *Haloquadratum walsbyi* and *Salinibacter ruber*.

Spatial and temporal changes on the prokaryotic community and retinal proteins bacteriorhodpsin and xanthorhodopsin along a salinity gradient in the course of one year.

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The aim of this work is to study the diversity of the retinal proteins bacteriorhodopsin (BR) and xanthorhodopsin (XR) in different ponds from Bras del Port solar salterns (Santa Pola) and correlate changes of these retinal proteins with microbial diversity and enviromental parameters.

Solar salterns consist of a series of ponds connected along a salinity gradient from seawater to saturation. In this study we chose five ponds of medium (ponds CM1 and CM2), high (CCAB) and extremely high salinity (C30 and C41). Each pond was sampled 9 times along the year. Thus, we harvested a total a 45 samples that were analyzed by denaturing gradient gel electrophoresis (DGGE) using different primer sets specific for *Archaea* and *Bacteria* 16S rRNA genes and for BR and XR coding genes. In parallel, a detailed chemical analysis of water samples and monitoring of environmental parameters were also carried out.

We observed that were fluctuations of differents phylotypes of *Archaea* and *Bacteria* in each saltern pond along the year. This temporal variation of the community diversity was higher in the ponds with medium and high salinity (CM2 and CCAB). In addition we observed considerable differences in the communities inhabiting the two very high salt ponds (C30 and C41) as well as different temporal variation patterns.

Regarding BRs, the strongest temporal variation was observed in the ponds of medium salinity, as happened with the archaeal community. BRs detected in C30 and C41 were different, in good agreement with the differences found in their microbial communities. On the other hand, XRs showed very low diversity and a very homogeneous distribution along the year.

Our results indicate that solar salterns harbour a microbial community more diverse and dynamic than expected. This community and their retinal proteins is clearly influenced by environmental parameters such as salinity, temperature, pH, solar radiation, rainfalls, as well as the operational cycle of the salterns.

An overview of the ecological distribution of the genus *Halomonas* studied using EnvDB, a database that correlates prokaryotic diversity and environmental information.

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Halomonas species are widely distributed throughout diverse environments, such as the sea, hypersaline sediments and soils, preserved foodstuffs, sewage farms and those contaminated with crude-oil products. In marine and hypersaline environments they represent a high percentage of the strains isolated or detected molecularly. Nevertheless, our knowledge about the global distribution of this genus or even about the existence of any biogeographic pattern is still to be explored.

EnvDB (web address http://metagenomics.uv.es/envDB) is a database that gathers all the available 16S rDNA sequences from sampling experiments deposited in GenBank and correlates them with taxonomic and environmental information. We have used the possibilities offered by this database to analyse the worldwide distribution of the species of *Halomonas*, focusing upon the habitats where they are normally found and also upon the other genera that tend to exist alongside *Halomonas* species.

Most ribosomal sequences of *Halomonas* stored in GenBank belong to aquatic environments, particularly saline ones, although this genus also occurs quite commonly in saline sediments. At family level *Halomonadaceae* are often found in the company of *Alteromonadaceae, Idiomarinaceae* and *Pseudoalteromonadaceae*. We have also undertaken tests to find out whether this pattern exists in other habitats such as saline soils, which contain over 8% of the total quantity of *Halomonas* sequences.

Microbial life in the high-altitude, saline, water bodies in the Andes of northern Chile

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Prokaryotes inhabiting athalassohaline environments are poorly known. We analyzed the planktonic bacterial and archaeal assemblages inhabiting several of these evaporitic basins in a remote and vast area in northern Chile by DGGE and sequencing of 16S rRNA gene fragments. Overall, we analyzed more than 25 samples from 19 different environments with strong gradients of altitude, ionic compositions and UV influence. Bacterial assemblages were dominated by the Cytophaga–Flavobacterium– Bacteroides (CFB) phylum and Proteobacteria. There was a tendency for increasing contribution of CFB with higher salinities and altitude. Lake Tebenquiche was selected for an in depth study. The bacterial assemblage (by DGGE) was quite variable both in space and time and community composition changes seemed to be related to the salinity of the particular sample. Clone libraries showed a predominance of Bacteroidetes (about one third of the clones) and Gammaproteobacteria (another third) with respect to all the other groups. The diversity of Bacteroidetes sequences in Lake Tebenquiche was large and showed a remarkable degree of novelty. In addition, a rich and diverse presence of *Salinibacter* relatives was found in the saltiest part.

Salar de Ascotán was selected to demonstrate the role of bacterial arsenic precipitation in the biogeochemical cycle of arsenic. Based on the microbiological and chemical evidence gathered, we concluded that bacteria contributed to the formation of the arsenic minerals. This evidence included: (1) enrichment and isolation of cultures with arsenic precipitation capacity from arsenic mineral samples, (2) high numbers of arsenic-precipitating bacteria in the Andean minerals and brines, (3) chemical and mineralogical properties of precipitates experimentally formed under biotic and abiotic conditions, (4) similarities in stoichiometry between natural and laboratory obtained minerals, and (5) the consistent depletion in $\delta^{34}S$ values for natural versus laboratory obtained sulfides. Thus, microbial precipitation of arsenic sulfides is a geochemically relevant metabolism. We also carried out a study of the presence of As processing genes in several environments. Surprisingly, the arsC gene necessary for detoxification was only present at low As concentrations while arsA, used for anaerobic respiration of arsenate was presten throughout the gradient.

