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Microbiology and Geochemistry of Smith Creek and Grass Valley Hot Springs: Emerging Evidence for Wide Distribution of Novel Thermophilic Lineages in the US Great Basin

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INTRODUCTION

The endorheic Great Basin (GB) region in the western US is host to a variety of non-acidic geothermal spring systems. Heating of the majority of these systems is due to their association with range-front faults, in contrast to caldera-associated hot springs in systems such as Yellowstone National Park and Kamchatka (Faulds et al., 2006). Previous characterization of two geothermal systems in the GB, Great Boiling/Mud Hot springs (Costa et al., 2009) and Little Hot Creek (Vick et al., 2010), indicated that they host several novel deep lineages of potentially abundant *Bacteria* and *Archaea*. To expand the knowledge base of the microbiology and geochemistry of GB hot springs and facilitate their comparison to other terrestrial geothermal systems worldwide, we present here the characterization of Smith Creek (SC1) and Grass Valley (GVS1) hot springs in central Nevada, US.

METHODS

Sampling was done at 39º18.816'N, 117º32.778'W (WGS84 datum) for SC1 and 39º56.462'N, 116º40.941'W for GVS1. SC1 is within the Southern Smith Creek Valley springs region and GVS1 is just southeast of Hot Spring Point, as designated by the Nevada Bureau of Mines and Geology website (http://www.nbmg.unr.edu/geothermal/gthome. tm). Sample collection, DNA extraction, field measurements and water chemistry analysis were performed with source pool samples essentially as described (Vick et al., 2010). 16S rRNA gene libraries were constructed using PCR forward primers 9bF (specific for *Bacteria*; L2) or 8aF (specific for *Archaea*; L4) in conjunction with reverse primer 1406uR as described in Costa et al. (2009). 48 clones from each library were sequenced using the Sanger method and the appropriate forward primer used in PCR. Low quality and chimeric sequences were removed, the remaining sequences were grouped into operational taxonomic units (OTUs) at the 3% level using DOTUR (Schloss and Handelsman, 2005), and representative sequences were assigned to named phylogenetic groups based on BLASTn analysis (Altschul et al., 1997) using the NCBI non-redundant nucleotide database.

RESULTS

Table 1 shows the temperature and selected chemical analyses of spring water at the sampling site. Both springs were circumneutral to alkaline with bicarbonate being the primary buffer, and had moderate amounts of dissolved silica and total ammonia (~80 μM). GVS1 was aerobic, with dissolved O_2 at about 1/4 saturation with respect to atmospheric O_2, and sul-

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fide was not detected. SC1 was apparently anaerobic with high levels of sulfide.

Table 1 Spring water temperature and selected chemistry[a]

	SC1	GVS1
Field measurements		
Temperature (℃)[b]	77.6±0.1	65–90[c]
Conductivity (mS/cm)[b]	0.75±0.02	n.a.[d]
pH	7.7[b]	n.a.
Alkalinity (ppm $CaCO_3$)[e]	200	360
Dissolved silica (mM)[e]	0.75	1.51
Total sulphide (μM)[e]	16.8	b.d.[f]
Total ammonia (μM)[e]	74	84
Nitrite (μM)[e]	18.2	21.7
Nitrate (μM)[e]	1.2	b.d.[f]
Oxygen (μM)[e]	b.d.[f]	43.8
Major ions (millimolal)[g]		
Na^+	7.19	5.46
K^+	0.189	1.05
Ca^{2+}	0.139	1.15
Mg^{2+}	b.d.[f]	0.465
Cl^-	0.67	0.781
SO_4^{2-}	1.06	0.517

[a] SC1 and GVS1 were sampled on 5/15/06 and 5/16/06, respectively; [b] Data not measured during sampling. Taken from the Nevada Bureau of Mines and Geology website (http://www.nbmg.unr.edu/geothermal/gthome.htm); [c] Sampled from a site with a steep temperature gradient; [d] n.a.. not analyzed; [e] Measured only once during this sampling trip; [f] b.d.. below detection limit for sulphide (0.3 μM), nitrate (0.7 μM), oxygen (3 μM) and Mg^{2+} (7×10^{-3} mmol/kg); [g] Measured by ion chromatography. Analytical error associated with duplicate measurements of a single sample was <10%. Phosphate (PO_4^{3-}) was below the detection limit of 4.7×10^{-4} mmol/kg.

Analysis of 16S rRNA gene libraries indicated the presence of a diversity of *Bacteria* (9 OTUs in SC1, 11 in GVS1) and *Archaea* (17 OTUs in SC1, 11 in GVS1). OTUs representing two or more sequences are listed in Table 2. All singletons failed to belong to formally described phyla except for two archaea in SC1 (one *Archaeoglobales* and one *Desulfurococcales*) and one archaeon in GVS1 (*Korarchaeota*). Almost 60% of OTUs had 16S rRNA gene sequences found in other GB hot springs as at least one of their two top BLASTn hits. In sum, only ~35% of the sequences could be affiliated to known phyla (for *Bacteria*) or orders (for *Archaea*). Of those that could be classified, the presence of members of *Thermodesulfobacteria*, *Archaeoglobales* and *Desulfurococcales* suggests the utilization of terminal electron acceptors such as sulphate or ferric iron in addition to or in lieu of O_2. *Thermus* was abundant in the clone libraries and may play an important role in coupling heterotrophy to oxygen or nitrate respiration. The abundance of sequences in GVS1_L2 closely related to moderately thermophilic *Cyanobacteria* and photosynthetic *Chloroflexi* as well as hyperthermophiles such as *Geothermobacterium* and *Korarchaeota* is consistent with the steep temperature gradient observed at the site during sampling (~65–90 ℃). All 16S rRNA gene sequences from this study will be deposited in Genbank.

