

The Phylogenetic Characterization of the *Crenarchaeal amoA* from an Alkaline Hotspring of the Lake Magadi Basin

K. Henderson ⁽¹⁾, J. Denson ⁽²⁾, and Mack Ivey ⁽²⁾, ⁽¹⁾ Southern University A&M College, Baton Rouge, Louisiana, Kiara_henderson_00@subr.edu ⁽²⁾ University of Arkansas Department of Biological Sciences Fayetteville, AR

Abstract Lake Magadi, Kenya, is an alkaline, "saline pan" lake system, and approximately 100 sq km in size being geographically the southernmost lake within the Rift Valley of Kenya. Located approximately 80 miles southwest of Nairobi, the lake contains concentrated brines of sodium carbonate as well as large precipitates of the mineral trona, hydrated sodium bicarbonate carbonate ($\text{Na}_3\text{HCO}_3\text{CO}_3 \cdot 2\text{H}_2\text{O}$). The lake water is primarily recharged by meteoric input as well as by a number of saline hot springs (up to 83°C) flowing into the lake's extremely alkaline system (pH values up to ~11). This creates a unique extreme environment, containing both thermal, highly saline, and extreme alkaline features all located in close proximity and overlapping to various degrees [1]. While some microbial diversity of this region has been characterized [2], many of the unique springs of the region have yet to be examined. Here we report the discovery of marine-related *Crenarchaeota* within a thermal feature and the initial phylogenetic placement of the archaeal monoammonium oxygenase (subunit amoA) from this location.

Background: (2° 00' 24.46" S, 36° 14' 05.80" E) The hot spring complexes of the Magadi region contain a wide variety of niches based on initial observations, including phototrophic mats as well as filamentous structures. One complex located at southern tip of the lake contained a unique structural feature associated with a thin (>1cm on average) biofilm (Fig. 1) This biofilm was extensively co-located with a "terraced" erosional structure occurring within the surface of the evaporitic deposit from which the

spring emerged, suggesting it potentially is involved in the formation of these structures. These features occurred both in the surrounding margins of the spring as well as within the spring as terraced mound-like features, with the spacing of the terraces appearing to correlate with the slope of the surface to which the biofilm is attached. These types of microbially associated structures have the potential to serve as biosignatures within the geological record both on Earth as well as Mars.



Fig. 1 Terraced evaporite deposit associated with Microbial Biofilm

Diversity Data: An initial survey of microbial diversity was conducted utilizing 16S rDNA amplicons obtained from bacterial (27F and 1525R, 342F and 1407R), archaeal (8F and 1041R), and nanoarchaeal (9F and 961R) universal primers, which were subsequently cloned and sequenced. 127 bacterial clones and 102 archaeal clones were analyzed utilizing an OTU definition of $\geq 97\%$ identity. Initial analysis suggests that this biofilm is composed predominantly of phototrophic bacteria as well as a number of

related archaeal species related to the marine *Crenarchaeota*. Among the bacterial OTUs obtained, two groups appear to dominate this system - a number of closely related sequences of the alphaproteobacteria, closely related to the photosynthetic genus *Rhodobacter*, and a single OTU most closely related to a *Fusibacter* sp. of the *Firmicutes* known for thiosulfate reduction. *Rhodobacter* sp. are known to possess an extensive range of metabolic capabilities, including photosynthesis, fermentation, aerobic respiration, lithotrophy to obtain reducing equivalents as well as nitrogen fixation [3]. Given the relative abundance of sulfate in this system, it can be envisioned that the sulfate-reducing *Fusibacter* sp. in combination with the photosynthetic alphaproteobacteria (utilizing sulfides produced through sulfate reduction) are potentially responsible for producing these biogenic erosional features [4]. Interestingly while Magadi is known for an abundance of halophilic *Euryarchaeota*, the dominant *Archaea* from this site were most closely related to marine *Crenarchaeota*.

Recently it was discovered that the *Crenarchaeota* possess the capacity for aerobic ammonia oxidation and recent studies suggest they are the dominant nitrifying organisms on the planet ($\text{NH}_3 \rightarrow \text{NO}_2 / \text{NO}_3$). Monoammonium oxygenase is composed of three subunits amoA, amoB, amoC. The amoA subunit has been utilized to phylogenetically classify the archaeal genes into two broad classes, one found predominately in marine environments and one found predominately in soils. In order to classify the amoA subunit from this unique location it was amplified via PCR (amoA_F and amoA_R), cloned, sequenced (96 isolates), and the peptide sequence were compared to representatives of the two broad classes of *Archaeal* enzymes.

Conclusions: These initial results lend support to the concept that a largely microbial biofilm is involved in the production of these these unique terraced features within this hot spring and evaporative basin. It contains a number of both bacteria and archaea known to be chemolithotrophs. Interestingly the dominant archaeal lineages fall within the uncultured marine *Crenarchaeota* in a location meters from Lake Magadi, which is famous for halophilic *Euryarchaeota*. The springs at this site are known to be nomadic, with locations of flow migrating with time. This could possibly lead to preservation of these structures as biosignatures of an active chemolithotrophic ecosystem from a thermal feature being preserved in the geological record. Phylogenetic characterization of the amoA subunit of the *Archaeal* monoammonium oxidase from this site shows that it is most closely related to the marine subgroup, but forms a unique cluster. In addition these sequences contain seven amino acids that are unique to this location, which is the most alkaline environment site reported to date in terms of ammonia oxidizing archaea (AOA) discovery. This could potentially provide structural and functional insight into this ubiquitous enzyme.

Acknowledgements: This work was funded by the National Science Foundation. I would like to thank the Center for Space and Planetary Sciences, and Mack Ivey of the Department of Biological Sciences at the University of Arkansas.

References: [1] Eugster H.P. (1980) *Hyper-sal. Brines and Evap. En*(Elsevier)[2] Rees H.C. et al. (2004) *Extremophiles*, 8, 63–71. [3] Mackenzie C. et al. (2007) *Ann. Rev. Micro.*, 61, 283-307. [4] Ravot G. et al. (1999) *Int. J. Sys. Bact.*49, 1141-1147