Finally, the El Litio salterns were chosen for an analysis of the most extreme salinity at which life is possible. Crystallizer ponds in lithium-salt recovery plants reach water activity levels below 0.6, while NaCl salterns only reach a w_a of 0.75. Prokaryotic diversity was studied by DGGE and sequencing in different ponds along the salinity gradient. Five halobacterial sequences were retrieved, which could be grouped into one cluster with 95 to 97% similarity to 16S rRNA sequences retrieved from Permo-Triassic salt. The similarity between these sequences could indicate ecophysiological similarities among them.

Microbial and lichen colonization of gypsum crust in the Atacama Desert

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The Atacama Desert is considered the driest desert on Earth. It is located along the eastern coast of Chile and Peru where dew and occasional marine fog from the Pacific Ocean are the only sources of liquid water in its hyper-arid region. Gypsum and anhydrite are widely distributed throughout the Atacama Desert and they represent potential lithic habitats for microorganisms.

The aim of this study was to characterize the structure and organization of novel endolithic microbial communities which have colonized the interior of gypsum crusts. The study was also intended to describe the diversity and the interactions of the components of the community with the abiotic surroundings. Samples were collected from several locations in the north and central part of the Atacama Desert and they were analyzed by a combination of microscopy (SEM-BSE, LTSEM, TEM, CSLM, LM) and molecular biology approaches.

Evaporite crusts in the hyper-arid core of the Atacama Desert harbour diverse and abundant forms of endolithic microbial colonization. These communities have shown a more complex structure and organization than previously thought.

In some areas of gypsum crust, algae and cyanobacteria cells were detected in endolithic positions inhabiting spaces between gypsum crystals. Heterotrophic bacteria were also frequently observed in their nearby surroundings. However, in other areas, free-living and lichenized fungi were the most common microbiota observed in endolithic positions (close to the surface) as well as in epilithic positions. Extracellular polymeric substances (EPS), which could be involved in the disintegration of the larger gypsum crystals, were frequently observed associating with these lithobiontic microorganisms.

Defined layers containing different types of microorganisms were observed in the gypsum crust just below the surface. It appears that the different microorganisms have distinct preferences for colonizing the gypsum and generating the distribution in colored thin layers. In addition, differences in the type of colonization have also been found between gypsum crusts from different locations. The environmental conditions and physic-chemical features of the gypsum crusts could be responsible for these differences, although conclusive results have not yet been obtained.

All these observations lead us to think that the interior of gypsum crusts provide protection for the microorganisms from the extreme aridity, hot temperatures and radiation that occurs in these regions. Endolithic colonization appears to permit the survival of microorganisms in one of the most extreme arid places of Earth.

Breathing the Unbreathable: Redox Potential as a New Form of Extremophilia

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Resistance to extreme values of temperature, pH, salinity, radioactivity and pressure are the most common forms of microbial extremophilia described so far. However, the recent discover of electrogenic bacteria able to respire insoluble electron acceptors polariced at accurate redox potential, revealed life around thermodynamic limit is possible. Those environments are present at microniches all along the anaerobic subsurface but can be simulated at the laboratory by using potentiostats to polarice electrodes at known redox potential.

Bacteria from the genus *Geobacter* are the best studied electrogenic microorganism and it has been shown to transfer electrons to electrodes at ranges from 600 mV till extremely low negative potential (<-300mV). The capacity for transferring electrons out of the cell to reduce insoluble substrates at very low redox potential makes microbial life possible under enviromental conditions where most of bacteria cannot survive. The mechanism that these bacteria use for dealing with such extreme form of respiration is based in a vast network of c-type cytochromes for transferring electrons from cytoplasm to the exocellular location of the electrode. *Geobacter sulfurreducens* genome shows more genes codifying for c-type cytochromes (more than 100 genes) than any other organism sequenced so far.

A recent study combining electrochemical techniques and surface-enhanced infrared reflection absorption spectroscopy (SEIRAS) allowed us to identify the outer-membrane c-type cytochromes as species responsible in the electron transfer from the bacteria to a gold electrode. This finding suggests the possibility of a specific interaction between outer-membrane c- type cytochromes and gold electrodes at nanometre scale. The dependence of that interaction on electrode surface structure is a crucial point to understand which mechanism control microbial respiration under redox extreme conditions.

A putative "penicillin acylase" from *Thermus thermophilus* HB27

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The penicillin G acylases (PGA) belong to the N-terminal nucleofile acylase family. The most studied PGA is that from *E. coli*, which is used mainly for the hydrolysis of penicillin G to get the penicillanic acid nucleus further used in the synthesis of semi-synthetic penicillins[1]. Enzymes from this family are also used in the synthesis of peptides and for the resolution of quiral compounds[2].

The *E. coli* PGA has two subunits that result from the processing of a pre-pro-protein translocated to the periplasm through the twin arginine transporter (TAT)- system. In the periplasm, the protein suffers a self-processing that eliminates an internal region (linker) leading to the formation of a hetero-dimer between a small (24 KDa) or α, and a large (62 KDa) or β subunits. This β subunit contains the catalytic N-terminal serine and other residues implicated in the catalysis[3].

In the sequenced strains of *Thermus thermophilus* a gene encoding a homologue to PGA was identified. In a previous work[4] this putative ThPGA was cloned from the genome of *T. thermophilus* HB27 and expressed in *E. coli*, where we could detect a low hydrolytic activity on penicillin G. However, nothing was known about its expression, function, or putative processing in the thermophile.

En this work, we have used policlonal antisera against the putative □ and □ subunits of ThPGA to analyze its expression and processing in the thermophile. We have found that the ThPGA is actually processed into N-terminal and C-terminal fragments of apparent mobility corresponding to 29 and 59 kDa from a precursor protein of around 87 kDa. We have also carried out complementation experiments in a *pga* mutant with PGA proteins lacking different C-terminal fragments to analyze the relevance of the C-terminal region on the processing. Finally, we have over-expressed and purified the protein from *Thermus thermophilus*.

1) Dürckheimer y cols (1985). Angew Chem, 97:183-205

2) Arroyo, y cols (2003). *Applied Microbiology and Biotecnology,* 60:507-514

3) Duggley y cols (1995). *Nature*, 273 264.

4) R. Moreno, Tesis Doctoral, UAM.

Implication of Omp85 on the secretion and insertion of the S-layer protein from *Thermus thermophilus*

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β-Barrel outer membrane proteins are present f Gram-negative bacteria, mitochondria and chloroplasts. It has been described the implication of proteins belonging to the Omp85, a beta-barrel protein itself, in the insertion and assembly of such β-Barrel outer membrane proteins (OMP) through a poorly known mechanism. In the systems so far analyzed, a C-terminal signature sequence found in many OMP beta-barrel proteins binds a N-terminal periplasmic extension of Omp85 which includes a domain called POTRA (polypeptide transport associated). Although this interaction could be species-specific, the C-terminal signature of the secreted OMP is usually well conserved, including a C-terminal Phe residue.

The cell envelope of the thermophile *Thermus thermophilus* is multilayered, comprising a cytoplasmic membrane, a thin cell wall layer, and an outer membrane/S-layer envelope bound to the secondary cell wall polymers. The S-layer is formed by subunits of SlpA (Surface layer protein A), a protein which has mixed characters common to both S-layers and outer membrane proteins. Although SlpA has a sec dependent signal peptide for its secretion, it is not known the mechanism underlying its secretion and precise integration in the OM envelope.

Analysis of SlpA protein sequences from different strains of *T. thermophilus* revealed a C-terminal signature sequence, similar to the consensus sequence used for secretion of OM proteins in Gram negative bacteria. On the other hand, a homologue to Omp85 has been described in this organism. Therefore, we hypothesized that SlpA could require this Omp85 homologue for its incorporation to the outer membrane. To analyze this hypothesis we have generated *slpA* and *omp85* mutants that produce proteins with changes or deletions at their respective C-terminal sequence. Our results show that proper location of SlpA depends on its C-terminal sequence, and that Omp85 is implicated in this process.

Comparative genomics of partial and complete denitrificant strains of *Thermus thermophilus*

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Although the two strains of *Thermus thermophilus* (HB27 and HB8) which genomes have been published are strictly aerobic, there are other strains of this species capable of growing under anaerobic conditions using some or all the reactions of the denitrification pathway. Strain NAR1 can grow by using a nitrate reductase (Nar) that uses the electrons from a new type of NADH dehydrogenase (Nrc), rendering nitrite as final product. The genes encoding these enzymes s are encoded within a conjugative element named NCE, which can be transferred by conjugation to aerobic strains of the same species[1]. Other strains, like PRQ25, can use the nitrite produced in this first step as an electron acceptor leading to the production of a gaseous species.

In order to know the genetic basis of this differential behavior, we have sequenced the strains NAR1 and PRQ25, and analyzed the potential genes and proteins involved in the denitrification pathway.

The comparison of the NCE sequence from both genomes shows the presence of unexpected differences at the level of the Nrc enzyme. Moreover, the characteristics of the flanking regions of the NCE element in both strains gave evidence of its location within a megaplasmid and not in the bacterial chromosome, as we had already deduced[2].In addition to NCE, we have found in PRQ25 a gene cluster that includes putative operons coding for a cd_1 type nitrite reductase (Nir) and for a nitric oxide reductase (Nor). In contrast, we have not been able to find any ORF with homology to any of the nitrous oxide reductases (Nos) so far described. This *nor-nir* cluster is located upstream from and very close to the NCE element, and flanked by putative transposases. This supports the idea of a frequent mobilization and horizontal transference of the denitrification genes between different strains of the genus. Actually, be have been able to transfer the whole denitrification capability of the PRQ25 strain to the aerobic strain HB27 through natural competence.

(1) Cava F and Berenguer J. *Biochem. Soc. Trans.,* 2006.

