

Interactive comment on “Increases in the abundance of microbial genes encoding halotolerance and photosynthesis along a sediment salinity gradient” by T. C. Jeffries et al.

A. Oren (Referee)

orena@cc.huji.ac.il

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The paper by Jeffries et al. explores the microbial communities in the sediments of the Coorong lagoon, South Australia, at a salinity range from 37 to 136 PSU (Practical Salinity Units), using metagenomic DNA libraries. From the analysis of the sequences obtained the authors draw conclusions about the changing community structure along the salinity gradient. They report an increase in cyanobacterial abundance at the higher salinities, and in the hypersaline part of the lagoon they also note an increased presence of genes that may participate in osmotic adaptation: genes encoding proteins involved in the biosynthesis or transport of glycine betaine, ectoine, and other com-

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pounds known to provide osmotic stabilization to different groups of halophilic and halotolerant microorganisms. Metagenomics has been used in recent years in the characterization of a number of hypersaline environments such as solar salterns, the microbial mats of Guerrero Negro, Baja California (references as cited by the authors), Great Salt Lake, Utah (Parnell et al., 2010), and the Dead Sea (Bodaker et al., 2010; Rhodes et al., 2010). The Coorong lagoon study is to my knowledge the first reported metagenomic analysis of microbial communities along a natural salinity gradient.

GENERAL COMMENTS

My main problem with the Jeffries et al. paper is the mode of sampling of the sediments at the four salinities studied. The authors have sampled from each site 10 g of sediment from a core representing the upper 10 cm of the sediment. Although the authors did not provide any information about the vertical structure of the sediment, it may be expected that the samples included aerobic surface sediment as well as anaerobic reducing mud. This is clear from the abundance of different groups of methanogenic Archaea, Clostridia, and (possibly sulfate-reducing) Deltaproteobacteria. Each sample is thus composed of a complex mixture of different microbial communities that had developed along the vertical gradients at each site. It is difficult to believe that the aerobic/anaerobic boundary at the four sampling sites was located at exactly the same depth. Therefore the changes detected in the community structure along the salinity gradient, as illustrated in Fig. 4, may well be due to factors unrelated to the salinity of the pore water: metagenomic analysis of a sample that consists for 90% of aerobic surface sediment will show dominance of very different taxa than a sample largely consisting of anaerobic mud. The study by Kunin et al. (2008) of a microbial mat at 90 PSU in Guerrero Negro showed sharp differences in microbial community structure on a millimeter scale. Jeffries et al. state that no such layered mats were evident in the Coorong lagoon, but still an understanding of the chemical gradients in the upper 10 cm of the sediments sampled is essential for a proper evaluation of the results. By pooling the upper 10 cm of the sediments much information about the structure of the

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microbial communities in the sediments was lost, and it therefore becomes very difficult to compare the metagenomic sequence data from the four sites in a meaningful way.

Metagenomics is an excellent tool to obtain information about the structure of microbial communities in different ecosystems, but it should always be combined with different, independent techniques. Jeffries et al. find evidence for an increased abundance of phototrophic microorganisms, especially cyanobacteria, at the elevated salt concentrations in the lagoon. One does not need metagenomics to draw such a conclusion: simple extraction of chlorophyll and its spectrophotometric or fluorimetric quantification is the first method of choice to compare the abundance of photosynthetic microorganisms. Microscopy can also add much important information. The metagenomic analysis showed apparent abundance of three groups of cyanobacteria in the sediments at the highest salinities: Nostocales, Chroococcales and Oscillatoriales. The latter two groups are the common types in hypersaline cyanobacterial mats (Oren, 2000 [erroneously cited as Oren, 2002 by Jeffries et al.]), but abundance of Nostocales, a group of heterocystous cyanobacteria, it not expected in hypersaline environments. Blooms of Nodularia occur the south arm of Great Salt Lake, Utah, at 60-100 PSU (Roney et al., 2009), but to the best of my knowledge massive occurrence of Nostocales was never reported at higher salinities such as found in the Coorong lagoon. Such heterocystous filamentous cyanobacteria can be easily recognized by their morphology, and a simple microscopic examination of the samples could have confirmed the occurrence of members of the Nostocales. Observation of heterocysts would have further strengthened the discussion about the possible role of the benthic cyanobacteria in the nitrogen cycle in the Coorong lagoon.

Cyanobacteria can be divided into three groups with respect to their modes of adaptation to elevated salt concentrations. The least halotolerant ones generally produce sucrose and/or trehalose as osmotic solutes under salt stress, the most salt-tolerant types use glycine betaine, and many cyanobacteria that grow in marine and slightly hypersaline waters synthesize glucosylglycerol as osmotic solute. The analysis of the

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metagenome of the Coorong lagoon has identified genes involved in sugar metabolism and in the synthesis of glycine betaine. In view of the apparent abundance of cyanobacteria in the more saline samples, a search for genes involved in glucosylglycerol metabolism may be of interest as well. The genes that participate in glucosylglycerol biosynthesis and degradation have been well characterized in model organisms (Hagemann, 2011).

In their discussion about the different organic compounds involved in osmotic adaptation and the genes for their biosynthesis or accumulation, Jeffries et al. also mention the glucans accumulated by some Proteobacteria in the periplasm under osmotic stress (Bohin, 2000). Whether or not the detection of genes potentially involved in the synthesis of such glucans may tell us anything about the mode of osmotic adaptation along the salinity gradient is not clear. Such genes are found in many non-halophiles, and appear to be especially important for bacteria such as *Rhizobium*, *Agrobacterium*, and related taxa that have to interact with a eukaryotic host, something not relevant in hypersaline sediments. I am not aware of any indications that genes for production of such glucans are prominently present in the genomes of salt-tolerant bacteria.

I agree with the assessment by the authors that in the salinity range examined, most microorganisms are expected to use organic 'compatible' solutes for osmotic balance rather than KCl, which is only used by a small number of specialized, mostly extreme halophiles: the archaeal family Halobacteriaceae and a few representatives of the Bacteria: the aerobic *Salinibacter* (Bacteroidetes) and the anaerobic Halanaerobiales (low G+C Firmicutes). Most members of the Halobacteriaceae require at least 150 g/l salt for growth. Therefore the finding of gene sequences assigned to the Halobacteriaceae in the hypersaline part of the Coorong lagoon is of interest and deserves a more in-depth discussion. The finding is not unique, and it may be interesting to compare the sequences obtained with those reported in a metagenomic study of Hamelin Pool, Shark Bay, Western Australia, an environment with a salinity about twice that of sea water (Allen et al., 2009), and with the 16S rRNA genes of *Haloferax* and *Halococcus*

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species recovered from that site (Allen et al., 2008; Goh et al., 2006).

As salt concentrations increase, more and more physiological types of microorganisms disappear, until only a few metabolic types remain active in NaCl-saturated environments. The decrease in metabolic diversity with increasing salt concentration has been evaluated in the past and a theory was presented to explain the observations (Oren, 1999) and recently refined (Oren, 2010). Although the salinities of the samples collected from the Coorong lagoon were all in the lower range, some interesting features relating to the apparent loss of metabolic functions at elevated salt concentrations deserve to be discussed. One is the elevated ammonium concentration in the high-salinity sediments, which may be caused by the inability of autotrophic nitrifying bacteria to function at high salt. Another interesting, and unexpected, observation is the apparent enrichment of methanogenic Archaea of the 'Methanomicrobia' (not a validly published name) at 136 PSU as compared to the 37 PSU sample. Like the Methanobacteria and Methanococci, the members of the order Methanomicrobiales produce methane almost exclusively from hydrogen and carbon dioxide, a process that ceases to function already at a relatively low salinity. At high salinity I rather expect to find enrichment in the Methanosarcinales, many of which metabolize trimethylamine and other methylated amines, formed as breakdown products of glycine betaine. This mode of methanogenesis can even function at salt concentrations above 200-250 g/l (Oren, 1999).

Halophilic prokaryotes that use KCl to provide osmotic balance all possess a high excess of acidic amino acids over basic amino acids in their proteins, and these acidic proteins require high salt concentrations for structural stability and activity (Lanyi, 1974). In contrast, organisms that use organic osmotic solutes do not need special modifications of most of their proteins, except for those extracellular and periplasmic proteins that are in contact with the saline medium, as shown in a study of the genome of *Chromohalobacter salexigens* (Gammaproteobacteria) (Oren et al., 2005). Jeffries et al. correctly state that the 'organic solutes in' strategy may be expected to be the

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most prevalent in the microorganisms inhabiting the Coorong lagoon sediments. The same is undoubtedly true for the Baja California microbial mat at 90 PSU analyzed by Kunin et al. (2008). Still, the average isoelectric point of the proteins predicted from the metagenomic analysis of that mat was conspicuously acid-shifted when compared with isolated non-halophiles and the proteomes of non-hypersaline microbial communities. Most of the acid shift could be attributed to an increased content of aspartate. Thus, already at a relatively low salinity the average isoelectric point of the proteins carries a clear signature related to salt adaptation of the community. Figure 3 in the recent paper by Rhodes et al. (2010) shows the general correlation between the ratio of acidic to basic amino acids encoded in the DNA and the salinity of the environment from which that DNA was extracted. It may be worthwhile to calculate the isoelectric point distribution of the proteins encoded by the DNA from the four sites of different salinities studied by Jeffries et al. and to compare the result with the data published by Kunin et al. (2008) and Rhodes et al. (2010). Such an analysis may well yield interesting new insights into the adaptation of the microbial communities in the Coorong lagoon to the increasing salinity along the gradient.

