Identification of the Microbial Life on the Bonneville Salt Flats

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Abstract

The Bonneville Salt Flats (BSF) are a 30,000-acre expanse of approximately 90% table salt in northern Utah (1). There is currently no research published on the evidence of life on the salt flats, yet despite the fact that the salt flats appear barren and void of life, there may be a microscopic community capable of surviving the harsh conditions. In numerous other "extreme" and high saline environments, such as Antarctica (2,3) the Dead Sea (4), Great Salt Lake (5), and Atacama Desert (6), halophilic organisms were discovered and identified, leading to the possibility that there may be similar organisms on the BSF. If extremophiles can be found on the salt flats, they may be useful in certain biotechnological processes (7). Microbial life that can sustain itself on the salt flats may also provide insight into the potential for life on other planets.

To determine if there is life on the BSF, samples were taken from brine in October 2011 and salt crusts in August 2012 and placed in broth and plate culture to observe if there was growth. In conjunction with the environmental samples, uninoculated plates and broth were stored to serve as negative controls. The presence of numerous colonies on the inoculated plates (and absence of growth on control plates) indicated the salt flats are inhabited by microbial life. Further tests were performed to determine the identities of the microbes inhabiting the BSF by extracting DNA from isolated samples. Purified DNA was then subjected to PCR amplification of the gene that codes for a 16s rRNA gene using either a 1HK and H589R or 8F and U1492 set of primers. The 16S DNA sequences revealed that at least two genera of Archaea inhabit the salt flats, including *Haloarcula species,* and *Halorubrum species*. Current studies are aimed at identifying other isolates and determining if cell population density changes based on the specific physical features of BSF: smooth surfaces of the flats, cracks in the crust, or regions of brine upwelling. Further studies will include looking at whether the population changes based on the season.

Introduction

The Bonneville Salt Flats are a large expanse of land 115 miles west of Salt Lake City composed of approximately 90% table salt (1). Despite the barren appearance of the salt flats, there may be microorganisms present in the brine or salt crystals. Given that halophilic organisms have been found in other high saline environments, such as Antarctica (2,3) the Dead Sea (4), Great Salt Lake (5), and Atacama Desert (6), there may be similar organisms on the salt flats. Halophilic organisms can be used in a variety of biotechnological applications. Some halobacteria can produce large amounts of polyhydroxyalkanoates, a family of polyesters that can be used in biodegradable plastics (7), delayed drug release (7), and bone replacement (7). *Halobacterium halobium* contains a protein that can be used to detect antibodies against an oncogene product and has been used to detect cancer (7), and halobacterial strains have been found to degrade toxic compounds in environmental sites (7). The microbial life that may be found on BSF could be used in a number of industrial applications.

Question

Is there life on the Bonneville Salt Flats? If so, what microbial life comprises the population?

Methods

Figure 1: Image of a "crack" sampling site in the Bonneville Salt Flats

Figure 2: Image of an "upwelling" sampling site surrounded by the surface

Figure 3: Photo of the Bonneville Salt Flats

- Samples were collected from BSF at one of three sampling site varieties: crack, upwelling, or smooth surface
- Salt crystals from each site were dissolved in either 23% MGM or modified R2A
- Samples were incubated either in broth media or on agar plates at 37 ^{o}C
- Individual colonies were isolated from plates and distinguished by colony morphology
- DNA was extracted and PCR was performed on purified DNA using either 1HK and H589R or 8F and U1492 primer sets
- The resulting 16S rna sequence was evaluated with BLAST to determine the isolate identity

Primer	Sequence 5' to 3'	Reference
8F	AGAGTTTGATCCTGGCTCAG	8
U1492	CGGTTACCTTGTTACGACTT	9
1HK	ATTCCGGTTGATCCTGCCGG	10
H589R	AGCTACGGACGCTTTAGGC	10

Table 1: Primers used in PCR and their respective sequences

Results

The following figures show the three sampling sites plated onto the two different types of media used in this study. The table lists the closest relatives to each isolate.

