RESEARCH ARTICLE

Microbial diversity of boron-rich volcanic hot springs of St. Lucia, Lesser Antilles

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Received 8 November 2008; revised 3 August 2009; accepted 15 August 2009.
Final version published online 30 September 2009.

DOI:10.1111/j.1574-6941.2009.00780.x

Editor: Michael Wagner

Keywords
sulfur springs; microbial communities; extreme environments; geochemistry; boron.

Abstract

The volcanic Sulphur Springs, St. Lucia, present an extreme environment due to high temperatures, low pH values, and high concentrations of sulfate and boron. St. Lucia offers some unique geochemical characteristics that may shape the microbial communities within the Sulphur Springs area. We chose six pools representing a range of geochemical characteristics for detailed microbial community analyses. Chemical concentrations varied greatly between sites. Microbial diversity was analyzed using 16S rRNA gene clone library analyses. With the exception of one pool with relatively low concentrations of dissolved ions, microbial diversity was very low, with *Aquificales* sequences dominating bacterial communities at most pools. The archaeal component of all pools was almost exclusively *Acidianus* spp. and did not vary between sites with different chemical characteristics. In the pool with the highest boron and sulfate concentrations, only archaeal sequences were detected. Compared with other sulfur springs such as those at Yellowstone, the microbial diversity at St. Lucia is very different, but it is similar to that at the nearby Lesser Antilles island of Montserrat. While high elemental concentrations seem to be related to differences in bacterial diversity here, similarities with other Lesser Antilles sites suggest that there may be a biogeographical component as well.

Introduction

The volcanic island of St. Lucia in the Lesser Antilles features the geothermally active area of Sulphur Springs Park in the town of Soufriere. The hot springs of St. Lucia exhibit high temperatures (some above 80 °C), low pH values (down to pH 2), and very high concentrations of sulfate ($> 6000\,mg\,mL^{-1}$) and boron ($> 3500\,mg\,mL^{-1}$; for comparisons with other sulfur springs, see Supporting Information, Table S1). While inhospitable to some organisms, here as in most extreme environments we find microorganisms that thrive and are adapted to conditions at these sites (Kushner, 1984).

Extreme environments are unique places to study how organisms interact with each other and their physical environment. These environments often serve as analogues that reflect early earth or extraterrestrial settings (Greenwood *et al.*, 2002). While hot spring microbial communities have been extensively studied in certain areas such as Yellowstone National Park (Barns *et al.*, 1994; Hugenholtz *et al.*, 1998; Reysenbach *et al.*, 2000; Blank *et al.*, 2002; Meyer-Dombard *et al.*, 2005), the islands of the Lesser Antilles share a different geology and geochemistry compared with other geothermal areas (Smith *et al.*, 1997; Burton & Norris, 2000; McCarthy *et al.*, 2005). Several of the Yellowstone studies (Siering *et al.*, 2006; Mathur *et al.*, 2007) have focused on abiotic factors and their effects on microbial communities. These studies as well as others have revealed surprising bacterial and archaeal diversity, including microorganisms that were previously unknown, and many of which have not yet been cultivated (Barns *et al.*, 1994; Hugenholtz *et al.*, 1998; Reysenbach *et al.*, 2000; Blank *et al.*, 2002; Meyer-Dombard *et al.*, 2005).

DNA-based molecular community analyses, despite their biases, may reveal more diversity than culture-based methods, since culture-based methods invariably select for

certain organisms (Amann *et al.*, 1995). These molecular methods as applied to the Sulphur Springs of St. Lucia serve as an ideal starting point for understanding the microbial diversity and ecology within and between pools.

Our goals for this study were to characterize the microbial diversity of pools with different geochemical characteristics across the St. Lucia Sulphur Springs and to relate community composition with the effects of specific elements such as boron on microbial communities. We hypothesize that high concentrations of boron and other elements limits microbial diversity at St. Lucia.

Materials and methods

Study site and sampling strategy

The Sulphur Springs represent the present-day activity of the Qualibou volcano (13°50′N, 61°03′W) located in the southwestern portion of St. Lucia near the town of Soufriere. St. Lucia is a volcanic island in the Lesser Antilles, which formed by the subduction of the Atlantic plate beneath the Caribbean plate. The Sulphur Springs are in the present-day caldera and are characterized by a number of hot, acidic pools and steaming fumaroles concentrated in a small area (~100 × 200 m; Fig. 1).

Sampling of water, sediment, and rock samples was undertaken during several field expeditions in November 2000, July 2001, and July 2004, allowing assessment of seasonal as well as temporal stability of spring water geochemistry. Temperature and pH at the sites ranged from 40 to > 80 °C and from 2 to 7, respectively, and the conditions and chemical composition of the largest, most stable pools was fairly constant from year to year. For the present study, we selected six sites displaying a range of geochemical characteristics for detailed microbiological analysis (SP2, SP5, SP12, SP13, SP14, and SPX; Fig. 1, Table 1) in order to survey microbial diversity and to assess relationships between microbial diversity and geochemistry. We used water samples collected in 2004 for these analyses.

