www.nature.com/jim

## HALOPHILES Preface

CD Litchfield

Department of Biology, George Mason University, Fairfax, VA 22030, USA

Keywords: halophiles; Archaea; Bacteria

Why a special section on halophiles? What is so special about halophiles? — logical questions given that there has been relatively little interest in this unusual group of organisms by the biotechnology industry and they are not known human or animal pathogens. To date, only one halophile has been sequenced [9], and there have been limited applications of these organisms in industry aside from the tanning and solar saltern production industries. It has been known for many years that halophiles, both Bacteria and Archaea, can produce extracellular enzymes [8,13-15], polysaccharides [11,12] and surfactants [2,5], but to date there has been little direct application of these organisms and/or their enzymes to industrial processes. The papers presented in this special section are the result of a symposium on Biology and Biotechnology of Halophiles held at the Annual Meeting of the Society for Industrial Microbiology in San Diego in 2000 to address these issues.

The five papers were selected to cover a broad range of habitats and activities of halophiles whether they be Bacteria or Archaea. For the purpose of these papers, halophiles are microorganisms which grow over an extended range of salt concentrations (>3% to 25-30% NaCl, w/v) and include the halotolerant Bacteria and the obligate halophilic Archaea. The first paper by Javor introduces solar salterns and the importance of microorganisms in salt production from the evaporation of sea water or natural brines. It is in these systems that we find the broadest range and types of halophiles, which include photosynthetic cyanobacteria as well as heterotrophs, the eukaryotic alga *Dunliella*, and fungi, in the sediments anaerobic Bacteria. Finally, Javor nicely correlates the microbial community with the nutrient status of the different salterns.

Because halophiles were first isolated from salted foods and hides [3,6,7], it has been generally assumed that their metabolic capabilities are limited to the degradation of a few simple sugars, amino acids and proteins [4,10]. An examination of salterns and a soda lake by Litchfield and Gillevet used BIOLOG to demonstrate greater metabolic diversity than was previously reported or expected. Along with this, these authors also report temporal and spatial diversity within a saltern using the amplicon length heterogeneity fingerprinting technique. The implications from these studies are that there is a great untapped potential for the discovery of new degradative capabilities under high salt conditions. It is also necessary that new culture techniques and

Received 28 July 2001; accepted 30 July 2001

more extensive temporal studies be performed in order to cultivate the strains appearing at different times and isolate special previously unknown strains.

The next paper by Vreeland and Rosenzweig delves into another interesting location where halophiles are found — salt mines. The authors' main interest, however, centers on the isolation and characterization of bacteria associated with the fluid inclusions in halite crystals. The authors have developed an exacting protocol for the isolation of bacteria from these fluid inclusions and have found that the ancient bacteria have remarkable similarity to today's isolates whether in salt mines or surface sources. Thus, their work has profound implications on evolution, phylogeny and long-term survival under stress conditions.

One fascinating aspect of halophile ecology and physiology has been the production and role of halocins. O'Connor and Shand summarize the literature on the production of these small protein antibiotics. Halocins are ribosomally synthesized by members of the Kingdom Archaea and appear to be specific for Archaea with no known effects on Bacteria. As these authors report, some of these halocins are temperature tolerant and are effective inhibitors of other members of the Archaea. In addition, two of the halocins are not cationic and do not form  $\alpha$ -helices, while most bacteriocins are cationic and form  $\alpha$ -helices or  $\beta$ -sheets. As O'Connor and Shand report, one of the halocins, HalH7, even has the same target in mammals as it does in the halobacteria, surely an area worth pursuing further. The authors make the case for further studies, too, on the basic mechanisms of protein synthesis, signal induction and the genetics of halocin production. Finally, the ecological role of halocins has yet to be defined, but presumably halocins could find applications in controlling the bacterial deterioration that can occur during the tanning of hides or the deterioration of salted foods.

In the last paper of this special section, Oren brings together many of the themes mentioned in the other papers to discuss the diversity of halophiles, including *Dunaliella*, and their possible and current biotechnology applications. These current applications range from the production of soy sauce, to  $\beta$ -carotene, to the organic compatible solute ectoine, which is used as an enzyme stabilizer, to encouraging the growth of bacteria in solar salterns to increase the temperature and hence speed water evaporation and increase salt production. Along the way, new microorganisms are being discovered such as the red halophilic *Salinibacter*, which requires at least 15% salt, but are in the Kingdom Bacteria [1]. The biotechnological potential of this new genus has not been investigated as yet, but it holds out the promise of not only the discovery of additional new organisms but also the discovery of new physiological properties.