(2) Ramírez-Arcos S and col. *J. Bacteriol.*, 1998.

Curated annotation and in silico reconstruction of the central metabolism of *Chromohalobacter salexigens* DSM 3043

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Systems biology is an emerging field that has contributed to understand more precisely data generated experimentally through the ability to obtain, integrate and analyze complex data from multiple experimental sources using interdisciplinary tools. A myriad of bioinformatic tools have been developed according to the needs of experimental biologists. The aim of our research group is to tackle the study of ectoine(s) synthesis in the moderate halophile *Chromohalobacter salexigens* with the global focus of Systems Biology, due to the complexity of this process in this bacterium, to finally optimize the production of ectoine and hydroxyectoine for biotechnological purposes (1). To achieve this, it is necessary to combine experimental data arising from global experimental analyses of the response to hyperosmotic and heat stress in *C. salexigens* (proteomics, transcriptomics) with genome-scale metabolic reconstructions, and the use of mathematical models that allow adequate predictions. As far as bioinformatics concern, the overall goal of our project is to rebuild completely the metabolism of *Chromohalobacter salexigens DSM 3043* based on genomic annotation, and the characterization and reconstruction of metabolic pathways of the compatible solutes ectoine and hidroxyectoine.

The assembled and automatically annotated complete genome of *Chromohalobacter salexigens DSM 3043* was done by the Joint Genome Institute (www.jgi.doe.gov). Currently, we are trying to curate the genome and to generate a more precise annotation. In order to visualize the data, a Generic Genome Browser, which is a combination of database and interactive Web page for manipulating and displaying annotations on genomes, has been designed (http://147.96.5.65/cgi-bin/gbrowse/biostab/). On the other hand, Pathway Tools, a useful systems biology software system (http://bioinformatics.ai.sri.com/ptools/), has been used to predict a partial metabolic network of *C. salexigens* through several powerful computational inference algorithms that extract more information from the previously curated genome. The results of the genome curated annotation in one hand, and the reconstruction of the targeted metabolic pathways on the other hand, will be used to develop a mathematical model in collaboration with the Department of Biochemistry, Molecular Biology and Immunology of University of Murcia (http://www.um.es/bbmbi/), in order to predict new strategies to optimize ectoine(s) production.

(1) Vargas et al. (2008). *Saline Systems.* 4:1

Involvement of the global regulators *rpoS* and *rpoD* in the synthesis of the bioestabilizers ectoine and hydroxyectoine in Chromohalobacter salexigens

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The moderately halophilic γ-proteobacterium *Chromohalobacter salexigens* shows a very broad salinity range of growth (0.5-3 M NaCl in minimal medium) due to the cytoplasmic accumulation of compatible solutes (mainly ectoine, hydroxyectoine, trehalose, and betaine). Ectoine and hydroxyectoine have biotechnological applications as biostabilizers of molecules, whole cells and tissues, and a promising potential for use in cryopreservation and neuroprotection (1, 2). By using qRT-PCR and transcriptional fusion analysis, we have previously reported that the genes encoding ectoine (*ectABC*) and hydroxyectoine (*ectD* and *ectE*) production are submitted to a complex regulation. Several transcriptional factors (σ^S, σ^{32}, Fur, EctR) were shown to be involved in responding efficiently to osmotic and temperature stress (3, 4, 5). The recent advances in proteomics and transcriptomics, and the availability of the *C. salexigens* genome sequence, prompted us to try a global expression approach to clarify this complex regulation. This objective is framed within a Systems Biology project, whose aim is to combine global expression data with a genome-based metabolic reconstruction (see communication by Garcia-Yoldi et al.) to generate a mathematical model that will be used to design optimal genetic manipulation and environmental conditions leading to ectoine(s) overproduction.

In this work we have generated and characterized mutants affected in the global regulators σ^S (*rpoS*) and σ^{32} (*rpoD*), in order to analyze the global transcriptional response of *C. salexigens* to hyperosmotic and heat stress, as these are environmental conditions triggering ectoine and hydroxyectoine synthesis, respectively. Single copies of orthologs to the *Escherichia coli rpoS and rpoD* genes were found in the genome of *C. salexigens*, and these were inactivated by insertion of a streptomycin- and a geneticin-resistance cassette, respectively. To generate the mutant strains, the inactivated genes *rpoS::*Ω and *rpoD::*Ω*aac* were cloned in the suicide vector pJQ200SK and integrated within the wild type chromosome by homologous recombination, resulting strains CHR196 (*rpoS*) and CHR198 (*rpoD*). Growth of the *C. salexigens rpoS* and *rpoD* mutants was compared with that of the parental strain at different salinities (from 0.6 to 3 M NaCl) and temperatures (37ºC or 45ºC). In addition, their compatible solute content was determined by ^{13}C-RMN analysis, and ectoine and hydroxyectoine was quantified by HPLC-MS under different growth conditions. Next step in this work is to perform a comparative proteomic and transcriptomic analysis of the both σ^S and σ^{32} mutants and wild type strain, to generate high-throughput data on the metabolic and regulatory circuits, as well as structural proteins, which are activated in response to salt and heat stress.

