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EVIDENCE FOR THE DISCHARGE OF HYDROTHERMAL WATER INTO LAKE LUCERO, WHITE SANDS NATIONAL MONUMENT, SOUTHERN NEW MEXICO

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Abstract.—Ground-water and soil samples from the western boundary of the playa Lake Lucero were analyzed for chemical and microbial composition. DNA analysis revealed the detection of *Methanosaeta thermoacetophila*, a microbe diagnostic for deep, hydrothermal water. This discovery led to the hypothesis that hydrothermal water discharges into ground water beneath Lake Lucero. Further support for this hypothesis is provided by (1) the presence of hydrothermal minerals in the soil, (2) abnormally high ground-water temperatures, and (3) a high ^{18}O isotope fractionation inconsistent with salinity effects alone. The inflow of hydrothermal water into an alkaline playa lake makes this area an extremely interesting study site for origin of life theories.

INTRODUCTION

Lake Lucero is a hypersaline, ephemeral playa that is the source of the most extensive gypsum dune field in North America (Allmendinger and Titus, 1973; LeMone, 1987). The playa is located on the western boundary in the Alkali Flat of White Sands National Monument (WSNM) (Fig. 1). Lake Lucero is flooded during the rainy season with fresh water, while during the dry season salts are deposited and the lake completely evaporates.

Lake Lucero is a remnant of Lake Otero that existed between 10,000 and 24,000 years ago during the Pleistocene covering an area of more than 4,000 km^2 (Herrick, 1904). The surface of Lake Lucero is a crust of white powdery gypsum with patches of halite. Below this crust are up to 10-cm thick beds of clay, gypsiferous clay, and coarse-grained gypsum crystals. The water table is approximately 1 m below ground surface, varying greatly with seasons, which occasionally leads to the flooding of the playa. Lake Lucero is the lowest topographic point of the Tularosa Basin with an elevation of 1,186 m above mean see level and the ground-water discharge point for the basin (Barud-Zubillaga, 2000).

Exploration of the deeper subsurface was conducted by installing various wells. The RATSCAT well at the northern boundary of WSNM within the Alkali Flat region penetrated about 49 m of recrystallized gypsum (Orr and Myers, 1986). Five wells were installed by the USGS approximately 10 km southeast of Lake Lucero and monitored for water quality (Basabilvazo et al., 1994). Total dissolved solid concentrations ranged from about 8,000 mg/l at a depth of about 30 m below ground surface (bgs) to values up to 111,000 mg/l at a depth of 250 m bgs.

The region is characterized by high heat flow and recent tectonism with the Rio Grande Rift providing a suitable setting for low-to-intermediate hydrothermal systems (< 150°C). The best local example is the Tortugas Mountain Geothermal Area, southwest of the San Andres Mountains, which is characterized by high a regional heat flow of > 84 mWm^{-2} (Decker and Smithson, 1975). Several hydrothermal wells are advanced in the geothermal area that supply New Mexico State University with heating during the winter months (Schulze-Makuch and Kennedy, 2000). Other evidence of thermal activity of the region are 1,000 – 5,000 year old basalt flows about 60 km north of Lake Lucero (USGS, 1965)

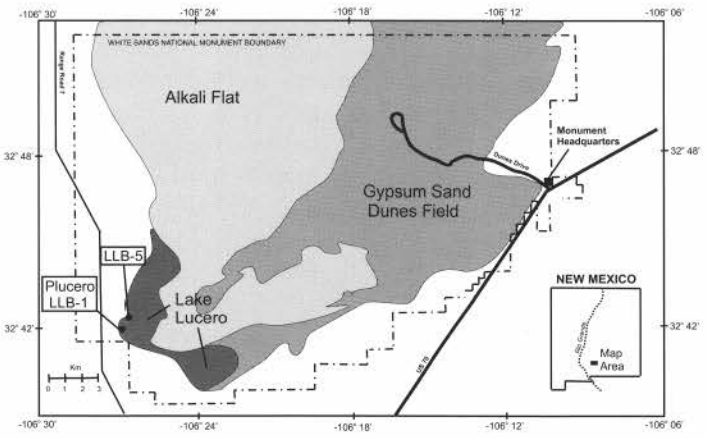

FIGURE 1. Study area with sampling locations (modified from Barud-Zubillaga, 2000).

