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The metagenomics of biosilicification: causes and effects

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ABSTRACT

In order to determine the links between geochemical parameters controlling the formation of silica sinter in hot springs and their associated microbial diversity, a detailed characterisation of the waters and of *in situ*-grown silica sinters was combined with molecular phylogenetic analyses of the bacterial communities in Icelandic geothermal environments. At all but one site, the microorganisms clearly affected, and in part controlled, the formation of the macroscopic textures and structures of silica sinter edifices. In addition, the class and genera level phylogenetic diversity and distribution appeared to be closely linked to variations in temperature, salinity and pH regimes.

Introduction

THE precipitation of silica in geothermal hot springs leads to the formation of silica sinters which incorporate microbial communities into their edifices. Fossilised microorganisms in active geothermal systems (e.g. Mountain *et al.*, 2003) provide a modern analogue to fossilisation/preservation in ancient silica sinters (Konhauser *et al.*, 2003 and references therein). Silica precipitation in geothermal systems is governed by purely abiotic processes linked to changes in silica saturation, which in turn are linked to changes in temperature, pH and silica concentration (Iler, 1979) upon the emergence of the geothermal waters at the Earth's surface. Microbes are ubiquitous in geothermal systems but what controls their phylogenetic abundance as well as the links between community diversity and geochemical regimes are still poorly understood (e.g. Hjørleifsdottir *et al.*, 2004). Here, results from a study aimed at elucidating the links between geochemical parameters and bacterial diversity in Icelandic geothermal environments, where silica precipitation leads to the preservation/fossilisation of microbes, are discussed.

Materials and methods

Temperature, pH and salinities, as well as *in situ* sinter growth rates, were quantified via field-based growth experiments in five geothermal areas in Iceland (Fig. 1), including Geysir, Hveragerdi, Reykjanes, Svartsengi and Krafla (Tobler *et al.*, 2008). At each site, geothermal waters were collected and analysed using ion chromatography (anions), mass spectrometry (cations) or molybdate yellow spectrophotometry (monomeric and total silica). *In situ* sinter growth rates were quantified from the time-resolved precipitation of silica (between 30 min and 25 months) on slides placed as substrates into the geothermal waters.

DNA was extracted from sediment samples at each site, followed by PCR amplification of the 16S rDNA (using bacterial primer sets Eu9f–Eu1492b), cloning and sequencing. Contiguous sequences were reassembled and aligned using Sequencher 4.7 (Gene Code Corporation), and the Ribosomal Database Project (RDP-II). Closest relatives and phylogenetic trees were constructed using the RDP-II Sequence Match and the Tree tool.

Results and discussion

The studied geothermal waters differed in silica content, pH, temperature and salinity, as well as microbial communities (prevalence, density, type;

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FIG. 1. Locations of the Icelandic geothermal environments studied.

Table 1). All these parameters affected the growth rates as well as the textures and structures of the precipitates, which in turn were affected by the precipitation mode (subaqueously and/or subaerially) and the presence or absence of microbial communities. At Reykjanes, extremely large subaqueous growth rates, which were primarily a consequence of the near neutral pH, as well as high salinity, temperature and silica contents were observed. The precipitates formed were porous and dominated by aggregates of amorphous silica nanospheres, however they were devoid of microorganisms. At Svartsengi (lower temperatures, high salinity) similar structures formed at

slower rates but microorganisms were abundant. Conversely, the geothermal waters at Hveragerdi and Geysir (Fig. 2a) had slightly alkaline pH and a much lower salinity and silica contents, resulting in undersaturated waters with small sinter growth rates. Subaerial silica precipitation close to the air-water interface (AWI) was dominated by evaporation and condensation which led to the formation of dense and heterogeneous crusts with well-defined terraces which were colonised by white and greenish biofilms (Fig. 2b; 8 months). In the submerged parts of the slides much higher degrees of colonization were observed (Fig. 2b) with the porous subaqueous silica precipitates fully encasing and preserving the biofilms (Fig. 2c,d). Finally, at Krafla, large sinter growth rates dominated, despite the waters being undersaturated with respect to amorphous silica. This was due to the pervasive presence of biofilms, which acted as substrates for silica deposition and which with time became fully silicified (Tobler *et al.*, 2008).

Bacterial DNA was successfully extracted and amplified from all sites apart from Reykjanes (Table 1), which was extremely poor or totally lacking in microbial activity. At Reykjanes the lack of microbes was clearly linked to the extremely large sinter growth rate, combined with the high salinity and temperature. Clone libraries for all other sites (4 libraries with a total of 283 clones) revealed a large biodiversity with

TABLE 1. Physicochemical conditions, sinter growth-rate and class-level diversity of bacterial communities at each study site.

	Geysir	Hveragerdi	Krafla	Svartsengi	Reykjanes
Physicochemical characteristics					
T ($^{\circ}\text{C}$)	Max. 96	Max. 74	80	42	75
Salinity (%)	0.05	0.04	0.06	2.56	4.67
pH	9	9.1	10	7.7	7.5
SiO_2 ($_{\text{aq}}$) (ppm)	363	304	603	250	695
Growth rate ($\text{kg y}^{-1} \text{m}^{-2}$)	0.3 ± 0.1	2.1 ± 0.7	19.5 ± 2.4	9.7 ± 3.5	304 ± 20
Phylogenetic diversity					
Type of classes	Aquificae (58)	Deinococci (45) Aquificae (20)	γ -Proteobacteria (21) Actinobacteria (9)	γ -Proteobacteria (70) α -Proteobacteria (10)	none
(# clones)	γ -Proteobacteria (1)	γ -Proteobacteria (4) β -Proteobacteria (3)	Flavobacteria (8) Aquificae (4)	Flavobacteria (4) Cyanobacteria (4)	
	Bacilli (2)	Nitrospira (4) Bacilli (1) unidentified (4)	α -Proteobacteria (3) β -Proteobacteria (1)	Sphingobacteria (3) β -Proteobacteria (2) unidentified (2)	

