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MICROBIAL IMPACTS ON GEOTHERMOMETRY TEMPERATURE PREDICTIONS

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ABSTRACT

Conventional geothermometry approaches assume that the composition of a water sample collected near the surface but with origins in a deep geothermal reservoir still reflects chemical equilibration with the deep reservoir rocks. However, for geothermal prospecting samples whose temperatures have dropped to <120°C, temperature predictions may be skewed by the activity of microorganisms as well as by changes induced by abiotic processes. Microbial metabolism can drastically and rapidly change water chemistry. We hypothesize that knowledge of microbial impacts on exploration sample geochemistry can be used to constrain input into geothermometry models and thereby improve the reliability of reservoir temperature predictions. To evaluate this hypothesis we have chosen to focus on sulfur cycling microorganisms, because they are known to induce significant changes in redox state and pH and therefore can impact the mineral-fluid equilibria that underlie solute geothermometry approaches. Initially we are focusing on the process of sulfate reduction. This paper reports on the development and initial testing of an assay for detecting sulfate reducing microorganisms in geothermal prospecting samples.

The assays rely on quantitative polymerase chain reaction (qPCR), which allows estimation of the number of target organisms in a particular sample without actual retrieval and characterization of the organisms themselves. We constructed a plasmid containing portions of two genes (*dsrA* and *dsrB*) directly involved with microbial sulfate reduction for use as a quantitation standard. Using the plasmid as well as DNA from other microorganisms known to be sulfate reducers or non-sulfate reducers, we developed qPCR protocols and showed the assay's specificity to sulfate reducers. A qPCR standard curve using the plasmid was linear over >5 orders of magnitude. The assay was applied to DNA extracted from water collected at surface springs located in and around the town of Soda Springs, Idaho. These springs produce water that is believed to be a mixture of groundwater and deep hydrothermal fluids. The qPCR results indicated that sulfate reducing genes were present in each of the samples tested, and consideration of the microbiological results together with geothermometry calculations suggests that microbial metabolism can influence reservoir temperature predictions.

INTRODUCTION

Chemical geothermometry is an important tool for geothermal resource exploration. The technique relies on the assumption that the chemical and isotopic composition of a water or gas sample derived from a deep reservoir continues to reflect chemical equilibration with the reservoir host rock at the reservoir temperature, even after the water has migrated to a shallower location, or to the surface. Various approaches have been developed to relate water composition to temperature. The most commonly employed methods use empirical correlations for different host lithologies or

theoretical predictions based on thermodynamic data for selected minerals. More recently, approaches that combine geochemical modeling of mineral-water reactions that may occur along the path from host rock to the point of sample collection with numerical methods for multi-parameter inverse optimization have been described (Cooper et al., 2013; Spycher et al., 2011). Such methods provide the ability to assess the impact of multiple processes on solution chemistry, and may improve the accuracy of geothermometry predictions. This in turn would enable better estimates of the production potential of geothermal reservoirs and in so doing reduce the risk of expensive exploratory drilling.

These new modeling approaches present an opportunity to account for the potential effects of microbial alteration of water chemistry. During transit from a geothermal reservoir to a surface or near surface sampling point, waters often cool to temperatures below ~121°C, generally considered a physiological threshold for microbial life (although recently microorganisms have been reported to survive at even higher temperatures (Takai et al., 2008)). Numerous studies have reported on the enormous biomass constituted by microorganisms in the terrestrial and marine subsurface (Whitman et al., 1998), and their occurrence and diversity in settings ranging from the vadose zone (Holden and Fierer, 2005; Kieft and Brockman, 2001) to deep (> 1 km) environments (Amend and Teske, 2005; Colwell and D'Hondt, 2013; Onstott et al., 2003; Pedersen, 2000). Geothermally derived waters in particular could be expected to be associated with microbial activity. Life depends on taking advantage of thermodynamic gradients, and the mixing of geothermal waters with cooler groundwaters, or migration of geothermal waters into geochemical environments different from the reservoir host rock would result in diverse and significant geochemical disequilibria that microorganisms can exploit for energy conservation (Reysenbach and Shock, 2002). Indeed, investigators have documented extensive phylogenetic diversity of microbial communities in both marine (Rogers and Amend, 2005; Takai and Nakamura, 2011) and terrestrial geothermal systems (Kimura et al., 2005; Marteinsson et al., 2001).

Microorganisms can impact water chemistry and geothermometry in both "passive" and active" ways. Mechanisms that would generally be considered "passive" include the release of biomolecules that can solubilize or chelate metals (Bennett et al., 2000; Ehrlich, 1996), or the sorption of ions on biological surfaces (Beveridge and Doyle, 1989; Schultze-Lam et al., 1996). For example, silica species are commonly employed in geothermometry (Arnorsson, 1975; Ferguson et al., 2009; Gunnarsson and Arnorsson, 2000), and microorganisms have the ability to mobilize silica through production of acids, alkali, and organic ligands (Rogers and Bennett, 2004). Microbial biomass can also contribute to removal of silica from solution by acting as a template for silica precipitation in hot springs (Konhauser et al., 2004). Rare earth elements, which are also often used in geoethermometry, have also been observed to be enriched in hot spring microbial biofilms and mats as compared to the surrounding water (Takahashi et al., 2005).