(1) Graf et al. (2008). *Clin. Dermatol.* 26: 326-333.

(2) Kanapathipillai et al. (2008). *Biochem. Biophys. Res. Commun.* 65: 808-813.

(3) Calderón et al. (2004). *Microbiology* 150: 3051-3063

(4) García-Estepa et al. (2006). *J. Bacteriol*. 188: 3774-3785.

(5) Vargas et al. (2008). *Saline Systems* 4: 14

Effect of knocking out the uptake of the ectoine and hydroxyectoine and co-expression of the synthesis genes to enhance hydroxyectoine production by *C. salexigens*

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Chromohalobacter salexigens is a moderately halophilic bacterium able to grow in a wide range of salinities (1) because its ability to synthesize and accumulate in its cytoplasm the compatible solutes ectoine and hydroxyectoine. These two compounds are of industrial interest as stabilisers of cells, enzymes and other biological molecules, used mainly in cosmetics and dermopharmacy. Since at present the chemical synthesis of ectoines is not available, their biological over-production is very interesting, especially in the case of hydroxyectoine, a minoritary product, due to its thermoprotective properties. The final objective of this work is the generation of *C. salexigens* strains showing enhanced hydroxyectoine production for subsequent industrial applications using different approaches. As a preliminary step, we are characterizing the systems involved in ectoine(s) uptake and its regulation, to impede ectoine(s) recovery from the external medium, and therefore enhancing the ectoine(s) production.

In the closely related *Halomonas elongata* an osmoregulated TRAP-type transporter with high affinity for ectoine and hydroxyectoine, encoded by the *teaABC* genes, was described (2) as a recovery system salvaging endogenous ectoine and hydroxyectoine leaking through the cell membrane (2). We have found orthologs to *teaABC* genes within the genome of *C. salexigens* and knocked out this transport system. The characterization of a *teaC::Ω* insertion mutant (CHR169) has demonstrated that TeaABC is the main uptake transport system of ectoines in *C. salexigens*. Besides, the synthesis and recovery of ectoine(s) of the CHR169 mutant is being investigated. On the other hand, we have previously isolated a *Tn1732*-induced mutant (CHR95), which, in contrast to the wild type strain, was able to use ectoine and hydroxyectoine as the sole carbon source at low salinity (0.6M NaCl) and showed higher ectoine transport rates than the wild type strain at any salinity tested. The *Tn1732* insertion generated a deletion covering two transcriptional regulators: *mntR* (manganese-uptake regulator) and *luxR* (transcriptional regulator). To elucidate if MntR or LuxR were involved in the transcriptional control of ectoine(s) transport, single *mntR::Ω* (Sm^r) and *luxR::ΩaaC* (Gn^r) insertion cassette mutants were generated but only the *luxR* mutant (CHR183) showed a deregulation in the capacity to use of ectoines as carbon source at low salinity. To demonstrate the involvement of LuxR in the regulation of *teaABC genes*, and therefore in the uptake of ectoines, we generated transcriptional fusions of the two promoter regions in pMP220 vector (P*teaA::lacZ* and P*teaB::lacZ*) and compared their activities in a *luxR* mutant and CHR95 strain to the wild type strain.

Finally, to test the effect of the supplying of *ectABC and ectD* genes in *trans* on multiple extrachromosomal copies and upon the control of *ectABC* promoter (*PectA*), we have designed P*ectA::ectABC::ectD* and P*ectA::ectABC::ectD::ectD* fusions, which were cloned in pHS15 plasmid and transferred to the *C. salexigens* wild type strain and a *ΔectABCΔectD* mutant (CHR137). The enhancement in ectoines production in these recombinant strains is currently under investigation in our lab.

1. Cánovas et al. (1997). *J. Biol. Chem*. 272: 7221-7226.

2. Grammann et al. (2002). *J. Bacteriol*. 184: 3078-3085.

Role of trehalose in the response to hypersaline, dehydration and heat stress in halophilic and drought-tolerant bacteria

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Accumulation of the disaccharide trehalose has been shown to be associated with increased osmo and thermotolerance of bacteria, yeast and fungi, as it protects proteins and cellular membranes against inactivation or denaturation caused by a variety of stresses, including salinity, desiccation, dehydration, heat, cold, and oxidation (1, 2). Trehalose is synthesized *de novo* from glucose through the *otsAB* pathway, the best known and most widely distributed one, which is catalysed, in its first step, by the enzyme trehalose-P-synthase, encoded by the gene *otsA*.