SPECIFIC MINOR COMMENT

The names of the classes Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria and Epsilonproteobacteria that appear in Fig. 4 were validly published in 2006 (Euzéby, 2006), and therefore these names are to be preferred over α -Proteobacteria, β -Proteobacteria, γ -Proteobacteria, δ -Proteobacteria, and ε -Proteobacteria, used in the text of the article.

REFERENCES

Allen, M. A., Goh, F., Leuko, S., Echigo, A., Kizuki, T., Usami, R., Kamekura, M., Neilan, B. A., and Burns, B. P.: *Haloferax elongans* sp. nov. and *Haloferax mucosum* sp. nov., isolated from microbial mats from Hamelin pool, Shark Bay, Australia. *Int. J. Syst. Evol. Microbiol.* 58, 798-802, 2008.

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Allen, M. A., Goh, F., Burns, B. P., and Neilan, B. A.: Bacterial, archaeal and eukaryotic diversity of smooth and pustular microbial mat communities in the hypersaline lagoon of Shark Bay. *Geobiology* 7, 82-96, 2009.

Bodaker, I., Sharon, I., Suzuki, M. T., Reingersch, R., Shmoish, M., Andreishcheva, E., Sogin, M. L., Rosenberg, M., Belkin, S., Oren, A., and Béjà, O.: Comparative community genomics in the Dead Sea: an increasingly extreme environment. *ISME J.* 4, 399-407, 2010.

Bohin, J.-P.: Osmoregulated periplasmic glucans in Proteobacteria. *FEMS Microbiol. Lett.* 186, 11-19, 2000.

Euzéby, J.: List of new names and new combinations previously effectively, but not validly, published. Validation list no. 107. *Int. J. Syst. Evol. Microbiol.* 56, 1-6, 2006.

Goh, F., Leuko, S., Allen, M. A., Bowman, J. P., Kamekura, M., Neilan, B. A., and Burns, B. P.: *Halococcus hamelinensis* sp. nov., a novel halophilic archaeon isolated from stromatolites in Shark Bay, Australia. *Int. J. Syst. Evol. Microbiol.* 56, 1323-1329, 2006.

Hagemann, M.: Molecular biology of cyanobacterial salt acclimation. *FEMS Microbiol. Rev.* 35, 87-123, 2011.

Kunin, V., Raes, J., Harris, J. K., Spear, J. R., Walker, J. J., Ivanova, N., von Mering, C., Bebout, B. M., Pace, N. R., Bork, P., and Hugenholtz, P.: Millimeter scale genetic gradients and community-level molecular convergence in a hypersaline microbial mat. *Mol. Systems Biol.* 4, 198, 2008.

Lanyi, J. K.: Salt-dependent properties of proteins from extremely halophilic bacteria. *Bacteriol. Rev.* 38, 272-290, 1974.

Oren, A.: Bioenergetic aspects of halophilism. *Microbiol. Mol. Biol. Rev.* 63, 334-348, 1999.

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Oren, A.: Salts and brines in: The Ecology of Cyanobacteria: Their Diversity in Time and Space, edited by: Whitton, B. A., and Potts, M., Kluwer Academic Publishers, Dordrecht, 281-306, 2000.

Oren, A.: Thermodynamic limits to microbial life at high salt concentrations. *Environ. Microbiol. / Microb. Biotechnol.* doi: 10.1111/j.1462-2920-2010.02365.x, 2010.

Oren, A., Larimer, F., Richardson, P., Lapidus, A., and Csonka, L. N.: How to be moderately halophilic with a broad salt tolerance: clues from the genome of *Chromohalobacter salexigens*. *Extremophiles* 9, 275-279, 2005.

Parnell, J. J., Rompato, G., Latta, L. C. IV, Pfender, M. E., van Nostrand, J. D., He, Z., Zhou, J., Andersen, G., Champine, P., Ganesan, B., and Weimer, B. C.: Functional biogeography as evidence of gene transfer in hypersaline microbial communities. *PLoS One* 5, e12919, 2010.

Rhodes, M. E., Fitz-Gibbon, S., Oren, A., and House, C. H.: Amino acid signatures of salinity on an environmental scale with a focus on the Dead Sea. *Environ. Microbiol.* 12, 2613-2623, 2010.

Roney, H. C., Booth, G. M., and Cox, P. A.: Competitive exclusion of cyanobacterial species in the Great Salt Lake. *Extremophiles* 13, 355-361, 2009.

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