More "active" mechanisms with potentially large impacts on geothermometry are reactions associated with microbial growth and energy conservation. For example, the commonly used Na-K-Ca-(Mg) geothermometer (Giggenbach, 1988) could be affected by reactions resulting in the production or dissolution of carbonate minerals. Microbial oxidation of reduced carbon for energy conservation can result in the precipitation of calcium carbonate ($CaCO_3$) (Ehrlich, 1996). Transformations of nitrogen compounds, as well as dissimilatory sulfate reduction, are also associated with $CaCO_3$ precipitation (Ehrlich, 1996; Wright and Oren, 2005). Besides calcium, other ions such as magnesium, iron, and manganese can be removed from solution by carbonate mineral precipitation (Ehrlich, 1996). Conversely, solution concentrations of these ions can also be increased by microbially induced dissolution of minerals via mechanisms such as organic acid production during the course of metabolism (Uroz et al., 2009; Welch et al., 2002).

Given the abundant evidence for the significant role of microorganisms in modulating water chemistry, we hypothesize that knowledge of microbial impacts on exploration sample geochemistry can be used to constrain input into geothermometry models and thereby improve the reliability of reservoir temperature predictions. As a first step toward addressing this hypothesis, we are developing methods to detect and estimate microbial activity in geothermal prospecting samples. We have chosen to focus initially on the process of sulfate reduction. As noted previously, microbial sulfate reduction is known to promote carbonate mineral precipitation through the production of alkalinity. Sulfate reduction is also associated with significant changes in redox state, which along with pH is a critical factor in general for defining the mineral-fluid equilibria that form the basis of solute geothermometry approaches.

We developed assays to detect the process of sulfate reduction in environmental water samples, using

knowledge of genes specific to sulfate reducing (SR) microorganisms. The assays rely on a common molecular biological technique known as quantitative polymerase chain reaction (qPCR), which allows estimation of the number of target organisms in a particular sample by enumerating genes specific to the organisms rather than actually retrieving and characterizing the organisms themselves. For quantitation of sulfate reducing genes using qPCR, we constructed a plasmid standard containing portions of two genes (*dsrA* and *dsrB*) directly involved with sulfate reduction and unique to sulfate reducing microorganisms. These genes code for the dissimilatory sulfite reductase enzymes that catalyze the conversion of sulfite to sulfide and are highly conserved between bacteria and archaea. The standard was constructed with *dsrA* and *dsrB* sequences amplified from the well known hyperthermophilic sulfate reducing archaeon *Archaeoglobus fulgidus* (Klenk et al., 1997). Using the plasmid as well as DNA from other microorganisms known to be sulfate reducers or non-sulfate reducers, we developed a quantitation protocol and showed the assay's specificity to sulfate reducers. As a first test with actual field samples, the assay was applied to DNA extracted from water collected at springs located in and around the town of Soda Springs, Idaho. Discharges from the springs are believed to be mixtures of shallow groundwater and deep hydrothermal fluids, with the proportions varying at individual springs (Lewicki et al., 2012). The results showed that the assays could indeed quantify microbial sulfate reducing genes in the samples, and consideration of the results together with geothermometric calculations suggests that microbial sulfur metabolism can influence reservoir temperature predictions.

METHODS

Genomic DNA

Genomic DNA (gDNA) was extracted from cultures using the UltraClean® Microbial DNA Isolation Kit (MoBio, Carlsbad, CA). *Escherichia coli* K12, JM109 cells were grown overnight on LB prior to harvest and gDNA extraction. *Alicyclobacillus acidocaldarius* cells were grown as described elsewhere for gDNA extraction (Thompson et al., 2011). *Nitrosomonas europaea* was provided by Daniel Arp and grown per his recommendations (http://nitrificationnetwork.org/Nerecipe.php) prior to gDNA extraction. *Desulfovibrio alaskensis* G20 gDNA was kindly provided by KL Reardon, USDA, Adams, OR, and *Archaeoglobus fulgidus* gDNA was obtained as previously described (Reed et al., 2001).

PCR primer selection and analysis

Previously reported PCR primers DSRQ2R (Chin et al., 2008), DSR1F and DSR4R (Wagner et al., 1998) and DSRp2060F (Geets et al., 2006) were ordered from Integrated DNA Technologies (Coralville, Iowa, USA). The specific primer sequences are presented parenthetically as follows:
DSRQ2R (5'GTTGA**Y**ACGCATGGT**R**TG),
DSR1F (5'AC**S**CACTGGAAGCACG),
DSR4R (5'GTGTAGCAGTTACCGCA), and
DSRp2060 (F5'CAACATCGT**Y**CA**Y**ACCCAGGG);
where S=C/G, Y=C/T, R=A/G. BioEdit software version 7.0 (Hall, 1999) was used to evaluate the primer binding site conservation and *dsrA/dsrB* PCR product lengths from the following microorganisms: *Archaeoglobus fulgidus, Archaeoglobus infectus, Archaeoglobus veneficus, Bilophila wadsworthia, Desulfatiferula olefinivorans, Desulfobacterium cetonicum, Desulfobacterium vacuolatum, Desulfohalobium retbaense, Desulfomonas pigra, Desulfonatronovibrio hydrogenovorans, Desulfosalsimonas propionicica, Desulfosarcina variabilis, Desulfovibrio desulfuricans, Desulfovibrio intestinalis, Desulfovibrio piger, Desulfovibrio vulgaris, Desulfovibrio termitidis* and *Thiobacillus denitrificans*.

Preparation of *dsrAB* standard pKN23

The *dsrAB* gene fragment was amplified from *A. fulgidus* gDNA using Platinum® *Taq* DNA Polymerase High Fidelity (Life Technologies, Carlsbad, CA) and the DSR1F and DSR4R primers. The PCR product was cloned into the pCR4 vector of the TOPO TA Cloning® Kit for Sequencing and transformed into One Shot® TOP10 Competent Cells according to the manufacturer's instructions (Life Technologies). Plasmid DNA was prepared using the Qiagen Plasmid Midi Kit (Qiagen, Germantown, Md). The concentration of plasmid DNA was initially quantified using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA) and then more rigorously quantified with the Quant-iT PicoGreen dsDNA Reagent Kit (Life Technologies) using a Rotor-Gene 3000 (Qiagen) with a FAM filter (absorbance at 470nm and emission at 510nm). The constructed plasmid, named pKN23, was sent for sequencing to Idaho State University's Molecular Research Core Facility (Pocatello, Idaho, USA) to confirm the desired construct, based on the Genbank sequence for *A. fulgidus* and the primer sequences.

qPCR amplification of *dsrB*

Using methods similar to those described previously (Agrawal and Lal, 2009), a qPCR assay was developed for amplification of the *dsrB* gene on the Rotor-gene 3000 real-time PCR thermal cycler. For qPCR thermal cycling, the final composition of each reaction was as follows: 1X Platinum qPCR SuperMix UDG (Life Technologies), 1mM additional $MgCl_2$ (Life Technologies), 400ng/µL BSA (Roche), Technologies), and DNA template. Dilutions of pKN23 representing 25 to 10^7 copies of *dsrB* were used for development of the standard curve for the assay. The following conditions were used: First, two minutes at 50°C to allow the UDGase to digest any contaminant DNA and then 5 minutes at 95°C to denature the UDGase and activate the polymerase. Next, forty cycles consisting of 95°C for 45 seconds, 58°C for 60 seconds, 72°C for 45 seconds, and 85°C for 15 seconds. During the 72°C and 85°C portions, measurements of fluorescence were taken on FAM (470nm for absorbance and 510nm for emission) and SYBR (470nm for absorbance and 585nm for emission) channels. Following completion of the amplification cycles, a melt curve was generated in order to confirm that the correct product was amplified. To prepare for the melt curve, the DNA was denatured at 95°C for 5 seconds and held at 55°C for 2 minutes. The melt curve was performed from 55°C to 99°C, with an initial wait of 60 seconds at 55°C and then 5 seconds at each degree on the increase.

Field site description and sample collection

Water samples were collected from three locations (Soda Geyser outlet, Hooper Springs, and Sulfur Springs; Figure 1) in and around the town of Soda Springs, Idaho. Soda Springs is located in the fold and thrust belt on the eastern boundary of the track of the Yellowstone Hotspot, and is known for the highly CO_2-charged waters that discharge from the many springs in the area. The CO_2 is believed to originate from the interaction of Paleozoic carbonates and acidic hydrothermal fluids at depth (McLing et al., 2012). Mixing of upwardly migrating deep waters (CO_2-charged) with the overlying aquifer waters (basalt-equilibrated) has created a system where the water chemistry within the Soda Springs area ranges from "no basaltic impact" (e.g., deep wells or water transported through faults) to "basalt modified" springs. The Soda Geyser, centrally located in the town of Soda Springs, was created in 1937 when a geothermal exploratory well penetrated into the CO_2 pressurized aquifer at approximately 100 meters below land surface. The City of Soda Springs subsequently capped the well with a timer, which allows the geyser to erupt hourly as a tourist attraction. Outflow water from the geyser has precipitated copious amounts of travertine. Hooper