and several hydrothermal water wells located about 50 km south of WSNM, which are used by the White Sands Missile Range. No hydrothermal activity, however, has been detected at WSNM previously. The exact route of hydrothermal upflow from the Rio Grande Rift source is largely unknown, but appears to be determined by the orientation and extension of fault zones within the hard rock that underlies the basin fill and alluvial sediments.

METHODS

One ground-water sample was extracted with a bailer from an installed piezometer (Plucero) at the western margin of Lake Lucero. One soil sample was collected next to the piezometer (LLB-1), and a second soil sample was collected about 1 km north at the fan delta of an arroyo that carries water and sediment into Lake Lucero (LLB-5). Sample locations are shown in Figure 1. The soil sample LLB-1 contained a powdery salt crust with moist mud beneath it, while LLB-5 contained dry brown coarse sand indicative for sediments that derived from the nearby San Andres Mountains, which are mixed in with evaporates from Lake Lucero. An X-ray diffractometer analysis was performed on the soil samples.

The bailed ground water was measured for field parameters such as electrical conductivity, pH, temperature, dissolved oxygen and redox-potential. A pair of sterile gloves was used to fill pre-prepared sampling bottles. A water sample collected for phospolipid fatty acid (PLFA) analysis had formaldehyde as preservative (20 ml of 37 % formaldehyde solution in a 2-L bottle), water collected for anion analysis had a chemical-specific preservative. Water samples collected for cation analysis were collected in sterile sampling bottles and acidified within 12 hours after sampling. Samples collected for Denaturing Gradient Gel Electrophoresis were collected in sterile 2-L sample containers and did not have a preservative. Soil samples were collected in 1-L sample containers. Samples collected for microbial and chemical analysis were put on ice and shipped overnight to commercial laboratories.

The PLFA analysis was used to measure viable microbial biomass and microbial community composition. The total amount of PLFA content provides a quantitative measure of the viable microbial biomass present in the sample. In addition, these fatty acids are suitable taxonomic markers, because different groups of microorganisms synthesize a variety of PLFA through various biochemical pathways. These correlations can be so strong that fatty acid biomarkers have been identified for particular organisms (Edlund et al. 1985; Dowling et al. 1986; White et al. 1980; Bhat and Carlson 1992). The lipids were analyzed using the modified Bligh and Dyer method, as described by Smith et al. (1986). Lipids were recovered, dissolved in chloroform, and fractionated on disposable silicic acid column into neutral-, glyco-, and polar-lipid fractions. The polar lipid fraction was trans-esterfied with mild alkali to recover the PLFA as methyl esters in hexane. PLFA were analyzed by gas chromatography with peak conformation performed by electron impact mass spectrometry (GC/MS). The method can distinguish among 6 structure groups: monoenoics, terminally branched saturated, branched monoenoic, mid-chain branched saturated, normal saturated and eukaryotic PLFA. In addition to the structure group, several biomarkers were identified which are typical for specific prokaryotic and eukaryotic microbes. For example, the biomarker 20:4w6 has a total of 20 carbons in the fatty acid, 4 double bonds from the aliphatic [w] end of the molecule, and the 6^{th} carbon atom is positioned from the aliphatic end before the double bond. This is characteristic for protozoa.

Denaturing Gradient Gel Electrophoresis (DGGE) was used as the nucleic-acid analysis technique, because it can be used to identify primary species of an ecosystem. The DGGE approach directly determines the species composition of complex microbial populations based on the amplification of 16S rDNA fragments in polyacrylamide gels containing a linearity-increasing gradient of denaturants. DNA fragments of the same length but with different base-pair sequences are separated based on their melting behavior in a polyacrylamide gel. The banding patterns and relative intensities of the recovered bands provide a measure of changes in the community. Although polymerase chain reaction (PCR) may not amplify each species equally, dominant species, which compose at least 1% of the total community in order to remain above the background level of minor bacterial amplification products, can generally be excised and sequenced. Fine-scale sequence analyses of individual bands are then used to infer the identity of the source organisms, based upon database searches and phylogenetic methods. The community microbial DNA was directly extracted from the sample, using the method described by Lehman et al. (1995). Polymerase chain reaction (PCR) amplification of 16S rDNA gene fragments was performed using the method described in Muyzer et al. (1993) with modifications as described in Schulze-Makuch and Kennedy (2000). The bands were sequenced to determine the exact bacterial and archaeal species. The intensity of each band provides a measure of the relative abundance of each species. This relatively novel technique is suited for sites (such as in this study) where only a few dominant microbial populations are hypothesized to exist (Muyzer et al. 1993, Ferris et al. 1996, Kowalchuk et al. 1997).

RESULTS

Results of the measured field and chemical parameters of the water sample from the piezometer are shown in Table 1. Results indicate that salt concentrations are very high at the southwest corner of Lake Lucero. Water is more dilute further north near LLB-5, probably due to shallow ground-water influx from the San Andres Mountains (Barud-Zubillaga, 2000).

X-ray diffractometer analysis of the soil samples taken at LLB-1 and LLB-5 indicated not only the presence of gypsum, but also minerals such as apatite, calcite, a mineral consisting of mixed layers of smectite and illite, zeolite, tosudite, and mirabilite. SEM images from one sample show unidentified microbes on mineral crystal faces. The detected minerals of zeolites, tosudite and the smectite/illite may suggest that some of the minerals in the soil had an association with hydrothermal origin.

TABLE 1. Field parameters and ion composition of ground water from sampling location Plucero.

Parameter	Plucero
Field Parameters	
Temperature (°C)	23.6
PH	8.1
Dissolved Oxygen	1.0
Redox-Potential (mV)	-67
Electrical Conductivity (microS/cm)	129,000
Major Cations (mg/L)	
Sodium	53,200
Magnesium	14,300
Potassium	2,240
Calcium	370
Iron	1
Major Anions (mg/L)	
Chloride	82,000
Sulfate	24,270
HCO_3^-	5,080
Nitrate	< 1
Phosphate	< 1

The microbial communities from the one ground-water sample and 2 soil samples were characterized using PLFA and DGGE. Biomass content in the soil samples was highest in LLB-1 with about 2 x 10^7 cells/g dry weight and somewhat lower at LLB-5. The water sample had the lowest biomass content with about 2.3 x 10^5 cells/mL filtered. All three samples contained relative diverse microbial communities composed primarily of Gram negative bacteria. Monoenoic, terminally branched saturated, branched monoenoic, mid-chain branched saturated, normal saturated and eukaryotic PLFA could be identified in each sample. Branched monoenoic and mid-chain branched saturated PLFA is indicative for anaerobic metal or iron reducing bacteria. Particularly, the Plucero and LLB-1 sample contained high proportions of biomarkers indicative of sulfate or iron reducing bacteria (e.g. biomarker 10me16:0). Biomarkers for fungi, algae and protozoa were also detected in all three samples, as well as a biomarker for diatoms (20:5w3) in samples LLB-1 and LLB-5.

Samples Plucero and LLB-5 contained amplifiable DNA (DNA results from LLB-1 could not be obtained due to some type of inhibition). The DGGE profiles for these two samples contained several dominant organisms (Fig. 2). Sequence results indicated that the Plucero sample was dominated by anaerobic sulfate or iron reducing bacteria such as *Desulfobulbus* and *Desulfobotulus* (Table 2). Band 5, 6 and 9 contained novel sequences loosely associated with Cytophaga. The majority of the organisms identified in sample LLB-5 were closely affiliated with the alpha subdivision of Proteobacteria such as *Sphingomonas, Methylomonas*, and *Hyphomicrobium*. Band 11 represents a novel sequence loosely associated with the genus *Sphingomonas*.

DNA profiles using Archaea primers could also be identified from Plucero but not from LLB-5 (Fig. 2). The majority of these organisms were closely associated with the genus Haloarcula. Haloarcula species thrive under hypersaline, playa condition and are associated with the Halobacteriales within the Euryarchaeota. Unexpectedly, however, a DNA profile from *Methanosaeta thermoacetophila* could also be sequenced and identified (Table 2). *Methanosaeta thermoacetophila* was formerly known as *Methanothrix*. They are thermophilic organisms that thrive at temperatures of at least 60°C, in a slightly acidic to neutral environment (Boone et al., 2001), and with methanogenesis as metabolic pathway.

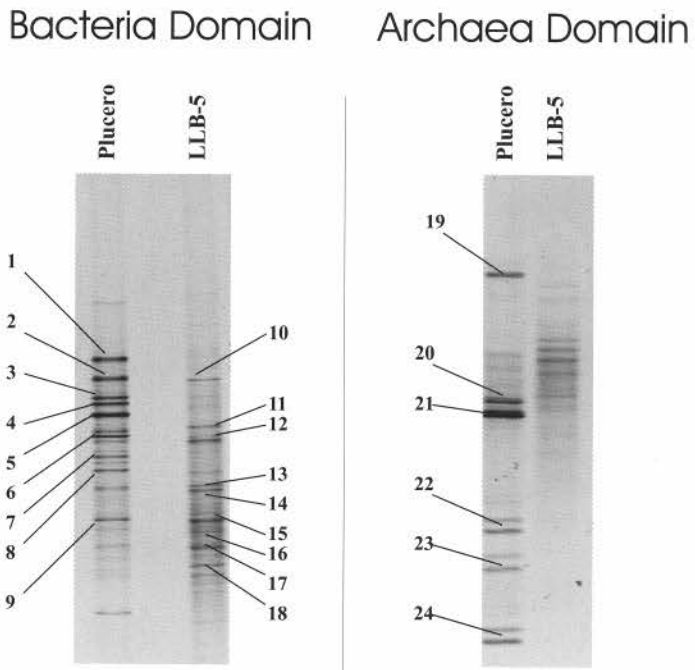

FIGURE 2. DGGE profiles for Eubacteria and Archaea. Band numbers are defined in Table 2.

DISCUSSION

Thermophilic microbial organisms, which prefer slightly acidophilic to neutrophilic conditions are not likely to be found in an alkaline, playa environment, such as Lake Lucero. The organisms may have derived from a source of water, which is acidic and at a temperature of at least 60°C. Geothermal sources in proximity to Lake Lucero are well known. Schulze-Makuch and Kennedy (2000) sampled the Tortugas Mountain Geothermal Area southwest of Lake Lucero and detected a related archaeal organism: *Methanobacterium formicicum*. Thus, it appears that the geothermal water evident to discharge at Lake Lucero may have the same source as the Tortugas Mountain Geothermal Area. Further evidence for the discharge of hydrothermal fluids are the minerals detected in the soil that are commonly associated with a hydrothermal origin. Also, Barud-Zubillaga (2000) sampled piezometer Plucero and showed abnormally high ground-water temperatures and a higher ^{18}O isotope fractionation than that would be expected based on salinity alone. These observations are consistent with hydrothermal upflow from the Rio Grande Rift into the southwestern part of Lake Lucero as illustrated in Figure 3.

The discharge of acidic, hydrothermal water into an alkaline gypsum-rich playa lake makes Lake Lucero and White Sands National Monument one of the most interesting places for studying the origin of life. There are a variety of environmental constrains which are needed for the formation of the first pre-cellular structures based on current knowledge. The Lake Lucero setting is one of a very small number of natural environments for which these constrains or conditions are present. They include (1) hydrothermal, acidic conditions, (2) alkaline, high-salinity conditions, (3) wetting and drying cycles, (4) freezing and thaw-