In this study we investigated the role of endogenous trehalose in osmo- and thermoprotection, as well as in desiccation tolerance, in two different model microorganisms, the halophilic bacterium *Chromohalobacter salexigens*, and *Rhizobium etli*, a soil bacterium which establishes symbiosis with common bean roots. We showed that *otsA* is the main gene responsible for trehalose synthesis from glucose in these bacteria, as a *R. etli* or a *C. salexigens otsA* mutant was unable to accumulate this compatible solute. Trehalose was involved in thermo- and osmoprotecion of *R. etli*, as judged by the fact that the *R. etli otsA* mutant was osmo- and thermo-sensitive. In *C. salexigens*, ectoines (the main compatible solutes) suppressed trehalose synthesis at 37ºC, but trehalose was accumulated in an ectoine-deficient mutant at this temperature, or in the wild type strain grown at 45ºC (3). As a *C. salexigens otsA* mutant grew similarly to the wild type strain at high salinity or high temperature, we generated double mutants affected in *otsA* and *ectABC* (encoding ectoine synthesis), or *otsA* and *ectD* (encoding hydroxyectoine synthesis). The purpose was to analyze the role of trehalose in osmo and thermoprotection in absence of ectoine or hydroxyectoine. Our findings suggest that trehalose accumulation in *C. salexigens* is up-regulated by high temperature but not by salinity.

To determine the effect of trehalose accumulation on desiccation tolerance of both bacteria, the wild-type strains and the different *otsA* mutants were grown in minimal medium and desiccation tests were performed as described by Manzanera *et al.* (2002) (4). *R. etli* cells incubated under osmotic stress conditions, which stimulated trehalose biosynthesis, were more tolerant to desiccation, if compared with cells not pre-treated with NaCl. In any growth condition tested, the *R. etli otsA* mutant displayed lower survival rates than the wild-type strain. The *C. salexigens* wild-type and *otsA* strains were more tolerant to desiccation than an *ectD* mutant, indicating that hydroxyectoine plays a major role in desiccation tolerance in *C. salexigens*. However, a double *ectDotsA* mutant showed lower survival rates than the *ectD* strain, suggesting that trehalose is also involved in desiccation tolerance in this halophilic bacterium.

1. Welsh (2000). *FEMS Microbiol. Rev.* 24: 263-290

2. Elbein et al. (2003). *Glycobiology* 13: 17R-27R

3. García-Estepa et al. (2006). *J. Bacteriol.* 188: 3774-3784

4. Manzanera et al. (2002). *Appl. and Environ. Microbiol.* 68: 4328-4333

Gene analysis and heterologous expression of PII proteins from the haloarchaea *Haloferax mediterranei*

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PII proteins are a protein family important to signal transduction found in all three domains of life. PII plays a critical role in regulation of carbon and nitrogen metabolism. Signals from the carbon, nitrogen and energy status of the cells are converted into different conformational (and modification) states of the PII proteins. Their functions are based in the modulation of glutamine synthetase activity in response to the nitrogen availability.

The variety of PII proteins, their different functions and their central role in nitrogen metabolism regulation have been extensively studied in many organisms, such as cyanobacteria [1,2] or *E. coli* [3] but has had less attention in the Archaea domain, where only PII from methanogens have been considered. Concerning PII nomenclature, proteins of the PII family can be classified into three closely related subgroups, the products of the genes *glnB*, *glnK* and *nifI* [4].

The protein family shows high conservation, with examples in eukaryota (plants and eukaryotic algae), archaea, and bacteria [5]. This distribution indicates that PII is one of the most ancient signalling proteins known.

This study contributes to the knowledge of the nitrogen regulatory system in this third domain by the sequencing, overexpression and purification of the PII proteins $GlnK_1$ and $GlnK_2$ from the extreme halophilic archaea *Haloferax mediterranei*.

1. Forchhammer, K. (2004) *FEMS Microbiol Rev* 28, 319.
2. Ying, Z. and Jindong, Z. (2008) *Sci China Ser C-Life Sci* 51, 1056.
3. Reitzer, L. (2003) *Ann Rev Microbiol* 57, 155.
4. Ehlers, C., Grabbe, R., Veit, K. and Schmitz, R.A. (2002) *J Bacteriol* 184, 1028.
5. Ninfa, A.J. and Jiang, P. (2005) *Curr Opin Microbiol* 8, 168.

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Characterization and cloning of the protease Salipro produced by the extremely halophilic bacterium *Salicola marasensis* IC10

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In recent years extreme halophiles have attracted great interest for their biotechnological and potential applications. Among these applications is the production of industrial compounds such as polysaccharides or organic compounds, called compatible solutes that accumulate in the citoplasm as a strategy for osmoadaptation. Moreover, these microorganisms play an important role in the biodegradation of waste and are of great interest as producers of extracellular enzymes that can be used as biocatalysts in extreme conditions (salinity, temperature, pH, etc….).

A screening program was performed in salterns of Huelva to determine the biodiversity of extreme halophiles able to produce hydrolytic enzymes (Moreno et al., 2009) and allowed the selection of a strain presenting proteolytic and lipolytic activity. This isolate has been taxonomically classified as *Salicola marasensis* IC10.

To determine the localization of hydrolytic enzymes, an activity test was performed in the intra and extracellular fractions that permitted us to conclude that *Salicola marasensis* IC10 is able of produce an extracellular protease, designated Salipro, and an intracellular lipase which was named LipL. The characterization of the extracellular fraction of strain IC10 during growth allowed us to identify the optimal conditions for protease production: pH 8.0, 40ºC and a medium with 15-20% (w/v) NaCl. Thus, there is a correlation between the optimal conditions for cultivation of the strain and the maximum production of the proteolytic enzyme.