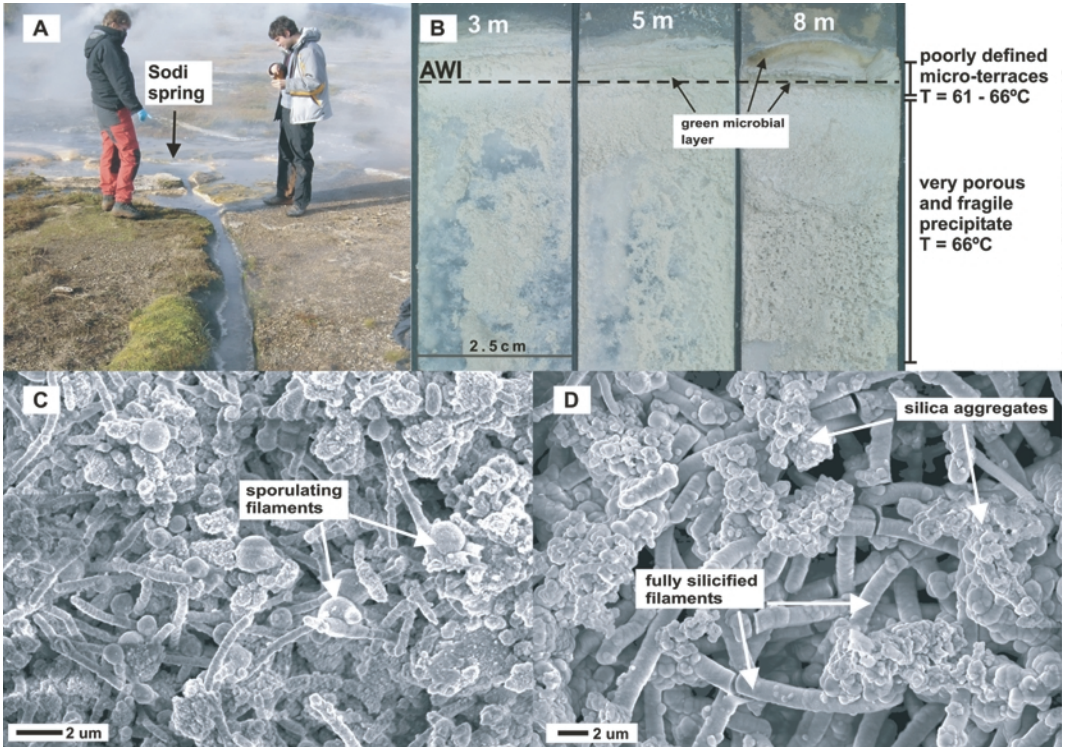


FIG. 2. (a) Sodi spring at Geysir; (b) slides collected after 3, 5 and 8 months showing progressive silicification and colonization. Photomicrographs show *in situ*-grown subaqueous sinters showing (c) microbial filaments and spores surrounded by silica aggregates; and (d) a mixture of fully silicified microbial filaments and silica aggregates.

specific links to various geochemical characteristics of each site (Table 1).

A distinct bacterial community structure dominated by one phylogenetic class (i.e., representing between 47 to 97% of the total clone library; Table 1) was typical for each site. The phylogenetic analyses showed the majority of

the clones affiliated to *Aquificae* (mostly related to *Thermocrinis*, most abundant at Geysir), *Deinococci* (*Thermus* species, most abundant at Hveragerdi) or γ -*Proteobacteria* (various genera, most abundant at Krafla and Svartsengi).

For example, the class-level diversity at Geysir and Svartsengi (Fig. 3) revealed that the high

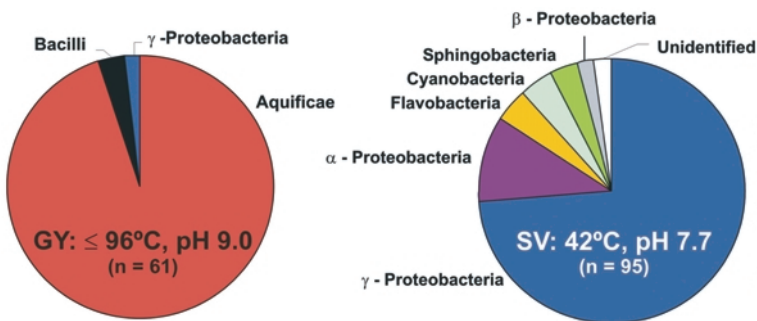


FIG. 3. Comparison between the class-level distribution and diversity of partial 16S rDNA sequences within the bacterial clone libraries for Geysir and Svartsengi.

temperature, low salinity and alkaline waters at Geysir were dominated by hyperthermophilic *Aquificae*. In contrast, at Svartsengi (low temperature, near neutral pH, high salinity) the class distribution was dominated by γ -*Proteobacteria* with the closest genus match being *Marinobacter* (marine species), which was not unexpected due to the low-temperature and the highly-saline geothermal waters.

Overall, at the class level, the *Proteobacteria* (with γ -*Proteobacteria* being most dominated) seemed less constrained by temperature, pH or salinity (Table 1). Interestingly, at the genera level among the γ -*Proteobacteria*, even small differences in geochemical parameters affected the species distribution (Fig. 4). At Krafla (KF) the γ -*Proteobacteria* were primarily related to isolates of the genus *Pseudomonas* (\pm *Lysobacter* and *Acinetobacter*) representative of freshwater and soil genera, which ties in with the meteoric nature of the waters and the proximity of common basaltic soils. At Geysir (GY) the γ -*Proteobacteria* clone was related to *Acinetobacter*, while the Hveragerdi (HV) clones belonged primarily to the genus *Stenotrophomonas*, again in both cases common bacteria in soils and freshwaters. One clone from Hveragerdi (HV 1A_20) branched well outside *Stenotrophomonas* and may thus represent a new phylotype within the γ -*Proteobacteria* (Fig. 4). Lastly, the *Marinobacter* genus found at Svartsengi (SV) was clearly linked to the high salinity of this site. This analysis showed that even relatively minor differences in temperature, salinity, or pH appeared to affect the bacterial community structure substantially (Tobler, 2008).