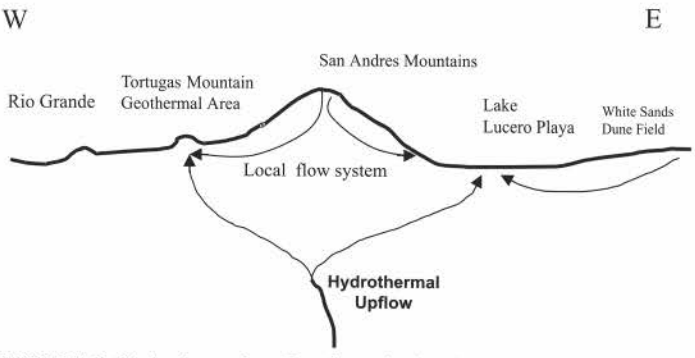

FIGURE 3. Hydrothermal upflow hypothesized to exist at Lake Lucero, schematic.

TABLE 2. Sequence results using eubacterial primers

Band	% Match	Best Match	Phylogenetic Affliation	Sequence (V3 region 3'-5' end)
1	95%	Pelobacter sp.	Delta Proteobacter	CCGTGCTTCCTTTNTGGACCGTAAACTANTNCTNTTAAACTGANNATTTTTTTCCNTTGACAGAGCTTTACgACTCgAAAGCCTTCaTCACTCACGCGGCGTTGCTGCGTCAGGGTTTCCCCATTGCGCAAAATTCTACTGCTGCCTCCCGTAGG
2	97%	Desulfobulbus sp.	Alpha Proteobacteria	CGGAATTAGCCGTGCTTCTCTGATGGTACCNTCAAANCACCTGCTGTTAACANGTATGAATTTATTCCCATCTGACAGAGCTTTACAACCCGAAGGCCTTCTTcACTCACGCGGCGTCGCTGcGTCAGGGTTTCCeCCATTGCGCAAtATTCTCACTGCTGCTCCCGTAGG
3	98%	Desulfobotulus sp.	Delta Proteobacteria	CGGAATAGCCGTGCTTACTNTGATGGTACCTTAAACACACNTGCTGTTAACACGAGTGAACTTATTCCCATCTGACAGAGCTTTACAACCCGAAGGCCTTCTTCACTCACGCGGCGTtGCTGcGTCAGGGTTTCCCCCATTGCGCAAAATTCCTCACTGCTGCCTCCCGTAGCCCCCCCG
4	95%	Desulfobotulus sp.	Delta Proteobacteria	CGGAATTAGCCCGTGCTTACTNTGATGGTACCGTCAAACACCCGTGCTGTTAACACGAATGAATTTATTCCCATCTGACAGAGCTTTACAACCCGAAGGCCTTCTTCACTCACGCGGCGTGGCTGAGTcAGGGTTTCCCCCATTGCGCAATATTCCTCACTGCTGCCTCCCGTAGG
5	-	Novel Sequence affliated with the Cytophaga group	Flexibacter-Cytoph-aga-Bacteroides	AGCCCGATGCTTATTCCTGCAGTACCGTCAATCCCGTATACATACGGGAGATTCTTCCTGCAGAAAAGCAGTTTACAACCCGTAGGGCTGTCATCCTGCACGCGGCATGGCTGCGTCAGAGTTTCCTCCATTGCGCAATATTCCTCACTGCTGCCTCCCGTAGG
6	-	Novel Sequence affliated with the Cytophaga group	Flexibacter-Cytoph-aga-Bacteroides	TAGCCGATGCTTATTCCTGCAGTACCGTCAAATCCGTACACGTACGGAAGGTTCTTCCTGCAGAAAAGCAGTTTACAACCCATAGGGCCGTCATCCTGCACGCGGCATGGCTGCGTCAGAGTTTCCTCCATTGCGCAATATTCCTCACTGCTGCCTCCCGTAGG
7	-	Failed	-	
8	-	Failed	-	
9	-	Novel Sequence affliated with the Cytophaga group	Flexibacter-Cytoph-aga-Bacteroides	AATTAGCCCGTGCTTATTCCTGCgGTACCGTCAAgCCCGTATACATACcGCAGGTTCTTCCTGCatAAAAGCAGtTTACAACCCGCAGGGCTGTcATCCTGCACGCGGCATGGCTGCGTCAGAGTTTCCTCCATTGCGCAATATTACTCACTGCTGCCTCCCGTAgG
10	99%	Bacillus benzo-evornas	Gram Positive bacteria	ACGTAGTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGGTACGAGCAGTTACTCTCGTACTTGTTCTTCCCTAACAACAGAGTTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGG
11	-	Novel Sequence affliated with the Sphingomonas group	Alpha Proteobacteria	GCCCGGaGCTTATTCTGCAGGTACCGTCATTaTCGTcGCTGGTaAAAGAGCtGTAcAACCCTaagGCCTTcATcAcTcACGCGGCGTtGGtGGaTCAGGCTTtCgCCcATTGTCCAaTATtAcCCACTGCTGCCTTcCGTAGG
12	99%	Sphingomonas sp.	Alpha Proteobacteria	TAGCCGGGCTTATTCTCCCGGTACTGTCATTATCATCCCGGGTAAAAGAGCTTTACAACCCTAAGGCCTTCATCACTCACGCGGCATTGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTcCCGTAGG
13	-	Failed	-	
14	97%	Methylobacterium sp.	Alpha Proteobacteria	ACGAAGTAGCCGGGGCTTCTTCTtCcgGTACCGTCATTATCGTcCCgGACGAAAGAGCTTTACAACCCTAAGGcCTTGATCACTcACGCGGCaTGGCTGGATcAGGCTTGCgCCCATTGTcCAATATTcCCCACTGCTGcCTTCCGTAGG
15	-	Failed	-	
16	98%	Hyphomicrobium sp.	Alpha Proteobacteria	TAGCCGGGCTTTTCTGCgGGTACCGTCATTATCGTcCCCGCCGAAAGAGCTTTACAACCCTAAGGCCTTcATCACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTTCCGTAGG
17	-	Failed	-	
18	-	Failed	-	
19	-	Failed	-	
20	94%	Haloarcula sp.	Halobacteriales within Euryarchaeota	CGGTCTTGCCCAGCCCTTATTcAtGtACcCTCTTACGGTTCAGAAAAGCGAGGGCTCTATGCCCTCGCACTCGGAGTCCCcATATCGCACTGTCgTGCaGTGTAAAGGTTTCGcGCcTGcTGCgCCCCGTaGG
21	95%	Haloarcula sp.	Halobacteriales within Euryarchaeota	GGTCTTGCCCAGCCCTTTTTCATGTACCACTCTTACGGTTCAGAAAAGCGAGGGCTCTATGCCCTCGCACTCGGAGTCCCCCTATCGCACTGTCGtGCaGTGTAAAGGTTTCGCGCCTGCTGCACCCCGTAGG
22	96%	Haloarcula sp.	Halobacteriales within Euryarchaeota	GGTCTTGCCCAGCCCTTTTNANNTACNCTCTTAcGGTTCAGAAAAGCGAGGAGCTTTaTGCCcTCACCACTcGGAGtCCCcCTATCGCACTGTCgtGCAGTGTAAAGGTTTCGCGCCTGCTGCACCCCGTAGG
23	-	Failed	-	
24	98%	Methanosaeta thermoacetophila	Methanosarcinales within Euryarchaeota	CACCGGTCTTGCCCGGTCCTTCCTAACCCAAGCTTTTTAGGCTCGGGGACAGCCCACGCAGTGCGCGGGCACTCGGGATACCCTTATCGCGGTTGCCCGCATTGTAAAGGTTTCGCGCCTGCTGCACCCCGTAGG

Note: The portion of the amplified 16SrDNA gene is the V3 region and sequences are listed from the 5' end. A = adenine, C= cytosine, G = guanine, and T = thymine.

ing cycles (e.g. during the winter months at Lake Lucero, and (5) availability of phosphate, which is present as apatite as an erosional product from the San Andres Mountains.

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A collection of namesakes; the White Sands in the foreground (title of this guidebook) and San Andres Peak (also the name of the mountain range) in the background. Photo by Virgil Lueth.