

Meeting report

## The life of brine: halophiles in 2001

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A report on the International conference on Halophilic Microorganisms, Sevilla, Spain, 23-27 September 2001.

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The 2001 halophiles meeting covered archaea, bacteria, fungi and algae adapted to living hypersaline environments. Despite the absence of some American colleagues as a result of the recent terrorist attack, the meeting was full of exciting advances (as well as pictures of salt lakes, salterns, brines and salt mines - see, for example, Figure 1). Only a few of the main themes - emerging organisms, genomics, enzyme function and biotechnology - can be mentioned here.

### Emerging organisms

One of the perks of working with extremophiles is the opportunity to visit extreme environments, and the plenary lecture was given by Bill Grant (University of Leicester, UK) who spoke on 'Half a lifetime in soda lakes', and related many of his adventures in the Great Rift Valley of Africa, where he identified new taxa of halophiles. Although the dominant haloarchaeal cell type in crystallizer ponds (which have more than 30% salt) is a flat, square-shaped organism that has still to be cultured, Aharon Oren (The Hebrew University of Jerusalem, Israel) and colleagues have isolated another common cell type, a slightly curved rod belonging to a new genus, *Salinibacter*. Sequencing of 16S rRNA and lipid analysis show it to be a member of the Bacteria, although like the haloarchaea, it has a red pigment and uses potassium ions as the internal compatible solute - hence its proteins are highly acidic. These similarities between members of the Bacteria and haloarchaea are so surprising that they would appear to be an example of convergent evolution.

Francisco Rodriguez-Valera (Universidad Miguel Hernandez, Alicante, Spain) described an intriguing new group of

haloarchaea found in salt ponds that have 8% salt (or 2.3-fold concentrated seawater) but are not detectable at the higher salinities usually associated with haloarchaea (20-35% salt). Discovered using 16S rRNA libraries, the new haloarchaea are distantly related to *Haloarcula*. None has been cultured but these results suggest an optimum salt concentration for growth far lower than that of known haloarchaea. These represent an intermediate group of haloarchaea, not previously suspected, and offer insight into the evolution of extreme halophiles. It will be important to isolate and compare them to the marine Archaea (as yet uncultivated!) and the extremely halophilic Archaea.

The presence of viable haloarchaea in rock salt from deep underground deposits was another important theme (mentioned, for example, by Helga Stan-Lotter, University of Salzburg, Austria). The culture media and growth conditions used can greatly affect isolation rates, but at least three independent groups have grown numerous different organisms,



**Figure 1**  
A salt lake in Australia.

some of which corresponded to the many new taxa of haloarchaea identified from DNA in the rock salt. This is a new and exciting area of study, but it is still difficult to understand how the organisms persist under such adverse conditions in these isolated formations. The salt is rock-hard, very dry, hundreds of meters thick, and looks to be geologically untouched (not heated, folded, fractured or recrystallized) since its deposition millions of years ago. Russell Vreeland (West Chester University, USA) presented evidence that bacteria trapped in the tiny fluid inclusions of salt crystals are relatively protected from damage by background radiation and oxidation. Can such organisms be preserved for millions of years? Have they been growing, albeit ever so slowly, or can they permeate through the strata? The answer to this mystery will be of considerable significance - on Earth and as we look elsewhere in the Solar System for possible life forms.

## Genomics

The genome sequences of two strains of *Halobacterium salinarum* were presented. Sean Kennedy (University of Massachusetts, Amherst, USA) and Dieter Oesterhelt (Max Planck Institute of Biochemistry, Martinsried, Germany) outlined some of the features of each strain, and described their efforts to annotate and make sense of their sequence data. Both groups have produced websites; the NRC-1 sequence has been available for a year now at the Halobacterium genome project [<http://zdna2.umbi.umd.edu/>] site, and the R1 sequence was made available at the time of the conference at the *H. salinarum* database, Halolex [<http://www.halolex.mpg.de/>]. The German project is in the process of adding proteomic and transcription data to the sequence database, a major undertaking that will make life easier for those groups wrestling with the largely 'hypothetical open reading frames' in these sequences. A comparison of the two sequences shows almost insignificant changes in the main chromosome but radical differences in their (large) plasmids. It is known that insertion sequences (ISs) play havoc in members of this genus and seem to prefer inserting into regions that are relatively AT-rich but poor in genes. Why the cells need (or tolerate) such high mutation rates, the factors regulating IS activity (perhaps in response to stress?), and the mechanism of target selection, are still matters to be resolved.