Geochemical analysis of water samples

Five hundred to 1000 mL of hot spring water was filtered using 0.2-μm Gelman Supor (polyethersulfone) filters that were presterilized by autoclaving. The filtrate was then split into several aliquots for major and trace element analysis. Filters used to process water samples were preserved using TE buffer, immediately shipped back to the laboratory on ice, and stored frozen at −20 °C until used for microbiological analysis. Water samples were analyzed by ICP-OES (inductively coupled plasma-optical emission spectroscopy) and ion chromatography for elemental chemistry.

DNA extraction from preserved filters

Filters were cut in half with one half kept frozen, and the remaining half was sliced into small sections with a sterile razor blade. Filter sections were placed into Power Bead tubes (Mo-Bio, Carlsbad, CA) along with 200 μL of excess TE buffer from whirl-pak bags. DNA was extracted from filter sections following the Mo-Bio Power Soil DNA extraction protocol for difficult-to-lyse cells (heating tubes to 70 °C for 10 min after addition of solution C1). DNA was quantified by electrophoresis through a 0.7% agarose gel, using 1 × TAE buffer, and comparison with a 1-kb DNA ladder (New England Biolabs, Ipswich, MA). DNA was stained with ethidium bromide and was visualized with a Bio-Rad Gel Documentation system (Bio-Rad, Hercules, CA).

PCR amplification and cloning of 16S rRNA genes

DNA extraction was followed by PCR using bacteria-specific primers 27F and 1492R, based on *Escherichia coli* numbering (Lane, 1991), and archaea-specific primers A751F and UA1406R (Baker *et al.*, 2003). PCR amplifications were prepared with 1 × buffer, 0.2 mM each dNTP, 1.5 mM $MgCl_2$ (all from Promega, Madison, WI), 0.5 μM of each primer (Integrated DNA Technologies, Coralville, IA), 1.5 U *Taq* polymerase (New England Biolabs), and ~40 ng DNA template in a final volume of 30 μL. Reactions were performed in a TGradient thermal cycler (Whatman Biometra, Goettingen, Germany). Amplification of 16S rRNA genes from bacteria consisted of 30 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s with an initial denaturation at 95 °C for 5 min and a final extension at 72 °C for 5 min. Amplification of archaeal 16S rRNA genes consisted of 30 cycles of 95 °C for 60 s, 62 °C for 60 s, 72 °C for 60 s, and a final extension at 72 °C for 10 min (modified from Baker *et al.*, 2003).

In order to minimize PCR bias in subsequent cloning steps (Stout & Nüsslein, 2005), three separate reactions were run for each sample and then pooled together before DNA quantification. Pooled PCR products were purified with the QIAquick PCR purification kit (Qiagen, Valencia, CA), and were quantified by comparison with a 1-kb DNA ladder (New England Biolabs) when run on a 1% agarose gel.

Cloning of mixed community 16S rRNA gene fragments was performed using the pGEM T-easy vector (Promega). Ligation into the vector at an insert to vector ratio of 1 : 1 followed the manufacturer's instructions.

Sequencing and phylogenetic analysis

For SP2, SP5, and SPX libraries, ligations were sent to Macrogen Inc. (Seoul, South Korea) for transformation, plasmid DNA extraction, and sequencing. Briefly, following

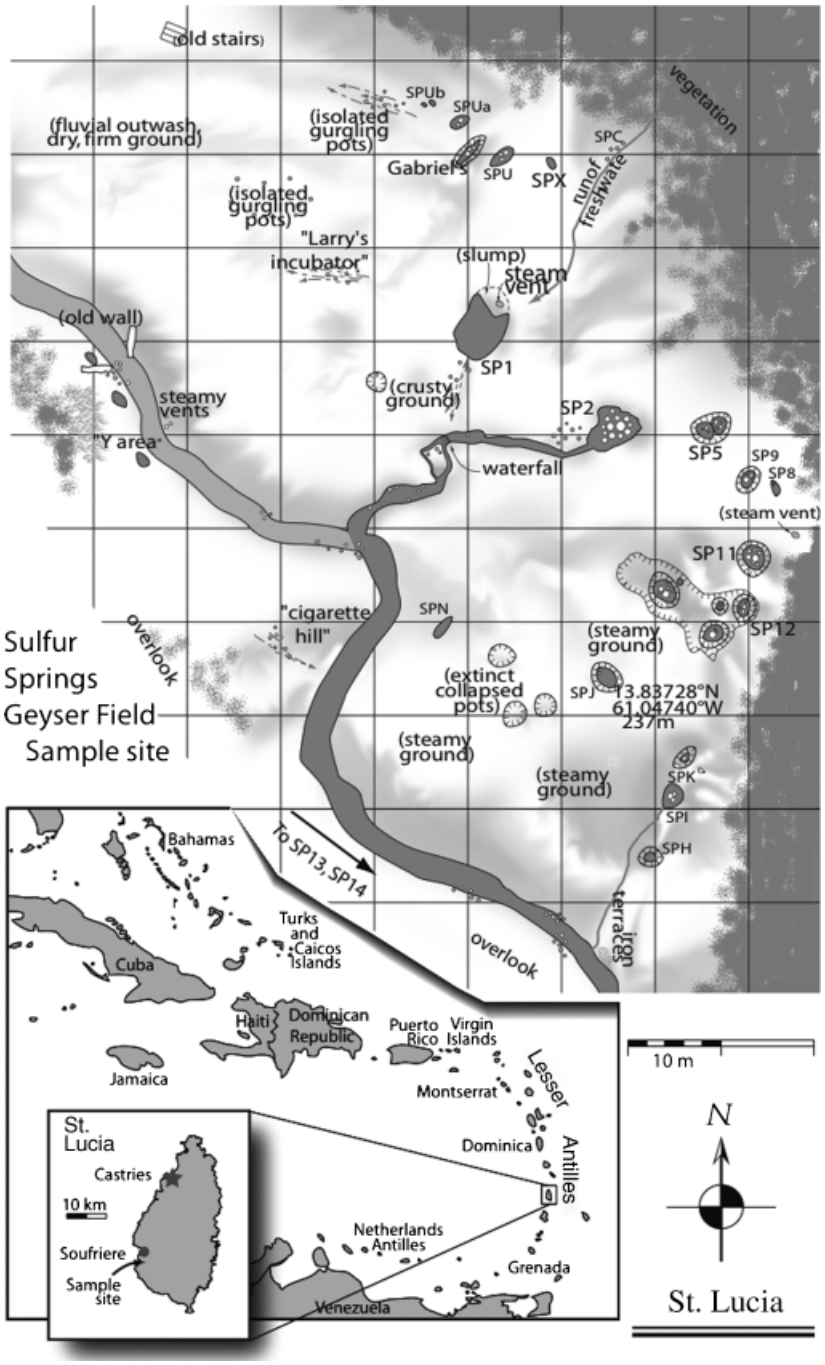

Fig. 1. Map of Lesser Antilles and St. Lucia showing sampling sites.

transformation, 50 positive clones were chosen randomly for community analysis. Plasmid DNA was extracted from these clones, and the M13 Forward vector-specific primer was used to sequence the 16S rRNA gene insert. All other libraries were constructed in our laboratory, where plasmids were extracted by alkaline lysis (Ausubel *et al.*, 1999) and were sent to the Yale University Science Hill DNA Analysis Facility for DNA sequencing.

Sequences were edited using the software Chromas (http://www.technelysium.com.au). rRNA gene sequences were searched against the Ribosomal Database Project II (Cole *et al.*, 2003) to determine nearest matches for bacterial sequences, and archaeal sequences were searched against the NCBI database using a BLASTN search (Altschul *et al.*, 1990).

Sequences were divided into two groups, based on whether orientation in the vector was in the forward or

Table 1. Water chemistry measurements from sample sites at St. Lucia

Sample	pH	Temperature (°C)	SO_4	Cl	Ca	Mg	Na	Si	B	K	Sr
SP2	5.5	70.8	1122.50	110.00	130.99	21.15	27.82	63.45	364.30	17.37	0.47
SP5	5.5	80.5	2135.00	188.25	246.13	45.76	46.04	58.58	937.26	28.40	0.52
SP12	7	> 80	1755.00	162.50	212.08	36.41	51.92	89.96	909.59	25.69	0.68
SP13	5.5	78.6	6237.50	61.50	282.76	25.27	26.25	38.68	3508.13	22.40	0.32
SP14	6.5	40	197.50	68.25	53.83	18.34	17.53	58.92	6.73	10.36	0.33
SPX	2	42.6	2740.00	14.00	33.42	6.52	11.17	98.10	3.06	16.03	0.23

All values are in $\mu g\,mL^{-1}$, except pH, which is in standard units, and temperature, which is in °C. For reference, values for $100\,\mu g\,mL^{-1}$ of each element in mM is given here: SO_4, 1.04 mM; Cl, 2.82 mM; Ca, 2.50 mM; Mg, 4.11 mM; Na, 4.35 mM; Si, 3.56 mM; B, 9.25 mM; K, 2.56 mM; Sr, 1.14 mM.

reverse direction, and for each sample, forward and reverse groups were analyzed separately when necessary. Multiple sequence alignments were created using the CLUSTALW interface within the software package BIOEDIT (Hall, 1999). BIOEDIT was used to create similarity matrices for all sequences and to determine phylotype groups (based on $\geq$97% similarity). Phylogenetic trees were created with MEGA 4 (Tamura *et al.*, 2007). Forward and reverse groups were highly similar for each sample; only forward-oriented sequence data are shown here). Possible chimeras were detected using the BELLEROPHON server (Huber *et al.*, 2004) and were removed from the analyses.

Diversity indices and statistics

Measurements of diversity ideally include richness, the number of different species or groups present, and evenness, the distribution of those groups (Hurlbert, 1971; Stirling & Wilsey, 2001). The Shannon–Wiener index, $H' = -\Sigma(pi)(\ln pi)$, and Simpson's reciprocal index, $1/D$, where $D = \Sigma(pi)^2$, and pi is the proportion of phylotypes i relative to the total number of phylotypes, both take richness and evenness into account (Stirling & Wilsey, 2001). The Shannon–Wiener index and Simpson's reciprocal index were calculated using ESTIMATES 8.0 (Colwell, 2006). Richness (S) and evenness ($E_H = H/\ln S$) were also calculated. ESTIMATES 8.0 was also used to calculate S_{ACE} and S_{Chao1}, coverage estimators that determine the number of probable phylotypes in the environment compared with the numbers observed in the sample.

Further analyses of libraries at the phylotype level included measurements of gene diversity, θ_π, using the software package ARLEQUIN (Excoffier *et al.*, 2005). θ_π is the average sequence divergence, or an estimate of the total genetic variation in a sample (Martin, 2002). Additionally, statistical analyses including principal components analysis, to determine the greatest contributors to variation between samples, and canonical correlation analysis, to determine correlations between microbial diversity and geochemical factors, were performed using the software XLSTAT (Addinsoft, New York, NY).

Nucleotide sequence accession numbers

Partial sequences of ~620 bp were deposited in GenBank with the accession numbers EU368977–EU368987 (bacterial sequences) and EU368964–EU368976 (archaeal sequences). SP14 sequences were deposited separately, with the accession numbers FJ379936–FJ379967.

Results

Elemental concentrations measured in pool water samples are listed in Table 1. With the exceptions of sulfate and boron (most likely as boric acid, found as the mineral sassolite in many volcanic hot springs; Dieter, 1994), concentrations of most elements were not exceptionally high, especially compared with other geothermal spring sites (Table S1). Sulfate ($> 6000\,\mu g\,mL^{-1}$) and boron ($> 3000\,\mu g\,mL^{-1}$) at site SP13 were the highest values observed at any site at St. Lucia. One site, SPX, had a much lower pH value (pH 2) than the other pools, but sulfate concentrations here were similar to the other sites ($2700\,\mu g\,mL^{-1}$). Site SP14 was an outlying site with moderate pH and temperature, and relatively low elemental concentrations. This site served as a control site in terms of relating geochemistry to diversity.

16S rRNA gene clone library analyses

DNA extraction and PCR was performed for water samples from six sites. Overall, diversity at each pool was very low, with the exception of site SP14 (Table 2). At sites SP2, SP5, and SP12, with high boron, high sulfate, and moderate pH, the bacteria library was dominated by sequences belonging to *Hydrogenobacter subterraneus* (Fig. S1a), a member of the *Aquificales*, with a few sequences belonging to other groups (Fig. 2). For SP13 samples, no PCR amplification was detected using primers for the domain Bacteria, even after several attempts; therefore, only archaeal clones were analyzed for this sample, while calculations of bacterial diversity assumed no bacterial sequences at SP13. Site SPX, with low pH, low temperature, high sulfate, and low concentrations of other elements, was, unlike SP2, SP5, or SP12 bacterial

Table 2. Diversity index scores for filter clone libraries from bacteria (bac) and archaea (arc) from sites SP2, SP5, SP12, SP13 (archaea only), SP14, and SPX

Sample	Shannon	Simpson	Rich	Even	S_{ACE}	S_{Chao1}	θ_π	% Coverage	n*
SP2 bac	0.28	1.14	3	0.23	4	3.13	24.85	84%	47
SP2 arc	0	1	1	0	1	1	0	100	43
SP5 bac	0	1	1	0	1	1	0	100	48
SP5 arc	0	1	1	0	1	1	0	100	47
SP12 bac	0.71	1.56	4	0.51	4.75	4.06	161.92	91	25
SP12 arc	1.27	2.64	6	0.71	7.43	6.44	78.14	87	23
SP13 arc	0.38	1.23	3	0.23	5	5	10.74	60	21
SP14 bac	2.86	15.68	24	0.9	63.9	41.71	70.47	45	46
SP14 arc	1.62	4	8	0.79	18.15	9.82	113.35	57	20
SPX bac	0.21	1.09	3	0.19	5	5	13.69	60	47
SPX arc	0.3	1.19	2	0.43	2	2	26.3	100	46

Diversity indices measured were Shannon–Wiener (Shannon), Simpson's reciprocal index (Simpson), Richness (Rich), Evenness (Even), the genetic diversity measurement θ_π, and the coverage estimators S_{ACE} and S_{Chao1}. Percent coverage was estimated by averaging results from S_{ACE} and S_{Chao1} and dividing these numbers, the number of expected phylotypes, by the number of observed phylotypes.

*The number of clones used for each clone library analysis, n, is indicated in the last column.

libraries, comprised predominantly of *Acidithiobacillus*, with several clones belonging to *Sulfobacillus* (Figs S1a and S2). The SP14 bacterial library, unlike those from other sites, was extremely diverse, with numerous sequences from the proteobacterial groups (Fig. 2). Shannon and Simpson diversity indices were extremely high here compared with other sites, but θ_π was lower than expected (Table 2), indicating that although sequences were distinct enough to be grouped into different phylotypes (97% sequence similarity), they were not highly divergent at lower phylogenetic resolution.

The SP2, SP5, and SP12 archaeal libraries were comprised entirely or almost entirely of one phylotype (Fig. 2), a member of the *Sulfolobales* most closely related to *Acidianus infernus*. The archaea clones from sample site SP13 were also closely related to *A. infernus*, as were most of the SPX and SP14 archaeal clones (Fig. S1b). There were several bacterial *Hydrogenobacter* sp. sequences detected in the SP12 archaeal library, although these did not match closely with those of *H. subterraneus* or other known *Hydrogenobacter* spp., so these may belong to another group, possibly archaeal (Fig. S1b). There were also some sequences related to uncultured *Crenarchaeota* from Yellowstone National Park in the SP12 archaeal sample (Fig. S1b). The SP14 archaeal library was the most diverse among the archaeal libraries, although it was still predominantly ($> 50\%$) composed of the same *A. infernus* sequence as the other archaeal libraries (Table 2, Fig. 2).