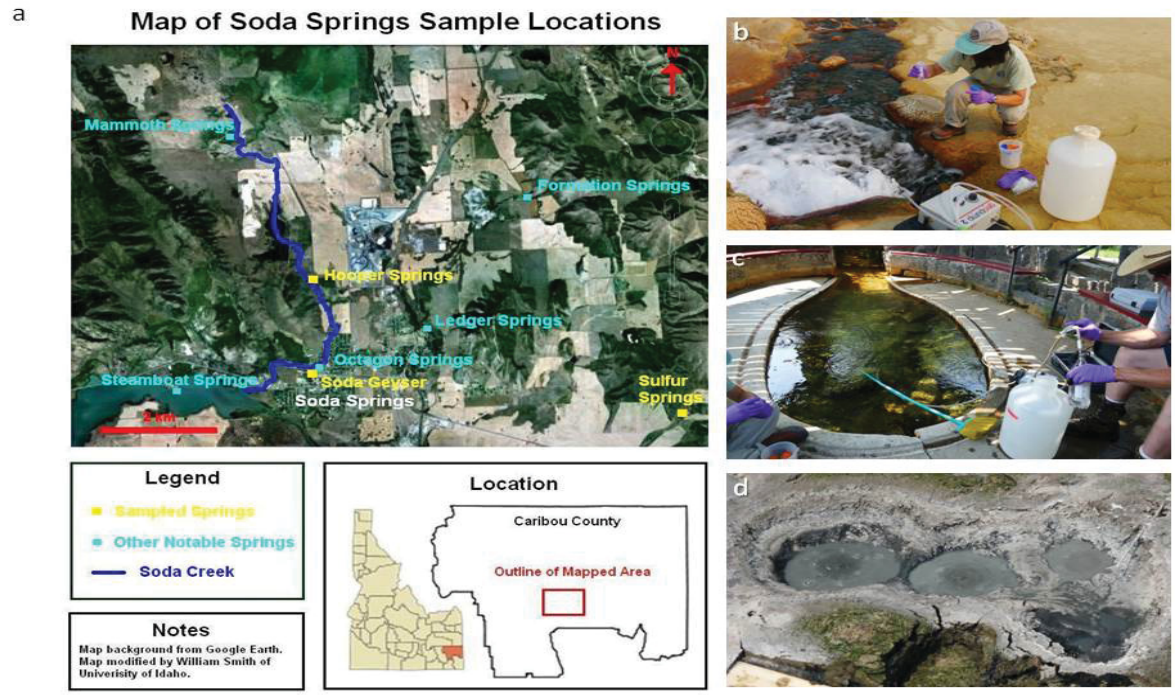

Figure 1: Map of Soda Springs area (a) and hot springs sampled: Soda Geyser outlet (b), Hooper Springs (c), Sulphur Springs (d).

Springs is a cold (11°C) CO_2 bubbling (70.0 vol.%) spring located north of the City of Soda Springs along the western margin of the Blackfoot Valley. Sulfur Springs is a series of CO_2 charged (99.6 vol.%; (Lewicki et al., 2012)), cold (14.2 °C) springs and pools located at the mouth of Sulfur canyon several kilometers east of the City of Soda Springs. A strong H_2S odor and the presence of elemental sulfur are characteristic of this location. In addition, diffuse, dry CO_2 and H_2S degassing occur in a 0.5 km radius around the spring (Lewicki et al., 2012). Sulfate and/or reduced sulfur species have been observed in samples collected previously at all three of these locations (Lewicki et al., 2012; Mayo et al., 1985; McLing et al., 2012).

At Soda Geyser and Hooper Springs, filtered water samples were collected for cation and anion analysis. The samples were filtered using a 0.45 micron pore size high–capacity, in-line filter capsule with a polyethersulfone membrane (Gelman Sciences), dispensing directly into acid-washed polyethylene bottles. The samples for cation analysis were then acidified to pH <2 using Optima-grade nitric acid and sealed with Parafilm. An additional non-acidified sample was collected at each site for anion analysis. For enumeration of cell numbers, water samples (40 mL; four replicates at each location) were collected at each of the three sites in polypropylene centrifuge tubes and cells were fixed in 2% formaldehyde directly after collection. Fixed cells were stored at 4°C prior to processing.

For DNA extraction the cells were concentrated by filtration (0.22 µm pore size) onto Sterivex™ GP Filter (EMD Millipore, Billerica, MA) units. At the Geyser and Hooper Springs sites, filtration of 10 L samples was conducted in the field, in triplicate, and the filters were immediately stored on dry ice for transport back to the laboratory, where they were stored at -80ºC until processing. Turbidity of the water samples at Sulfur Springs precluded filtration in the field; instead water was collected and stored on ice and then at 4ºC until the water could be filtered similarly in the laboratory the following day, after gravity settling of much of the suspended solids. Only 50-75 mL could be processed through an individual filter; additional time for settling did not increase this filterable volume.

Geochemical analysis

Temperature and pH were measured onsite at Soda Geyser and Hooper Springs. Major metals (e.g. Ca, Mg, K, Na, Si) were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) using standard methods recommended by the instrument manufacturers and major anions (F^-, Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}) were measured by ion chromatography, again using standard methods.