Smaller genomes were also presented: Angela Witte (University of Vienna, Austria) and one of us (M.D.S.) described the first complete genome sequences of two haloviruses, PhiCh1 (58.5 kilobase genome, and *Natrialba magadii* as its host) and HF2 (77 kb genome, host *Halorubrum coriense*), respectively. Others focused on specific genes or operons. For example, Felicitas Pfeifer (Technical University of Darmstadt, Germany) described the elegant regulatory system of gas-vesicle genes (coding for the gas-filled vesicles that allow cells to float in the water column) using the *bgaH* ( $\beta$ -galactosidase) reporter, and Dick Shand (Northern Arizona University, Flagstaff, USA) described the halocin

story - the production of a diverse range of natural antibiotics by haloarchaea. Given that much is not yet known about the Archaea, and that genetic experimentation remains harder for Archaea than in organisms such as *Escherichia coli*, it is not surprising that providing experimental evidence to identify the functions of gene products can be a challenge, even when they show sequence similarity to well studied enzymes. For example, the operon coding for pyruvate-dehydrogenase-like subunits in *Haloferax volcanii* is transcribed and at least one component (dihydrolipoamide dehydrogenase) is translated, but no 2-oxoacid dehydrogenase activity has yet been detected (presented by M.D.). The *kaiC* gene of cyanobacteria is involved in maintaining a circadian rhythm but the haloarchaeal homolog of this gene (*hkaiC*) could not be shown to have this role, and its function remains obscure (Kunio Ihara, Nagoya University, Japan). DNA microarrays to study expression from genomes whose sequences have been completed, and bioinformatics comparisons (for example, that presented by Michael Galperin, National Center for Biotechnology Information, Bethesda, USA), should help in future studies of gene expression and provide clues to function.

## Enzyme function

A fascinating aspect of halophilicity addressed by Giuseppe Zaccai and his group (Institut de Biologie Structurale CFA-CNRS, Grenoble, France) concerns the question of how enzymes from haloarchaea maintain their structure and function at near-saturating concentrations of salt, at which most 'normal' proteins precipitate out of solution. Zaccai described an impressive array of biophysical techniques to monitor protein structure and flexibility in solution, and to study the effects of salt concentrations on these parameters. Coupled with X-ray structural data and mutagenesis studies of specific halophilic proteins, including a new enzyme structure from the group of Maria-Jose Bonete (Universidad de Alicante, Spain), a detailed picture is emerging of how the high negative charge found on the surface of these proteins serves to maintain a hydration layer by attracting water to those surfaces in the form of hydrated potassium and sodium cations. In addition, it is clear that it is not just the charge density that is important, but that hydration (and therefore solubility) relies on the coordinate interaction of these charges in three-dimensional space.

## Biotechnology

The final session of the conference turned to biotechnological applications of halophiles. Erwin Galinski (University of Münster, Germany) reported on the pharmaceutical applications of the (bacterially produced) compatible solute ectoine, and Brian Jones (Genencor International, The Netherlands) gave impressive examples of the use in the cleaning industry of enzymes from soda-lake organisms. A century ago clothes were washed in near-boiling water, but now washing

powders are designed to work in cold water, saving enormous amounts of energy. This change has been made possible by the use of enzymes that are rugged enough to be stored for long times at room temperature, and are active at high pH and in the presence of bleaches. Bioremediation in salty or desert areas (Heiko Patzelt, Sultan Qaboos University, Al-Khod, Oman) is another possibility, but in general the applications are few. Future funding for research on halophiles may well be determined by their biotechnological potential, and it was clear that it is this area that needs the greatest input of ideas and imagination before we meet again for 'Halophiles 2004' in Ljubljana, Slovenia.