Coverage estimators provide an estimate of the number of phylotypes expected in the sample source and allow us to determine how well the sample represents that environment. The coverage estimators S_{ACE} and S_{Chao1} suggest that most of the diversity present in our samples was covered, and we calculated percent coverage by dividing the number of phylotypes we observed by the average of estimates given by S_{ACE} and S_{Chao1} (Table 2). These estimators were used periodically to measure coverage as libraries were sampled, resulting in somewhat smaller libraries as sampling progressed, especially for those samples with very low diversity.

Geochemistry and microbial diversity correlations

A matrix was created based on Pearson's correlation coefficients (r) calculated from measurements at these sites (Table 3). Several geochemical factors were correlated with each other. Temperature was strongly correlated with the cations Ca, Na, K, and Mg, and moderately correlated with Sr and Cl. SO_4 and B were strongly correlated with each other. Principal components analysis of site variables showed that axes F1 and F2 accounted for 84.4% of the variation between sites; temperature, Cl, Ca, Mg, Na, K, and Sr contributed about equally to PC1, while SO_4 and B contributed about equally to PC2. Sites SP2, SP5, SP12, and SP13 were high-temperature sites with high elemental concentrations, and variations at SP2, SP5, and SP12 appeared to be explained best by PC1, but variations at site SP13, with extremely high SO_4 and B concentrations, were best explained by PC2 (Fig. 3). The moderate-temperature sites SP14 and SPX fell outside of these groups. SPX was characterized by low pH and high SO_4 concentrations, whereas SP14 had moderate pH values and elemental concentrations.

When studying correlation patterns between microbial diversity indicators and element concentrations in pools, since archaeal diversity did not appear to show much variation between sites (Fig. 2), we investigated only bacterial diversity measures and their correlation with geochemical factors. We examined correlations based on diversity

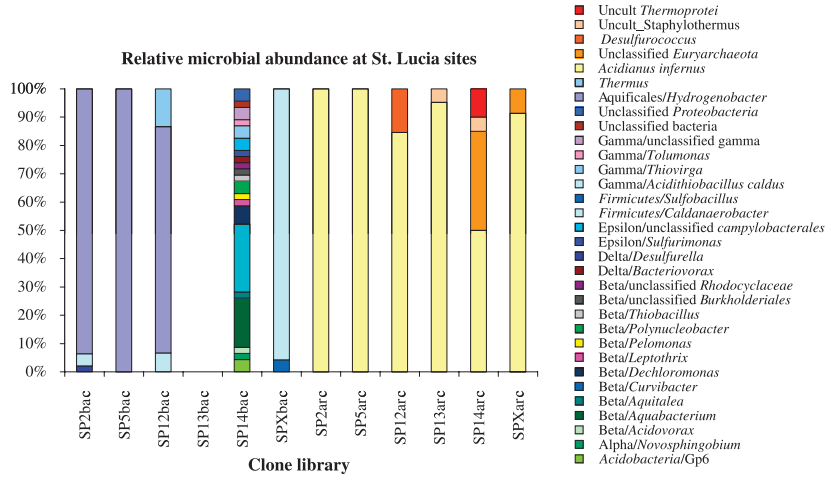

Fig. 2. Relative abundance of clones in each library (as percentage of total library) from bacterial and archaeal libraries.

indices (Table 3) as well as proportions of bacterial groups with geochemical factors (Fig. 4). Using Shannon, Simpson, Richness, or Evenness diversity indices, bacterial diversity was negatively correlated with B and Ca, but the strongest negative correlations were with K, temperature, and SO_4. Weak to moderate positive correlations were seen when microbial diversity was correlated with pH values. In the cases of temperature, calcium, and potassium, the correlations with microbial diversity were fairly consistent between bacteria and archaea across all diversity measures except bacterial genetic diversity, θ_π. However, the genetic diversity measure θ_π was positively correlated with Sr, Si, Ni, and pH (Table 3). θ_π may show much different results from other diversity indices due to the low genetic diversity at site SP14 compared with other diversity measures, indicating that many of the phylotypes we observed were distinct enough to be different phylotypes but still closely related. Canonical correlation analysis showed that some specific bacterial groups did correlate with a particular geochemical factor, such as the *Firmicutes* with Si, and the *Aquificales* and *Thermus* groups with temperature and numerous other ions, but most of the bacterial groups did not specifically correlate with any geochemical factors (Fig. 4, Table S1).

Discussion

We have investigated microbial diversity at the volcanic Sulphur Springs in Qualibou Crater at St. Lucia, a site that, compared with other geothermal springs with high temperatures and low to moderate pH values, has not been well characterized.

Diversity in both the bacterial and archaeal domains at St. Lucia is low compared with the well-characterized pools of Yellowstone (Meyer-Dombard *et al.*, 2005; Mathur *et al.*, 2007), or the more recently described sites at Kamchatka (Belkova *et al.*, 2007), Solfatara Crater, Italy (Glamoclija *et al.*, 2004), New Zealand hot spring sinters (Mountain *et al.*, 2003), Lassen Volcanic hot springs, CA (Siering *et al.*, 2006), or acidic sulfur springs in New Mexico (Rzonca & Schulze-Makuch, 2003). However, diversity and types of microorganisms found at St. Lucia appear similar to some sites on the nearby island of Montserrat (Atkinson *et al.*, 2000; Burton & Norris, 2000). For example, Burton & Norris (2000) found that at least one site on Montserrat, Galway's Soufriere, had an archaeal community composition of entirely the same sequence related to *A. infernus* that we found to dominate the pools at St. Lucia. Some of the representatives of the bacterial communities at Montserrat matched *Acidithiobacillus* sequences that we found at St. Lucia. Overall, the Montserrat archaeal communities appeared to be similar in composition and diversity, but the bacterial communities were slightly more diverse than those at St. Lucia, and there was not a dominance of *Hydrogenobacter* or any *Aquificales* like we saw at most of the St. Lucia sites. The *Aquificales* have been found previously in other hot spring sites with moderate pH values (between pH 5 and 7) such as those found at most of our sites (Spear *et al.*, 2005). Atkinson *et al.* (2000) reported isolation of microorganisms from Montserrat that were common to well-studied geothermal sites such as Yellowstone, as well as previously undescribed microorganisms, which may be unique to the area due possibly to the geographic isolation and the transient nature of the thermal pools at Montserrat, similar to the pools at St. Lucia.

The microbial DNA sequences that were dominant at St. Lucia sites match well with microorganisms that have been found at other geothermal areas, indicating that these microorganisms are cosmopolitan. However, while we do find these few ubiquitous geothermal bacteria, the extremely low diversity here could also suggest that many bacteria found in an environment such as Yellowstone may be absent from St. Lucia due to geographic isolation. *Hydrogenobacter*

Table 3. Correlation matrix showing *r* values for Pearson's correlation

	1/*D*	pH	Temperature	SO_4	Cl	Ca	Mg	Na	Si	B	K	Sr
1/*D*	**1.00**	0.33	−0.66	−0.57	−0.21	−0.53	−0.24	−0.36	−0.13	−0.42	−0.70	−0.24
pH	0.33	**1.00**	0.46	−0.24	0.63	0.46	0.62	0.62	−0.42	0.20	0.22	0.67
Temperature	−0.66	0.46	**1.00**	0.40	0.73	**0.93**	0.80	**0.82**	−0.33	0.60	**0.88**	0.71
SO_4	−0.57	−0.24	0.40	**1.00**	−0.27	0.60	0.01	−0.05	−0.38	**0.90**	0.38	−0.28
Cl	−0.21	0.63	0.73	−0.27	**1.00**	0.59	**0.94**	**0.94**	−0.09	0.01	0.74	**0.91**
Ca	−0.53	0.46	**0.93**	0.60	0.59	**1.00**	0.78	0.71	−0.52	0.81	**0.84**	0.51
Mg	−0.24	0.62	0.80	0.01	**0.94**	0.78	**1.00**	**0.93**	−0.28	0.28	**0.84**	0.80
Na	−0.36	0.62	**0.82**	−0.05	**0.94**	0.71	**0.93**	**1.00**	0.00	0.19	**0.85**	**0.95**
Si	−0.13	−0.42	−0.33	−0.38	−0.09	−0.52	−0.28	0.00	**1.00**	−0.64	−0.06	0.14
B	−0.42	0.20	0.60	**0.90**	0.01	0.81	0.28	0.19	−0.64	**1.00**	0.46	−0.02
K	−0.70	0.22	**0.88**	0.38	0.74	**0.84**	**0.84**	**0.85**	−0.06	0.46	**1.00**	0.66
Sr	−0.24	0.67	0.71	−0.28	**0.91**	0.51	0.80	**0.95**	0.14	−0.02	0.66	**1.00**

Values in bold are different from 0 with a significance level $\alpha = 0.05$. Diversity indices showed very similar correlations with each other and environmental variables: only one index for bacterial clone libraries. Simpson's reciprocal index (1/*D*) is shown.

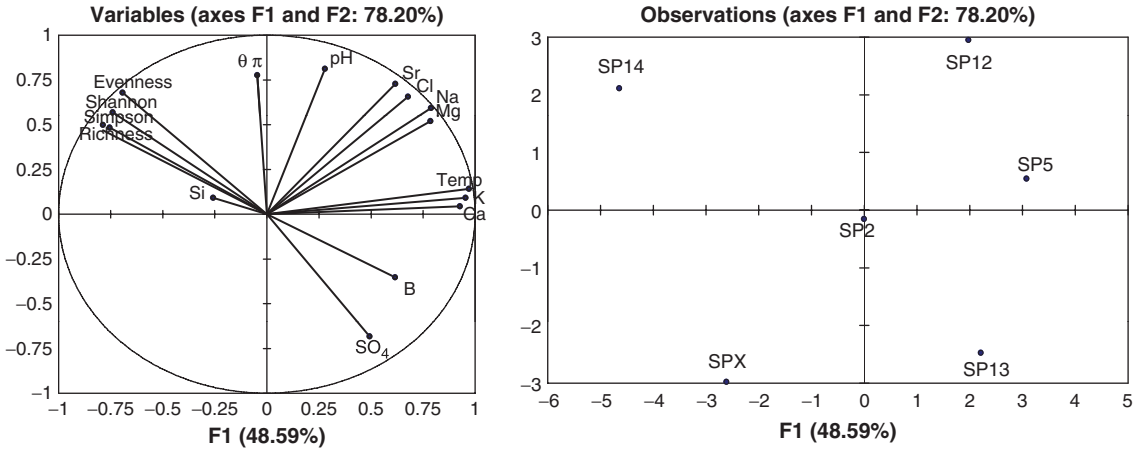

Fig. 3. Principal components analysis showing loadings on principal components 1 and 2 for geochemical factors at sites, and the relationship to each site.

subterraneus, the dominant clone at sites SP2, SP5, and SP12, was originally isolated from a deep geothermal pool in Japan. It grows between 60 and 85 °C, at pH between 5.5 and 9, with sulfur, sulfide, or thiosulfate (Takai *et al.*, 2001). This bacterium is not an acidophile, but a neutrophilic moderate thermophile, which fits with the profile of the chemistry at these pools, where the pH is between 5.5 and 7. The presence of elemental sulfur and high concentrations of sulfate at most of the St. Lucia sites indicates that these *Aquificales* may be utilizing reduced sulfur compounds as electron donors and sulfate as an electron acceptor, such as has been shown in a *Hydrogenobacter* sp. from neutral hot springs in Iceland (Skirnisdottir *et al.*, 2001).

Hydrogen may also play a major role in geochemical cycling at hot springs, based on thermodynamic modeling (Spear *et al.*, 2005), and the discovery of many *Hydrogenobacter* sequences in these environments. These bacteria could be utilizing hydrogen rather than sulfur compounds, as was found at Yellowstone by Spear (Nealson, 2005; Spear *et al.*, 2005).

Hydrogen concentrations were not measured at our St. Lucia sample sites, although Chiodini *et al.* (1996) measured gas concentrations at various sites around nearby Montserrat, and found variable H_2 concentrations from 2 to 4500 μmol mol^{-1}, and found these fumarolic vapor concentrations in line with other hydrothermal discharges, where hydrogen is often a major constituent.

Acidithiobacillus caldus (originally *Thiobacillus caldus*), which dominated at site SPX, grows optimally at 40 °C, pH 2–2.5, and oxidizes sulfur (Kelly & Wood, 2000). It has been found at other thermally active sites, including Montserrat (Burton & Norris, 2000). Site SPX, with a much lower pH value of 2, is an ideal habitat for *Acidithiobacillus* over *Hydrogenobacter*. Archaea in the *Sulfolobales*, including *Acidianus*, which dominated at all pools, are thermoacidophilic sulfur-oxidizing microorganisms that have been

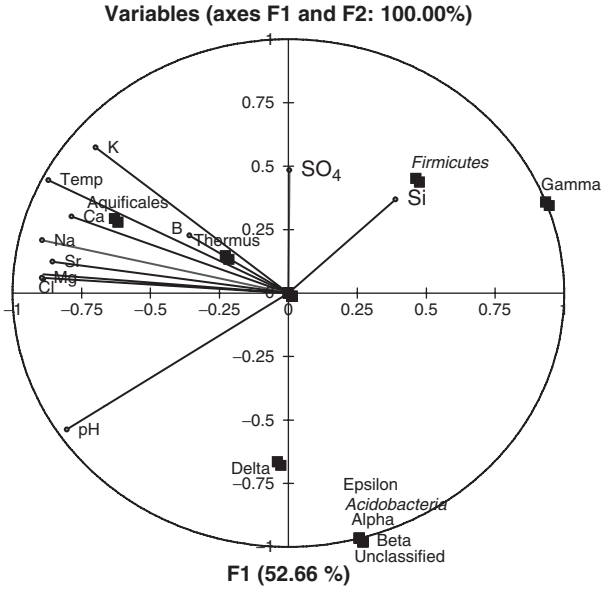

Fig. 4. Canonical correlation analysis showing correlative relationships between geochemical factors and proportions of individual bacterial groups. Bacterial group name abbreviations are shown. The proteobacterial groups alpha, beta, delta, epsilon, and gamma are shown with those names.

found almost exclusively at solfataras (Huber & Prangishvili, 2006), including those in New Guinea (Plumb *et al.*, 2007) and Montserrat (Burton & Norris, 2000).

Site SP14 featured lower temperature and elemental concentrations than the other sites, and much higher microbial diversity. The bacterial community here was more similar to bacterial communities found in other freshwater aquatic systems (Stout & Nüsslein, 2005; Escalante *et al.*, 2008), rather than those found in geothermal systems.

All sites except site SP14 contained high numbers of sulfur-oxidizing microorganisms, indicating that the high concentrations of sulfur and sulfur compounds are important microbial metabolites here, as expected based on previous studies of solfatara environments. We found that unlike the Bacteria, the archaeal component of our clone libraries did not show much variation between pools, despite the changes in temperature and pH that in some cases were outside of the known growth range of the *Sulfolobales*. For instance, pools SP2, SP5, SP12, and SP13, with more moderate pH and higher temperatures than pool SPX, were similar to SPX in archaeal composition. Even the very moderate site SP14 contained many of these sequences. We cannot be sure that these sequences came from active community members, or if the sequences may be from resting or dead cells from warmer, more acidic parts of the pools or even from other pools, although SPX is not connected to any other nearby pools.