However, at each site it seems that the combination and specificity of temperature, pH and salinity, combined with the precipitation rate, governed both the sinter types and growth rates as well as the biodiversity patterns.

Conclusion

The microbial community structure and phylogenetic diversity in the studied Icelandic geothermal environments was clearly affected by variations in the physicochemical conditions of the geothermal waters with clear connections between specific geochemical characteristics (i.e. temperature, pH or salinity) and bacterial genera. In most cases, however, the bacterial diversity was influenced by a combination of geochemical factors with specific bacterial phyla and genera exploiting particular metabolic niches constrained by the prevailing geochemical and hydrodynamic parameters. Furthermore, the presence and abundance of microbial colonisation clearly affected the resulting textures and structures of the forming silica sinters. Together, these observations can aid the search for biomarkers in rocks in ancient geothermal systems, which in turn can be useful to derive the geochemical parameters at the time of sinter deposition and colonization, both on Earth and possibly also in the recently discovered siliceous deposits on Mars (Squyres and Team, 2007).

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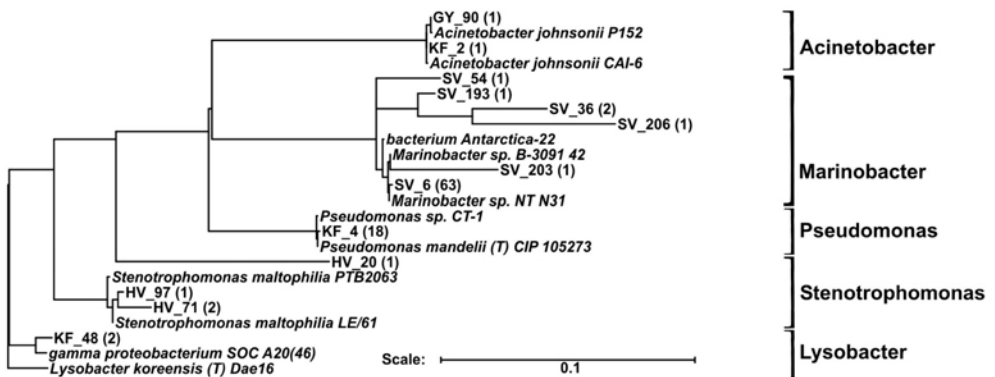


FIG. 4. Evolutionary distance dendrogram of γ -*Proteobacteria* 16S rDNA gene sequences in the context of currently recognized bacterial divisions, with *Lysobacter koreensis* used as an outgroup.

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References

- Hjorleifsdottir, S., Skirnisdottir, S., Hreggvidsson, G.O., Holst, O. and Kristjansson, J.K. (2001) Species composition of cultivated and noncultivated bacteria from short filaments in an Icelandic hot spring at 88°C. *Microbial Ecology*, **42**, 117–125.
- Iler, R.K. (1979) *The Chemistry of Silica*. John Wiley, New York.
- Konhauser, K.O., Jones, B., Reysenbach, A.-L. and Renaut, R.W. (2003) Hot spring sinters: keys to understanding Earth's earliest life forms. *Canadian Journal of Earth Science*, **40**, 1713–1724.
- Mountain, B.W., Benning, L.G. and Boerema, J. (2003) Experimental studies on New Zealand hot spring sinters: rates of growth and textural development. *Canadian Journal of Earth Sciences*, **40**, 1643–1667.
- Squyres, S.W. and Team, A.S. (2007) Recent results from the Spirit Rover at Home Plate and 'Silica Valley'. *EOS Transactions*, AGU, **88**, P21C-01 (abstr).
- Tobler, D.J., Stefánsson, A. and Benning, L.G. (2008) In-situ silicification experiments in Icelandic hot springs. *Geobiology* (in press).
- Tobler, D.J. (2008) *Molecular pathways of silica nanoparticle formation and biosilicification*. PhD thesis. University of Leeds.