Correspondence: CD Litchfield, Department of Biology, George Mason University, Fairfax, VA 22030, USA

It is often said that working with such high salt concentrations is difficult and destructive of equipment, but with current abilities to genetically engineer strains, these cease to be valid reasons. Much work needs to be done on the genetics of these bacteria, but some progress is being made. In actuality, it is easy to work with the extreme halophiles as contamination is seldom a problem, and some investigators do not even autoclave their media. Thus, there is a large untapped reservoir of microbial diversity in these hypersaline environments; from these papers, it becomes clear that simply studying only one environment will not reveal all of the metabolic and biotechnological potentials that halophiles possess. Even with our current limited knowledge, the halophilic Bacteria and Archaea constitute an underutilized resource for industrial, food and biotechnological applications ranging from drug delivery systems to biotreatment of saline industrial waste streams.

## References

- 1 Antón J, A Oren, S Benlloch, F Rodríguez-Valera, R Amann and R Rosselló-Mora. 2001. Salinibacter ruber gen. nov., sp. nov., a novel extreme halophile from saltern crystallizer ponds. Int J Syst Evol Microbiol. In press.
- 2 Azizova I. 2000. Halobacteria: a new source of bioactives. *Cosmet Med* (Russian Journal with web page translation: www.cmjournal).
- 3 Brown WW. 1922. Halophilic bacteria. Proc Exp Biol Med 19: 321-322.
- 4 Gibbons NE. 1969. Isolation, growth and requirements of halophilic bacteria. In: Norris JR and DW Ribbons (Eds), Methods in Microbiology, Vol. 3B. Academic Press, New York, pp. 169–185.
- 5 Grant WD. 1999. Halobacteria: ecophysiology, diversity and applications. Abstr Soc Gen Microbiol 143 Meet.: 11.00, p. 21.

- 6 Hof T. 1935. Investigations concerning bacterial life in strong brines. *Recl Trav Bot Neerl* 23: 92–173.
- 7 Larsen H. 1962. Halophilism. In: Gunsalas IC and RY Stanier (Eds), The Bacteria. A Treatise on Structure and Function: Vol. IV. The Physiology of Growth. Academic Press, New York, pp. 297–342.
- 8 Nachum R. 1970. Studies of the extracellular amylase isolated from an extremely halophilic bacterium. PhD Dissertation, University of Southern California. University Microfilms, Ann Arbor, MI, 148 pp. Dissertation No. 70–5225.
- 9 Ng WV, SP Kennedy, GG Mahairas, B Berquist, M Pan, HD Shukla, SR Lasky, NS Baliga, V Thorsson, J Sbrogna, S Swartzell, D Weir, J Hall, TA Dahl, R Welti, YA Goo, B Leithauser, K Keller, R Cruz, MJ Danson, DW Hough, DG Maddocks, PE Jablonski, MP Krebs, CM Angevine, H Dale, TA Isenbarger, RF Peck, M Pohlschroder, JL Spudich, KH Jung, M Alam, T Freitas, S Hou, CJ Daniels, PP Dennis, AD Omer, H Ebhardt, TM Lowe, P Liang, M Riley, L Hood, S DacSarma. 2000. Genome sequence of *Halobacterium* species NRC-1. *Proc Natl Acad Sci USA* 97: 12176–12181.
- 10 Oren A, P Gurevich, M Azachi and Y Henis. 1992. Microbial degradation of pollutants at high salt concentrations. *Biodegradation* 3: 387–398.
- 11 Pfiffner SM, MJ McInerney, GE Jenneman and RM Knapp. 1986. Isolation of halotolerant, thermotolerant, facultative polymer-producing bacteria and characterization of the exopolymer. *Appl Environ Microbiol* 51: 1224–1229.
- 12 Quesada E, V Bejar and C Calvo. 1993. Exopolysaccharide production by Volcaniella eurihalina. Experientia 49: 1037–1041.
- 13 Ramos-Cormenzana A. 1989. Ecological distribution and biotechnological potential of halophilic microorganisms. In: Da Costa MS, JC Duarte and RAD Williams (Eds), Microbiology of Extreme Environments and Its Potential for Biotechnology. Elsevier, New York, pp. 289–309.
- 14 Van Qua D, U Simidu and N Taga. 1981. Purification and some properties of halophilic protease produced by a moderately halophilic marine *Pseudomonas* sp. *Can J Microbiol* 27: 505–510.
- 15 Ventosa A, J Nieto and A Oren. 1998. Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev* 62: 504–544.