By using inverse PCR we have isolated the gene *prot*S, which encodes the protease Salipro, consisting of 817 amino acids. This protease belongs to the S16 family (Merops) and shows a 75% homology with an ATP-dependent protease of *Marinobacter aquaeolei*. Salipro presents the specific conserved domains of this family: walker A, GxxGxGK [S/T], walker B, ATP binding and arginine finger. In order to purify and characterize the enzyme, the gene *prot*S will be expressed in the pET22b vector to obtain the fusion protein in *Escherichia coli*.

Reference:

Moreno M.L., García M.T., Ventosa A. and Mellado E. (2009). Characterization of *Salicola* sp. IC10, a lipase-and protease- producing extreme halophile. *FEMS Microbiol Ecol* **68**: 59-71.

Determination and analysis of the three-dimensional structure of a new D-2-hydroxyacid dehydrogenase NAD(P)H-dependent from *Haloferax mediterranei.*

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D-2-hydroxyacid dehydrogenases catalyze the stereospecific NADH-dependent reduction of 2-ketocarboxylic acids into their corresponding 2-hydroxy carboxylic acids. A new D-2-hydroxyacid dehydrogenase with dual coenzyme specificity from the haloarchaeon *Haloferax mediterranei* (D2-HDH) has been isolated and characterizated in our laboratory [1]. Crystallization assays of the enzyme were performed in collaboration with the group of Prof. D.W. Rice and Dr. P. J. Baker at the Department of Molecular Biology and Biotechnology in the University of Sheffield (England) obtaining two different crystal forms. Crystals of form I were obtained with the free enzyme using ammonium sulphate as precipitant agent. These crystals diffracted beyond 3.0 Å resolution and belonged to the monoclinic space group $P2_1$ [2]. Crystals of form II were obtained with the ternary non-productive complex enzyme-ketohexanoate-NAD^+ using PEG 3350 as precipitant. These crystals diffracted to 1.35 Å and belonged to the space group *P*1. Both crystal forms contain two dimers of the D2-HDH related by translational pseudosymmetry. These crystals have allowed us to obtain the three-dimensional structure of the protein which represents the highest resolution structure for any family member and halophilic proteins to date. This has proved possible to describe the protein monomeric and oligomeric structure and the substrate and dinucleotide binding sites in high detail. The high resolution achieved has allowed us to obtain a better knowledge of the molecular basis of specificity of D2-HDH and enhance our understanding of the structural factors of halophilic adaptation. This information will contribute to the development of enzymes to function efficiently in other dehydrating conditions such as organic solvents with industrial interest.

[1] Domenech J. and Ferrer J., *Biochim. Biophys. Act.* **1760** (2006) 1667-1674.

[2] Domenech, J., Baker, P.J., Sedelnikova, S.E., Rodgers, H.F., Rice, D.W. and Ferrer, *J. Acta Cryst.* **F65** (2009) 415–418.

B100S, a highly sulphated exopolysaccharide produced in its native state by the bacterium *Halomonas maura*, causes the death of hematopoietic tumour cells by apoptosis via the mitochondrial pathway.

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Exopolysaccharides have aroused great interest among biotechnologists because of their wide potential range of applications in such fields as medicine, pharmacy, foodstuffs, cosmetics and the petroleum industry. The advantages of using microbial EPSs are becoming clear in that they are more versatile and efficient than vegetable and synthetic polymers.

The EPSs produced by *Halomonas* species are anionic polymers composed mainly of carbohydrates, but as well as their various neutral sugars they also contain other organic components and an inorganic fraction which, particularly in the case of *H. maura* strain B-100*,* incorporates a large quantity of sulphates. Sulphated EPSs may be used as blood anticoagulants, insulinotropic, antitumoral and antiviral agents.

We have found that modification of EPS 100 by adding extra sulphate groups to the native polymer gives a polymer (B100S) with a vigorous antiproliferative activity in several haematopoietic tumour cell lines. By cell cycle analysis we have determined that EPS B100S induces apoptosis of Jurkat and MOLT-4T cell leukemias via the mitochondrial pathway. Polymer B100S also induces apoptosis in primary leukemic T cells obtained from the peripheral blood of patients.

Homologous expression: A new and simple alternative for halophilic enzymes production

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The homologous and heterologous expression of genes is the first step for most biochemical studies of protein function. Many systems have been carried out for protein production in members of the *Bacteria* and *Eukarya*, however members of the *Archaea* are less amenable to genetic manipulation. Because of this, mesophilic hosts, in particular *Escherichia coli*, have been used to produce halophilic proteins for biochemical characterization and crystallographic studies. Expression in *E. coli* has been employed in our lab for the expression and refolding of simple halophilic proteins. Enzymes recovered from inclusion bodies were solubilized and rightly refolded in high salt buffers (1). However, severe difficulties were encountered for the refolding of metalloenzymes or complex proteins. In this case, the overexpressed product can`t be reactivated due to the incorrect thermodynamic conditions for the cofactor insertion. For this reason, we have explored the homologous expression of two nitrite reductases (assimilatory and respiratory) from *Hfx. mediterranei* using *Hfx. volcanii* as halophilic host (2). Recombinant enzymes were expressed and spontaneously refolded inside the cell. Clear evidences as the high levels of activity detected in crude extracts from complex medium or overexpressed protein bands with a correct molecular weight in SDS-PAGE gels suggest a proper homologous expression (3). This alternative technique allow obtains directly the fully active enzyme from the host avoiding refolding steps. Nevertheless, low expression levels were achieved for assimilative NiR and the improvement of the process is necessary.

