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François Orange [a] , Stefan V. Lalonde [b] & Kurt O. Konhauser [a]

[a] Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton, Alberta, Canada

[b] UMR 6538 Domaines Océaniques, Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale, Technopôle Brest-Iroise, Place Nicolas Copernic, Plouzané, France

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The Formation and Preservation of *Synechococcus elongatus* Cell Molds in Simulated Silica Sinter: Implications for the Identification of Microfossils

FRANÇOIS ORANGE[1]*, STEFAN V. LALONDE[2], and KURT O. KONHAUSER[1]

[1]*Department of Earth and Atmospheric Sciences, University of Alberta, Edmonton, Alberta, Canada*
[2]*UMR 6538 Domaines Océaniques, Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale, Technopôle Brest-Iroise, Place Nicolas Copernic, Plouzané, France*

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Siliceous sinters that precipitate around modern hot spring systems are able to fossilize the indigenous microbial communities, forming molds that accurately outline the shape of the microorganisms. Over time, the biomass decays, and only silica molds or their infill may remain as evidence of the former living cells. However, little is known regarding the fidelity of such silica molds in terms of size and morphology, and the preservation of critical parameters for the identification of ancient silicified microorganisms by silica molds remains untested. Here we report experiments examining the formation of microbial molds of the cyanobacterium *Synechococcus elongatus* in silica gel. We demonstrate that post-depositional processes, primarily desiccation, are crucial for obtaining accurate and robust molds, and that initial desiccation acts to strengthen cell molds against further alteration. However, all silica gel treatments systematically created preservational biases (changes in size, additional structures) that may be misleading and may complicate the identification of fossil microorganisms.

Keywords: early diagenesis, microfossils, silica gel, siliceous sinter, silicification, *Synechococcus elongatus*, taphonomy

Introduction

Siliceous sinters form in many hot springs and geysers following the precipitative loss of dissolved silica from geothermal waters (Cady et al. 1995; Walter 1976a, 1976b; Walter et al. 1972, 1976). Silica mineral precipitation may be the result of several abiotic drivers, including cooling, pH change, or evaporation after subaerial exposure (Fournier 1985; Jones and Renaut 1996; Jones et al. 1998, 2005; Renaut et al. 2002; Rimstidt and Cole 1983; White et al. 1956). Silicification can also be promoted by reactive mineral and microbial cell surfaces that serve as reactive substrata for heterogeneous nucleation (Benning et al. 2004a, 2004b; Konhauser et al. 2004; Lalonde et al. 2005, 2008a, 2008b; Phoenix et al. 2003; Yee et al. 2003). In the case of the latter, the microbial communities often become encrusted in amorphous silica. This fossilization process has been studied in depth during the last decades through *in situ* studies (Cady and Farmer 1996; Handley et al. 2005, 2008; Jones et al. 1998, 2001, 2003, 2004; Konhauser and Ferris 1996; Konhauser et al. 2001; Schultze-Lam et al. 1995; Tobler et al. 2008; Walter et al. 1972, 1976) and fossilization experiments (Benning et al. 2004a, 2004b; Birnbaum et al. 1989; Ferris et al. 1988; Francis et al. 1978; Lalonde et al. 2005; Oehler 1976; Oehler and Schopf 1971; Orange et al. 2009, 2011; