The Biogeography of Great Salt Lake Haloarchaea: Testing the Hypothesis of Avian Mechanical Carriers

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Abstract

To explain the vast diversity of microorganisms and their overlapping geographic distributions, Baas Becking famously postulated that “everything is everywhere, but the environment selects.” Recent studies have disputed this idea and suggest other mechanisms of distribution. Haloarchaeal archaea inhabit hypersaline ecosystems all around our planet, and they are highly adapted to desiccating conditions, surviving in salt crystals over long time periods. Genetically similar strains have been found in locales that are geographically isolated from one another. We sought to test the hypothesis that small salt crystals could be carried on bird feathers and that these were the driving force of these distributions through their migration patterns. We collected salt from the shores of the north arm of Great Salt Lake (GSL), then isolated microorganisms from the salt. Subsequently, microorganisms were isolated from salt crystals located on pelican feathers from Gunnison Island in the north arm of GSL. Archaea isolated from the shore and the feathers crystals were primarily Halorubrum genus, with a small portion being Halibaccula genus. Archaea from the feathers showed to be strictly Halorubrum. These species were identified through PCR amplification and sequencing of the 16S RNA gene and compared to similar strains in the GenBank database. We found there to be five geographical locations that Halorubrum and Halocarcula had in common as well as GSL. To evaluate the hypothesis that nearly identical Halorubrum strains exist in salt sites around the globe due to “hitchhiking” on the exterior of birds, we compared these sites against different bird migration patterns that included GSL as a stop-over.

Introduction

- Halophilic archaea inhabit hypersaline environments and have adapted to desiccating conditions which allows for them to survive in salt crystals for long periods of time (e.g. Vneeland et al., 2000).
- Cultivation of Haloarchaebacteriae has shown overrepresentation of Halorubrum and Haloarcula (Benotch et al, 1995; 2001) with 25 known species of Halorubrum and 10 Haloarcula (Oren, 2014). Both Halorubrum and Haloarcula reside in the salt-saturated regions of Great Salt Lake (GSL), a natural hypersaline terminal lake in Utah (Baxter et al., 2005; Tazi et al., 2014; Almeida-Dalmet et al., 2015)
- GSL is the largest lake in the western United States and the fourth largest meromictic lake in the world (Keck and Hassibe, 1979) making it a critical stop on the Pacific flyway for around ten million waterbirds; approximately 250 species (Bellrose, 1989; Ortig et al., 2000; Paul and Manning, 2001; Aldrich and Paul 2002; Neill et al., 2016).
- Halorubrum and Haloarcula have been identified in various geographic locations around the world (Ochsenreiter et al., 2002) and we wanted to test the hypothesis that birds could transport halorubrum in salt crystals on feathers from site to site, as they utilize highly saline lakes and salterns as stopovers during migration.
- We collected chest feathers from American White Pelicans from the Gunnison Island breeding colony and halite from Rozel Bay in the north arm of GSL. We then analyzed the 16S RNA gene and global distribution patterns.

Methods

Collection, cultures, and DNA extraction. Chest feathers were collected from American White Pelicans from the Gunnison Island breeding colony under the migratory bird permit MB105510-0, which allowed for salvage and scientific collection of feathers by migratory birds at GSL. Feathers were individually placed in 8 ml of 23% MGG media, such that the feathers were submerged, for three weeks at 37˚C until cultures were turbid. Salt crystals were collected from the shore at Rozel Bay in the north arm of GSL and dissolved and cultured with shaking in 5 ml of 23% Minimal Growth Media (MGM) (Dyak-Smith, 2018) for three weeks at 37˚C.

Dilutions of each culture were spread on 23% MGM agar plates and incubated at 37˚C for three weeks. Colonies were selected and sub-cultured, into 23% MGM liquid broth, from each plate. We extracted DNA from 1 ml of turbid culture with the FastDNA Spin Kit for Soil (MP Biomedicals, Santa Ana, California). The kit protocol was followed by an ethanol precipitation to further clean the DNA.

PCR amplification, DNA sequencing and GenBank comparisons. To amplify a partial sequence of the archaeal 16S rRNA gene, we used the Tax PCR kit (New England Biolabs, Ipswich, Mass.) The 1HK (5` ATTCGGTGTATCGCTGCCG3`) (Martinez-Murcia et al., 1995; Mankin et al., 1985; DeLong et al., 1994; Mankin et al., 1998) and H589R (5` AGCTACGGCGTTTASGGC3`) primers were used (Almeida-Dalmet et al., 2018). Initial denaturation was at 94˚C for 5 minutes. Then, 45 cycles of denaturation were at 94˚C for 30 seconds, annealing at 58˚C for 30 seconds, elongation at 72˚C for 60 seconds followed by 72˚C for 5 minutes (Almeida-Dalmet et al., 2018; Litchfield et al., 2006). Amplification of this ~550 bp product was observed by analysis on a 2% agarose gel. For successful amplifications, the PCR products were cleaned with a QiAquick PCR Purification Kit (Qiagen, Venlo, Netherlands) and then submitted to the Center for Integrated Biosystems at Utah State University for DNA sequencing. Sequence data were analyzed by the Basic Local Alignment Search Tool (National Library of Medicine, 2018).

Results and Discussion

To investigate the possibility of microorganisms living on the feathers or in the halite on the feathers, we incubated the feathers in broth media and then plated cultures on solid media (Figure 3A). DNA was extracted from isolated strains. We amplified a partial 16S rRNA gene sequence, which should result in a 550 bp PCR product. This confirmed the microorganisms were haloarchaea (Figure 3B).

To compare the cultures from the feathers with those from halite, we collected halite crystals from Rozel Bay, Great Salt Lake (Figure 4)

Conclusion

- Species from the Halorubrum genus were present in both shore halite and feather halite, although the genus Halorubrum was only found in shore halite samples.
- Distribution patterns of Halorubrum and Haloarcula were very similar, and shared several of the same isolation locations.
- The only significant correlation between climate and distribution patterns was salinity (DWS).
- Based on the distribution patterns of Halorubrum and Haloarcula, and the significance of salinity selection, we can provide evidence that birds may act as mechanical carriers of microorganisms through salt crystals, thus contributing to geographic distribution and diversity.

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References

Table 1. Cultured isolates from Great Salt Lake, including closest matched species, percent identity in 16S rRNA gene sequence, and geographic location of match.

Table 2. Cultured isolates from Gunnison Island American White Pelican feathers, including closest matched species, percent identity in 16S rRNA gene sequence, and geographic location of match.

Figure 1. Map of Great Salt Lake depicting the two sampling sites for White American Pelican feathers (left), and halite (right).

Figure 2. A. American White Pelican on Gunnison Island. B. Halite encrusted on American White Pelican feather collected from the Gunnison Island breeding colony.

Figure 3. A. Culture of a Haloarcula species isolated from American White Pelican feathers. B. PCR amplification of the 16S rRNA gene extracted from isolates from feather cultures.

Figure 4. A. Shoreline of the north arm of Great Salt Lake. B. Collected halite from Rozel bay.

Figure 5. Geographic distribution of Halorubrum (red) and Haloarcula (green) based on DNA sequence data collected from GenBank and 16S rRNA sequencing.