[1] J.M. Esclapez, M.J. Bonete, M. Camacho, C. Pire, J. Ferrer, V. Bautista, R.M. Martínez-Espinosa, B. Zafrilla, F. Pérez-Pomares y S. Díaz (2006). An optimized method to produce halophilic proteins in *Escherichia coli*. Microbial Cell Factories **5,** 1-2.

[2] R.M. Martínez-Espinosa, F.C. Marhuenda-Egea, M.J. Bonete (2001). Purification and characterization of a possible assimilatory nitrite reductase from the halophile archaeon *Haloferax mediterranei.* FEMS Microbiol Lett. **196(2)**: 113-118.

[3] F. Pfeifer, S. Offner, K. Krueger, P. Ghahraman, C. Englert (1994) Transformation of halophilic Archaea and investigation of gas vesicle synthesis. System Appl Microbiol. **16**,569-567.

Purification and characterization of *LipBL*, a lipase with high homology to the class C beta-lactamases

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Moderately halophilic bacteria are a group of halophilic microorganisms that grow optimally in media containing between 3 and 15 % sodium chloride. Many industrial processes are carried out under specific conditions which do not meet the currently available enzymes. Thus, the search for novel enzymes showing optimal activities in a wide range of temperature, pH and salt concentration is of great industrial importance.

Marinobacter lipolyticus is a moderately halophilic bacterium isolated from saline environments, showing lipolytic activity (Martin et al., 2003). There is good correlation between the optimum conditions of growth and the conditions for the maximum production of the enzymes, these being 1 M NaCl, pH 7.5 and 37 ºC with good aeration. The construction of a gene library of *M. lipolyticus* in *Escherichia coli* allowed us to isolate the gene that encodes one of the intracellular lipolytic enzymes. The gene named *LipBL* encodes a protein LipBL, consisting of 404 amino acids with a molecular mass of approximately 45 KDa. This lipolytic enzyme presents high homology with the class C beta-lactamases, showing all the typical motifs of this family of enzymes. In terms of its biochemical characteristics, LipBL presents maximum activity at pH 7, 80 ºC and is stable in the presence of a large number of organic solvents showing enantioselective hydrolysis of fats.

The immobilization of enzymes presents significant advantages to the use of enzymes as it allows a significant improvement in its stability and allows the reuse of the enzyme, so it can use in the production of industrial chemicals, pharmaceuticals, food, in waste treatment, and many other applications. In order to study the stability of the enzyme, several immobilized derivatives have been prepared and we will present the most recent results obtained.

Reference:

Martín, S., Márquez, M. C., Sánchez-Porro, C., Mellado, E., Arahal, D. R. y Ventosa, A. (2003).

Marinobacter lipolyticus sp. nov., a novel moderate halophile with lipolytic activity. *Int J Syst Evol Microbiol* **53**: 1383-1387.

Transcript levels of glutamine synthetase and glutamate synthase genes in response to the nitrogen source in *Haloferax mediterranei*.

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Glutamine synthetase (GS) and glutamate synthase (GOGAT) play a key role in ammonium assimilation processes among many organisms by forming the GS/GOGAT pathway. The total reaction incorporates ammonium into 2-oxoglutarate at the expense of ATP and reducing power. The GS/GOGAT pathway is particularly important because in many organisms it allows ammonium assimilation into L-Glutamate at low intracellular ammonium concentrations and, in this conditions, it efficiently substitutes the glutamate biosynthetic reaction catalyzed by glutamate dehydrogenase.
Haloferax mediterranei (*Hm*) is able to grow in controlled media containing different nitrogen sources, as NO_2^-, NO_3^-, amino acids or NH_4^+ but the mechanisms of the nitrogen assimilaton in this organism still unknown in detail. The glutamine synthetase from the haloarchaeon *Haloferax mediterranei* has been purified and characterized [1], the enzyme consists of eight subunits of 51.7 kDa, suggesting that this enzyme belongs to the glutamine synthetase type II. The *Hm* glutamate synthase consist in a unique polypeptide of 1513 amino acids that showed high similarity with the α subunit of the bacterial enzyme and as ferredoxin dependent glutamate synthase it lacks the small β subunit. The current study shows the different transcript levels of glutamine synthetase and glutamate synthase genes in response to the different nitrogen sources tested by mRNA quantification using real time reverse transcription PCR (qRT-PCR). The enzyme displayed maximal activity with ferredoxin from *Haloferax mediterranei* and it is also able to use methyl viologen as electron donor.

[1] Martínez-Espinosa, R.M., Esclapez,J., Bautista, V. and Bonete, M.J. 2006. An octameric prokaryotic glutamine synthetase from the haloarchaeon Haloferax mediterranei. FEMS Microbiol. Lett. 264, 110–116

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