Direct cell enumeration and microscopy

Total microbial cells were enumerated using acridine orange (AO) staining and epifluorescence microscopy (Nikon Eclipse E800, 1000X, filters: excitation 440±12.5 nm, dichroic mirror 565 nm, emission 605±27.5 nm) using standard protocols (Kepner and Pratt, 1994). Triplicates were counted for the water samples. Pictures were taken with an Olympus American camera and MagniFire 2.1A software.

Field sample DNA extraction and *dsrB* quantitation

DNA was extracted from the Sterivex filters using the PowerWater® Sterivex™ DNA Isolation Kit (MoBio) according to manufacturer recommendations and the DNA was suspended in 100µL of 10mM Tris pH 8.0. For the Geyser and Hooper Springs samples two µL of extracted DNA were used as template per qPCR reaction, performed in duplicate from each of the triplicate samples. For the Sulphur Springs samples only two DNA extraction preparations were used, and the DNA was diluted 10-fold prior to inclusion in the qPCR reaction mix.

Geothermometry calculations

Consideration of the thermal history of the water in the Soda Springs system is important for assessing the degree to which microorganisms may have influenced the chemistry. Because there are no deep wells into the system, the thermal history of the water can only be estimated using geochemical thermometers. The degree of accuracy of these geothermometers depends on many factors, including mineral precipitation and dissolution, groundwater mixing, and the applicability of the geothermometer used (Fournier, 1977). Of the geothermometers commonly employed to estimate maximum geothermal reservoir temperatures, silicate geothermometers (quartz and chalcedony) are considered the most applicable to the study area (Mitchell, 1976). The models as described by Fournier (1977) were applied to available water chemistry data for the sampled springs. The same data were also used for preliminary geothermometric predictions generated using the inverse geochemical modeling approach described by Cooper et. al. (2013), with an equilibrium-controlling mineral assemblage of siderite, calcite, anhydrite, dolomite, and chalcedony.

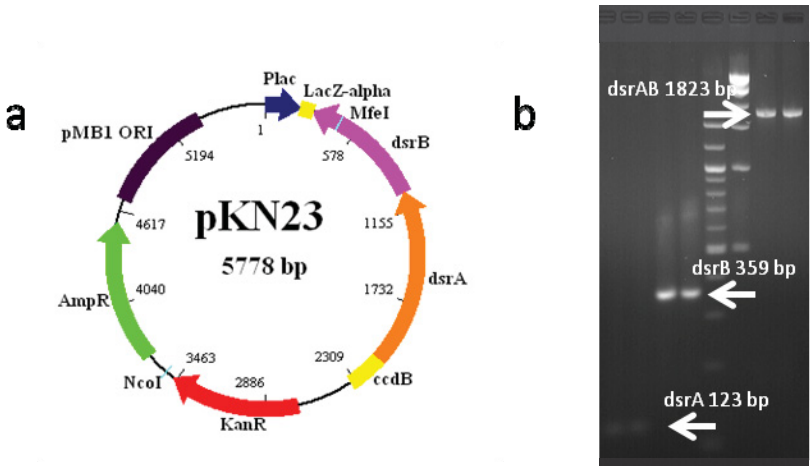

Figure 2: Construct of pKN23 plasmid including gene sequence from A. fulgidus (accession M95624), dsrA nucleotide 181 to dsrB nucleotide 746 (a), and electrophoresis gel showing amplification products dsrAB, dsrA, and dsrB from pKN23 (b).

RESULTS

Evaluation of *dsr* gene primers and standard

The *dsrA* and *dsrB* genes code for the dissimilatory sulfite reductase enzymes that catalyze the conversion of sulfite to sulfide and are highly conserved between bacteria and archaea. The primers used for this study were chosen because they had been used by several groups for detection of *dsrAB* genes and had high target specificity to the *dsrB* genes from a number of representative sulfate reducing bacteria and archaea (Agrawal and Lal, 2009). Our primer verification alignments conducted against *dsrB* gene sequence from known SR bacteria and archaea also suggested a general applicability of the primers (data not shown). Of course, as is the case whenever specific PCR primers are applied to environmental samples, ensuring both definitive detection of all sulfate reducers present and the absence of false positives is not possible. However, because our ultimate intention is not to identify all of the individual sulfate reducing species but rather to estimate the likelihood of sulfate reducing activity to have occurred in a sample, this is not a significant concern.

The plasmid pKN23 containing the highly conserved sulfate reduction genes *dsrA* and *dsrB* from *Archaeoglobus fulgidus* was created for use as a quantitative external standard for estimating the number of SR bacteria and SR archaea in a sample. The *dsrAB* genes were amplified in tandem by PCR from adjacent genes in *A. fulgidus* using the degenerate primers DSR1F and DSR4R and ligated into the host vector. Sequence analysis confirmed that the desired primer annealing sites were conserved in pKN23. Figure 2a shows the structure of the plasmid, consisting of the target gene fragments *dsrA* and *dsrB* as well as other specific DNA sequences used for characterization and sequencing purposes.