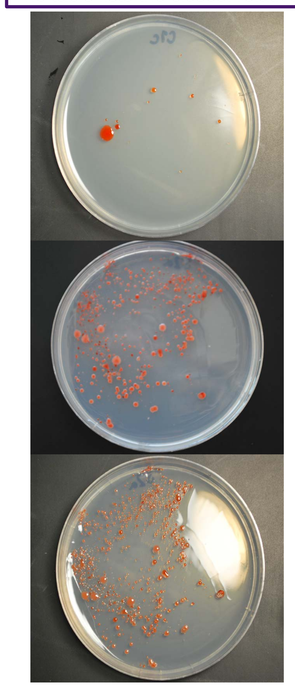

Figure 4: From top to bottom: crack, surface, and upwelling samples streaked onto modified R2A plates.

Table 1- 1st Round Colony Isolation Sequence Results		
Culture	Closest Relatives	% Similarity to Known Species
White	Haloarcula argentinensis strain JCM9737	99%
	Haloarcula Tradensis strain HST03	99%
Pink	Archaeon BC32	98%
	Haloarcula japonica strain JCM7785	99%
Red	Halorubrum sp. SYM	99%
	Halorubrum trapanicum	99%
Pink	Haloarcula sp. A283	99%
	Haloarcula sp. PV7	99%
Red	Halorubrum ejinorense	99%
	Halorubrum ejinorense	99%
White	Haloarcula sp. SP2(1)	99%
	Haloarcula sp. PV7	99%
Pink	Halorubrum sp. SYM	99%
	Archaeon GS11A	99%
Red	Haloarcula argentinensis strain SD45-2_DGR	99%
	Haloarcula argentinensis strain SD45-2_DGR	99%
White	Salicola marasensis strain 7Sm5	98%
	Salicola salis strain B2	97%
Pink	Haloarchaeon SC8	98%

Table 2: DNA sequencing results from the first round of colony isolation and identification. These colonies were all grown on 23% MGM.

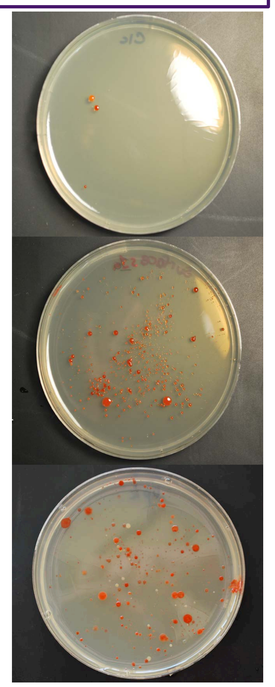

Figure 5: From top to bottom: crack, surface, and upwelling samples streaked onto MGM plates.

Results

Turbidity in the broth and visible growth on the plates was seen after about 1 week of incubation. Lack of growth on uninoculated control plates indicated that the growth we saw on plates that contained BSF samples demonstrated that microbes came from BSF and not contamination. Multiple colony morphologies could be seen, as evidenced by figures 4 and 5. Colonies usually appeared to be either red, pink/orange, or white, and ranged from 0.1 mm to approximately 2 mm in diameter after 1 week.

The colonies formed in varying time sequences, with new colonies appearing weeks after the original colonies have formed. Initial findings indicate that there is a greater number of culturable microbes in the cracks and upwellings than the surface. The results from the first round of DNA sequencing are shown in table 2. The majority of the isolates were either *Halorubrum* or *Haloarcula*.

Conclusions

- There is microbial life on the Bonneville Salt Flats
- There are multiple species of halophiles, including *Halorubrum* and *Haloarcula*.

Future Work

We continue to work on colony isolation and identification by selecting for growth of BSF isolates on different kinds of media. Culture independent methods are being developed to determine the identity of all microbial life inhabiting BSF including both culturable and unculturable microbes.

Future goals include determining whether the species composition of BSF changes seasonally and whether the differences in the numbers of microbes we are finding between cracks, smooth surfaces, and upwellings provides clues about the origin of the microbes or overall viability of these communities.

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