CONCLUSIONS

SC1 and GVS1 were broadly similar to other high-temperature (>75 ℃) GB springs. The majority of the 16S rRNA gene sequences had top BLASTn hits to sequences detected in other GB springs, supporting the hypothesis that geothermal springs in this region have microbial lineages that are specific to the region. These springs are also similar to other Great Basin hot springs in the relative abundance of deep, novel phylogenetic lineages (i.e. candidate classes and phyla). OTUs GVS1_L2_F02 and SC1_L2_F10 showed high identity (>97%) to uncharacterized, deeply branching members of the *Chloroflexi* that are dominant bacterial phylotypes in clone libraries in other GB hot springs (e.g. GBS_L1_A03 in Great Boiling Spring) but rare elsewhere (Vick et al., 2010; Costa et al., 2009). Members of the candidate phylum OP1 dominated the bacterial library of SC1, similar to GB spring LHC4, and both springs share broadly similar water chemistry. Archaeal communities appeared to be dominated by novel *Crenarchaeota*, similar to both GB and many Yellowstone National Park hot springs (Vick et al., 2010; Costa et al., 2009; Huang et al., 2007), with only singletons representing the *Korarchaeota* and the *Euryarchaeota* (*Archaeoglobales*).

Table 2 OTUs from GVS1 and SC1 representing more than one sequences

Representative sequence	# in OTU[a]	Cultured microorganism whose 16S rRNA gene has the highest BLASTn hit to the OTU representative sequence			Phylogenetic affiliation of OTU[b]
		Organism name and strain	Accession #	id.[c]	
GVS1 Bacteria					
GVS1_L2_E01	12	*Synechococcus* sp. JA-3-3Ab	CP000239.1	98%	*Cyanobacteria*
GVS1_L2_B06	7	*Thermus* sp. A03C	EF204914.1	98%	*Thermus-Deinococcus*
GVS1_L2_C05	5	*Chloroflexus* sp. 396-1	AJ308498.1	96%	*Chloroflexi*
GVS1_L2_A04	4	*Thermus oshimai* SPS-17	NR_026503.1	99%	*Thermus-Deinococcus*
GVS1_L2_G02	3	*Thermocrinis* sp. P2L2B	AJ320219.1	95%	*Aquificae*
GVS1_L2_F03	2	*Geothermobacterium ferrireducens* FW-1a	AF411013.1	98%	*Thermodesulfobacteria*
GVS1_L2_A01	2	*Synechococcus* sp. C9	AF132773.1	98%	*Cyanobacteria*
GVS1_L2_A05	2	*Geothermobacterium ferrireducens* FW-1a	AF411013.1	82%	Unaffiliated
GVS1_L2_F02	2	*Thermomicrobium roseum* DSM 5159	CP001275.1	81%	*Chloroflexi*
SC1 Bacteria					
SC1_L2_G09	19	*Thermoleiphilum minutum* ATCC 35268T	AJ458464.1	79%	Candidate Phylum OP1
SC1_L2_F10	6	*Dehalococcoides* sp. BHI80-15	AJ431246.1	80%	*Chloroflexi*
SC1_L2_F07	4	*Geothermobacterium ferrireducens* FW-1a	AF411013.1	92%	*Thermodesulfobacteria*
SC1_L2_E09	4	*Thermotogales* sp. SRI-15	AF255594.1	81%	Unaffiliated
SC1_L2_G08	3	*Geothermobacterium ferrireducens* FW-1a	AF411013.1	97%	*Thermodesulfobacteria*
GVS1 Archaea					
GVS1_L4_D04	16	*Vulcanisaeta distributa* IC-017	AB063630.1	88%	Unaffiliated *Cren*.
GVS1_L4_B05	6	*Pyrodictium occultum* PL-19	NR_025933.1	91%	*Desulfurococcales*
GVS1_L4_F05	4	*Hyperthermus butylicus* DSM 5456	CP000493.1	87%	Unaffiliated *Cren*.
GVS1_L4_C03	2	*Geogemma indica* 296	DQ492260.1	86%	Unaffiliated *Cren*.
GVS1_L4_C06	2	*Thermofilum* sp. 1505	GU187356.1	86%	Unaffiliated *Cren*.
GVS1_L4_E03	2	*Staphylothermus* sp. 1633	GQ292555.1	90%	*Desulfurococcales*
GVS1_L4_G03	2	*Vulcanisaeta distributa* IC-135	AB063641.1	82%	Unaffiliated *Cren*.
SC1 Archaea					
SC1_L4_G07	10	*Staphylothermus* sp. 1633	GQ292555.1	86%	Unaffiliated *Cren*.
SC1_L4_H12	5	*Geogemma indica* 296	DQ492260.1	83%	Unaffiliated *Cren*.
SC1_L4_F07	5	*Thermofilum sp.* 1505	GU187356.1	83%	Unaffiliated *Cren*.
SC1_L4_F12	2	*Pyrodictium occultum* PL-19	NR_025933.1	88%	Unaffiliated *Cren*.
SC1_L4_C08	2	*Stetteria hydrogenophila* 4ABC	Y07784.1	94%	*Desulfurococcales*

[a] Number of sequences in the 16S rRNA gene library represented by the OUT; [b] Affiliation at the level of phylum (for *Bacteria*) or order (for *Archaea*). *Cren*.. *Crenarchaeota*; [c] Percent identity to the OTU representative sequence.

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