Development of qPCR assay

The entire *dsrAB* fragment was too large for use in qPCR, and preliminary studies comparing *dsrA* and *dsrB* amplification indicated that the *dsrB* assay would be more sensitive to low gene copy numbers than the *dsrA* assay. Figure 2b illustrates the relative sizes and abundances of the PCR amplification products, as determined by gel electrophoresis. Therefore primer pair DSRp2060F/DSR4R, targeting an approximately 360 bp fragment of the *dsrB* gene, was chosen for quantitative studies. This primer pair amplified a single product from the pKN23 *dsrB* gene with optimum standard efficiencies ranging from 0.91 to 0.93. Correlation coefficients (R^2) were greater than 0.99, indicating an efficient amplification (100% DNA doubling per cycle). The standard assay curve was linear over >5 orders of magnitude, from $<10^2$ to 10^7 gene copies per reaction.

The qPCR assay was tested against positive genomic DNA (gDNA) from *A. fulgidus* and the SRB *D. alaskensis* G20. Examination of the temperature at which the double stranded DNA product "melted" (separated into two strands) showed that the product from the *A. fulgidus* melted at ~88°C, the same value as for the DNA product from pKN23. This was expected since the melting temperature is a function

of the specific DNA sequence, and the pKN23 *dsrB* sequence was the same as the *A. fulgidus dsrB* sequence. In contrast, the DNA from the SRB (*D. alaskensis*) melted at 92°C. The higher melting temperature indicates that the two DNA strands are more strongly bound together, because of specific nucleotide interactions and/or a longer overall length of the hybridized region. Calculated sizes of known bacterial *dsrB* fragments are generally larger than the calculated sizes of known archaeal fragments (calculations and alignments of *dsrB* gene sequences retrieved from GenBank not shown), potentially contributing to the difference in peak temperatures. A size differential was also detected by gel analysis, as shown in Figure 3; the *A. fulgidus* archaeal product in lane 6 migrates farther in the gel, consistent with a smaller size, than the *D. alaskensis* bacterial product in lane 7. This attribute may prove useful for differentiating archaeal and bacterial *dsrB* genes in studies where the composition of the sulfate reducing community is of interest, although additional experiments should be conducted to more rigorously assess its utility for this purpose.

The qPCR assay was also applied against genomic DNA from organisms in which the *dsrAB* gene clusters are known to be absent (*E. coli*, *A. acidocaldarius* and *N. europaea*). *E. coli* and *N. europaea* each produced minor fluorescent signals but melt curve (data not shown) and gel analyses (Figure 3, lanes 3 and 5) suggested that the PCR products arose from non-specific priming events. *A. acidocaldarius* did not produce a fluorescent signal and the minor products observed by gel electrophoresis and staining were likewise probably due to non-specific priming. Based on these results real-time fluorescence of the amplified DNA was ultimately analyzed spectrophotometrically with the FAM filters at 85°C, which eliminated interference from the non-specific primer binding observed in the negative control template reactions at lower temperatures (72-84°C).

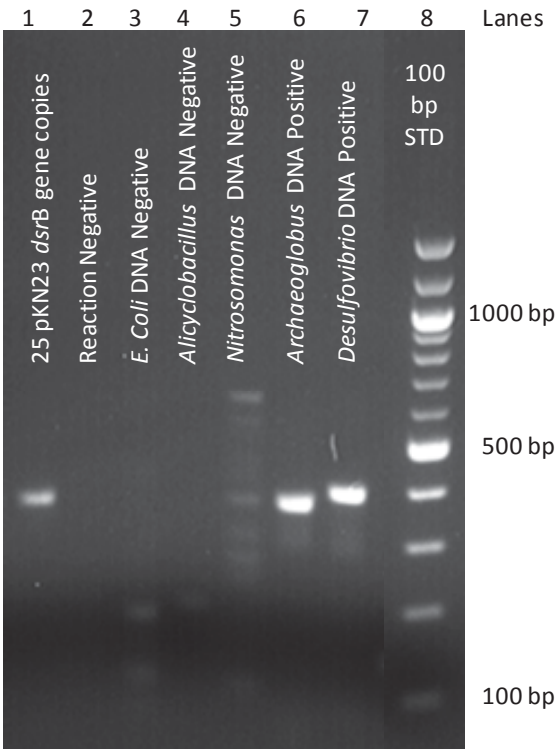

Figure 3: Ethidium bromide stained qPCR products separated by agarose gel electrophoresis. Lane 1, pKN23 standard DNA; lane 2, no DNA control; lanes 3-5, DNA negative controls E. coli, A. acidocaldarius and N. europaea, respectively; lanes 6-7, DNA positive controls A. fulgidus and D. alaskensis, respectively; lane 8, NEB 100 base pair DNA standard.