A recent study (Aller & Kemp, 2008) examined a number of rRNA gene studies of bacteria and archaea, and found that in general, archaeal communities tend to be less diverse than bacterial communities in the same environment, which we also observed at our St. Lucia sites. The authors suggest that archaea may use more energy in maintaining metabolism in extreme environments and are thus less able to diversify; another suggestion is that archaea are highly physiologically diverse, and archaea living in 'nonextreme' environments may be able to perform different functions or already possess adaptations to survive over a large range of conditions. This may be one explanation for the dominance of *Acidianus*-like sequences at site SPX, despite water temperature that would be suboptimal for most *Sulfolobales*. These sequences could have also come from hotter pools, although it is unlikely given that SPX is not connected to any other pools in the area. In the case of the SP12 archaeal library, several sequences most closely matching *Hydrogenobacter* sp., a bacterium, were detected in the archaeal library. While this finding may point to limitations of the archaeal primer set we used, it is also noteworthy that *Hydrogenobacter* is believed to be one of the earliest branching bacteria, sharing phylogenetic similarities with the archaea (Shima *et al.*, 1994), and so may be more easily detected with archaea-specific primers than other bacteria. At site SP13, no bacterial sequences were detected. While this could also point to limitations with our PCR primers, it is possible that this site with high temperature and extremely elemental concentrations supports mostly archaea. High concentrations of these elements are most likely not due to evaporation of the sample, since other elemental concentrations at this site are not exceptionally high compared with other pools.

Relative to other comparable hot spring sites, pH, temperature, and sulfate concentrations at St. Lucia are not exceptional. Boron levels at several St. Lucia sites, however, are some of the highest values measured anywhere in the world. Boron concentrations in surface waters around the world average 0.1 $\mu g\,mL^{-1}$, with higher concentrations in seawater and geothermal waters (Koc, 2007). Water from geothermal sources and from subduction zones tends to have high boron concentrations due to the common occurrence of the boron-rich mineral tourmaline (Johannesson *et al.*, 2000). Some sites within the Lesser Antilles islands have measured boron concentrations between 10 and 20 $\mu g\,mL^{-1}$, which are considered high (Chiodini *et al.*, 1996; Smith *et al.*, 1997; McCarthy *et al.*, 2005). However, the values observed at several St. Lucia sites are as high as 3500 $\mu g\,mL^{-1}$, and thus, while we did not see a strong correlation between boron and microbial diversity as we had hypothesized, we may yet find that boron plays some role in restricting microbial diversity here and across the Lesser Antilles. While the strongest correlations we observed

were between bacterial diversity and temperature, we did see some negative correlations between bacterial diversity and boron, as well as sulfate, potassium, and calcium.

While boron has been shown to be necessary for certain microbial processes in low amounts, such as quorum sensing (Semmelhack *et al.*, 2004) and antibiotic production (Rezanka & Sigler, 2008), little is known about the effects of higher concentrations of boron on microorganisms (Steiman *et al.*, 2004). There have been a handful of studies on bacteria that grow in high boron concentrations (Dhakephalkar & Chopade, 1994; Baldi *et al.*, 1995), and more recently, several bacteria have been described that tolerate high concentrations of boron and even require high concentrations of boron for growth (Ahmed *et al.*, 2007a, b, c); while no resistance mechanism has yet been described, plasmids from *E. coli* and *Pseudomonas aeruginosa* that confer boron tolerance have been discovered (Summers & Jacoby, 1978). The toxic effects of boron on microbial communities have only been studied in a few cases so far, but exposure to high concentrations does appear to limit microbial diversity (Nelson & Mele, 2007).

Molecular analyses give more description of the diversity of a particular environment since culture-based studies may only detect a small fraction of the bacteria present. Despite biases associated with molecular studies (von Wintzingerode *et al.*, 1997), these currently offer the most complete picture of community composition and thus are an ideal starting point for microbial community studies. These analyses, combined with water chemistry measurements, allow us to begin tracing trends in geochemical influence on microbial diversity; however, linking these types of studies with culture-based studies such as microbial isolation and microcosms will give more insight into how specific elements affect microbial communities. Additional comparisons from other sites across the Lesser Antilles are needed to establish features that are unique to the microbial diversity of St. Lucia and the island arc.

Acknowledgements

The authors thank Alex Surkov, Lawrence Feinberg, and Howard Hughes summer research students from Amherst College Helen Dole, Phil Cameron, and Chantae Sullivan for help with field sample collection and laboratory work. We thank Paul Turner for DNA sequencing support. This research was supported in part by the Yale Institute for Biospheric Studies Center for Microbial Diversity. L.M.S. was supported by a Yale Endowed Fellowship in the Biological Sciences. We also acknowledge and thank the Soufriere foundation of St. Lucia for allowing access to the Sulphur Springs study site.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Phylogenetic relationships of bacteria (a) and archaea (b) detected at St. Lucia sites inferred from 16S rRNA gene sequence analysis.

Table S1. Published geochemical data for other thermal springs.

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