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Peer-Review Record:

Halophilic Archaea: Life with Desiccation, Radiation and Oligotrophy over Geological Times

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Reviewer 1: Anonymous
Reviewer 2: Tim Lowenstein

Editors: John A. Baross and William Bains (Guest Editor of Special Issue "The Physico-Chemical Limits of Life")

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First Round of Evaluation

Round 1: Reviewer 1 Report and Author Response

This is a highly readable and very well written review, drawing together material that has not been seen in this collated form before and as such will be the general review that the ancient DNA people and the astrobiologists will refer to for the next few years.

The authors note the ubiquity of Haloquadratum walsbyi in neutral hypersaline hypersaline habitats and its unique features such as flat cells and the production of halomucin that might particularly predispose towards long term survival. However, as far as I am aware, this haloarchaeon has never been isolated from ancient halite, nor has it's DNA been detected. For completeness, I would have thought that a comment about might have been appropriate?

Response: It is true that Haloquadratum walsbyi was not isolated from ancient halite so far. A reference stating this (Gramain et al. 2011) was pointed out now in line 84. However, it has been suggested by Oh et al. (2010) that the nearly ubiquitous global distribution of Haloquadratum walsbyi might be due to its survival in fluid inclusions. This information was now included and the reference Oh et al. (2010) was added.

Round 1: Reviewer 2 Report and Author Response

This is a fascinating review of the halophilic archaea and their adaptations to extreme conditions of desiccation, salinity, radiation, and oligotrophy. Earlier reviews by Grant, 1999 and 2004, for example, have become dated so this manuscript fills in some of the new ideas of the last 10 years. I suggest the following minor revisions:

Lines 38f: The statement about the dating and skepticism of the age of cultured halophiles is not quite right. We never determine the age of organisms (except using radiocarbon, up to about 40,000 years) so dating a single microorganism is not really the problem. The problem with the microbes is contamination, which in macrofossils is much easier to deal with (see for example Satterfield, C.L., Lowenstein, T.K., Vreeland, R.H., Rosenzweig, W.D., and Powers, D.W., 2005, New Evidence for 250 Ma age of halotolerant bacterium from a Permian salt crystal. Geology, v. 33, pp. 265–268.)

Response: The statement was modified to include the contamination issue. The phrase about determination of microbial age was deleted.

Line 45: Please add the reference: Lowenstein, T.K., Timofeeff, M.N., Schubert, B.A., 2011, Microbial communities in fluid inclusions and long-term survival in halite. GSA (Geological Society of America) TODAY, v. 21, no 1, pp. 4–9.

Response: The reference was added (# 18).

Line 68–69: discussion of activity of water: "indicates pure water, whereas a value of 0 indicates the total absence of free water molecules". Water activity of 0 is not possible—water activity is defined as the vapor pressure or fugacity of a water or brine divided by the vapor pressure of pure water. So pure water by definition has activity of 1; since all waters have a measurable vapor pressure, activity of 0 is not possible.

Response: The half sentence containing, value of 0 was deleted.

Line 84: "In any case, *Hqr. walsbyi* is the most dominant microorganism in highly salty ecological niches [29]". This may be an overstatement - we have never observed *Hqr. Walsbyi* in any of the hypersaline lakes in the western US.

Response: The statement was rephrased to include the countries where Hqr. walsbyi was found so far, and the reference of Oh et al. (2010) was added. The most (dominant) was deleted.

Lines 142–144: "An explanation for the spherical particles, which were observed in fluid inclusions of ancient and well-dated halite [13,16], could thus be the transformation from former rod-shaped haloarchaea to roundish forms as a response to lowered external a_w."

This is an interesting explanation, but the a_w inside a fluid inclusion does not change with time and the a_w in the fluid inclusion was probably the same as the brines from which the halite grew. So lowered a_w is probably not the explanation for the rounded cells found in natural fluid inclusions.

Response: In fluid inclusions which contain active microorganisms, metabolism is going on for at least a while, polymers are synthesized, water is being adsorbed to those and other molecules, and

conceivably, the overall water activity in this non-homogenous liquid might change. Of course, this has not been tested yet. We would like to draw attention to the problem, and we have rephrased the statements to make them even more cautiously than before.

We feel this would be an interesting area of future research.

Line 204–205: "Fluid inclusions were also found in billion year old meteorites and represent apparently a very old type of structure in the universe [51]."

Suggest new sentence: "Fluid inclusions with high salt concentrations from a dated meteorite show that water trapped in halite can be preserved for billions of years".

Response: The sentence was modified as suggested.