Water chemistry and geothermometry calculations for field samples

Field measurements for temperature and pH at the three Soda Springs sites are shown in Table 1, along with available data on major ion concentrations. The temperature at the Soda Geyser outlet is significantly higher (30°C) than at Hooper (11°C) and Sulfur Springs (14.2 °C, historical data). This is consistent with the fact that the Soda Geyser well accesses water from below the regional aquitard, and therefore mixing with the local shallow groundwater is expected to be minimal (Lewicki et al., 2012). In contrast, the water discharging at Hooper Springs exhibits signs of equilibration with basalt and is considered to be "well mixed" with the local groundwater (Lewicki et al., 2012; McLing et al., 2012). The water chemistry at Sulfur Springs indicates that it is sourced, like Soda Geyser, in deep Paleozoic carbonates (McLing et al., 2012). However, unlike the Soda Geyser and Hooper Springs to the west, Sulfur Springs shows no evidence, either in the local outcrops or chemically that the water has had any of the interactions with basalt that are so important to the water chemistry at Soda Geyser and Hooper Springs (McLing et al., 2012). Additionally, sulfate concentrations are particularly elevated at Sulfur Springs, indicative of thermal water interaction with carbonates.

Table 1. Temperature, pH, and major ion chemistry for Soda Springs samples.

			(mg l^{-1})											
	T (°C)	pH	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Fe^{2+}	SO_4^{2-}	Cl^{-}	F^{-}	NO_3^{-}	PO_4^{3-}	HCO_3^{-}	SiO_2
Geyser[a]	30.0	6.3	925.7 (42.3)	177.7 (7.9)	13.1 (0.4)	23.5 (1.2)	4.9 (1.0)	762	4.95	< 1	< 1	< 1	2613	34.1 (2.7)
Hooper Springs[b]	11.0	6.0	133.5 (8.2)	138.2 (5.8)	34.0 (1.4)	13.0 (0.2)	8.0 (0.8)	50.9	12.2	< 1	< 1	< 1	101	71.7 (4.5)
Sulfur Springs[c]	14.2	4.8	599.5	53.4	12.8	7.8	101.2	1593	6	0.4	0.12	0.21	67	97.9

[a]Geyser values for Ca, Mg, Na, K, Fe, HCO_3^{-} and SiO_2 are average values from four past sampling events.
[b]Hooper Springs values for Ca, Mg, Na, K, Fe, HCO_3^{-} and SiO_2 are average values from three past sampling events.
[c]Sulfur Springs values for T and pH are averages from 6 past sampling events. Values for Ca, Mg, Na, K, Fe, HCO_3^{-} and SiO_2 are from one past sampling event. Values for anions are from Mitchell (1976).

Using the data shown in Table 1, the reservoir temperature was calculated using the quartz geothermometers as well as the multi-parameter inverse optimization approach described by Cooper et al. (2013). The predicted temperatures are shown in Table 2. Using the quartz geothermometer the predicted temperature of the deep geothermal system ranges from 86 °C at the Geyser well to 128 °C at Hooper Springs. Using the chalcedony geothermometer the calculated reservoir temperature ranges from 57 °C at Geyser to 99 °C at Hooper Springs. Typically, quartz geothermometers are considered to be the most accurate for geothermal systems in southeast Idaho (Mitchell 1976). The results from the calculated temperatures are consistent with values given in Mitchell 1976 and seem to indicate that the deep geothermal system feeding the features at Soda Springs is relatively cool. Preliminary calculations using the inverse geochemical modeling approach described by Cooper et. al. (2013), using an equilibrium-controlling mineral assemblage of siderite, calcite, anhydrite, dolomite, and chalcedony and optimizing to CO_2, temperature, and H_2S (for Sulfur Springs) provide estimates of 61 °C at Geyser, 81 °C at Sulfur Springs, and 136 °C at Hooper Springs. These results are generally consistent with the results from the quartz and chalcedony geothermometers. Note however that all of these predictive values may underestimate the true temperature of the deep reservoir, as the high concentrations of Mg^{2+} and the presence of a productive freshwater aquifer provide evidence for the mixing of multiple source waters.

Table 2. Geothermal reservoir temperature predictions based on water composition at Soda Spring sites.

	Quartz Geothermometer (°C)	Chalcedony Geothermometer (°C)	Inverse Modeling (°C)
Geyser	86.0	57.0	61.1
Hooper Springs	128.0	99.0	136.1
Sulfur Springs	121.0	93.0	81.0

Cell enumeration and quantification of SR microorganisms

Cell counts (shown in Table 3) obtained by fluorescent staining and microscopy indicated that significantly fewer cells were present in the Geyser and Hooper Springs samples (average $<10^5$ cells/mL) compared to the Sulfur Springs samples (average 8.5 x 10^7 cells/mL). Cells at Geyser and Hooper Springs were typically very small (~1 μm) and mostly cocci-like whereas cells from Sulfur Springs were generally larger (1-3 μm) and mostly bacilli-like (Figure 4).

Table 3. Cell counts and dsrB gene numbers for Soda Springs samples (standard deviation in parentheses).

	Cells (ml^{-1})	*dsrB* (ml^{-1})
Geyser	9.3(3.1)E+4	3.1 (2.6)E+1
Hooper Springs	4.9(1.6)E+4	1.9(1.3)E+1
Sulfur Springs	8.5(3.4)E+7	9.0(3.4)E+5

The *dsrB* gene qPCR analyses suggested that SR microorganisms are present at all three of the Soda Springs sites sampled, but Sulfur Springs has by far

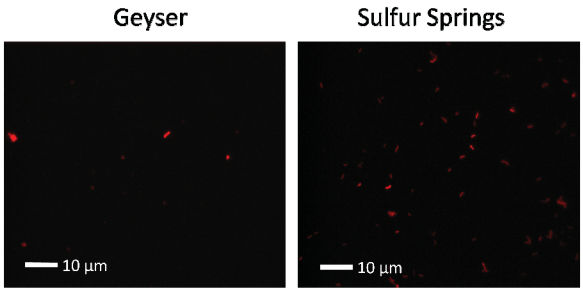

Figure 4: Epifluorescent microscopic images of representative samples from Geyser and Sulfur Springs; cells were stained with acridine orange dye and visualized at 1000X magnification. Note: Although cell densities in the images look similar, the Sulfur Springs sample was diluted one thousand-fold compared to the Geyser sample to facilitate counting. Cells from Hooper Springs (not shown) appeared very similar in size and shape to those from Geyser. Scale bars are 10 microns.

the largest populations (Table 3). On average <100 SR gene copies were present per ml of water from Geyser and Hooper Springs, whereas 9 x 10^5 gene copies ml^{-1} were present in samples from Sulfur Springs. Higher numbers of SR microorganisms might be expected given the ~3 orders of magnitude higher total cell counts at Sulfur Springs, but SR also appeared to be enriched at Sulfur Springs compared to the two other locations. Normalizing to total cell numbers, Geyser and Hooper Springs *dsrB* gene copies constituted 0.03-0.04% of the total. At Sulfur Springs the number of *dsrB* gene copies constituted 1.1% of the total cells counted.

Melt curve analysis of the qPCR products showed peaks for Hooper Springs at 91°C and Geyser at 91.5°C (Figure 5), similar to that observed for the SRB positive control *D. Alaskensis* (not shown in Figure). For the Sulfur Springs samples the peaks occurred near 89.5°C, between the peaks observed for the SRB and SRA positive controls (92°C and 88°C, respectively). As noted previously, the differences in melt peak temperatures may suggest that the communities of sulfate reducers differ, with sulfate reducing archaea playing a larger role at Sulfur Springs than at the other two locations, where bacteria predominate.

CONCLUSIONS

As a first step in assessing the potential implications of microbial activity on geothermometry, we developed and tested a quantitative assay for genes

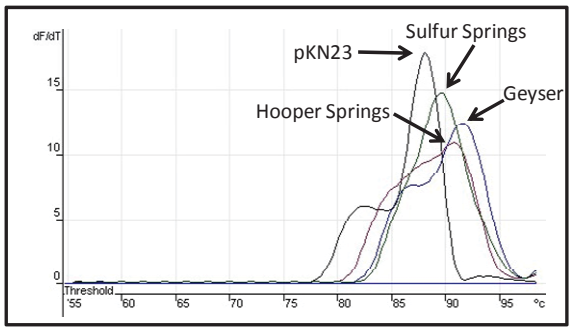

Figure 5: Representative melt curves for field sample qPCR products, showing first derivative of fluorescence with respect to time (Y-axis) and PCR product complete denaturation temperature (X-axis). Black, pKN23; Sulphur Spring, Green; Hooper Springs, Red; Geyser, Blue.

specific to sulfate reducing bacteria and archaea. We applied it to water samples collected from a "blind" geothermal system in southeastern Idaho and obtained evidence indicating that these samples did indeed harbor sulfate reducing microorganisms. In particular, samples from a very S rich site (Sulfur Springs) exhibited both high biomass and high concentrations of sulfate reducing genes. These results indicate that this is an environment conducive to sulfur metabolizing activity, and thus microorganisms may play an important role in mediating water chemistry at this site. The inverse modeling calculations for geothermometry could not resolve a temperature solution without including sulfide in the geochemical calculations at levels ≥ 5 mM, and the resulting temperature prediction for Sulfur Springs (81 °C) was significantly lower than predictions using the chalcedony (93 °C) and quartz (121 °C) geothermometers. These observations provide preliminary evidence suggesting that microbial sulfur metabolism may influence geothermometry predictions in the Soda Springs system, and indicate that further investigation is merited. Future work will include the development of quantitative assays for other functional genes involved in sulfur cycling, and application of the assays in laboratory and/or field studies of microbial sulfur transformation and impacts on geothermometric predictions.

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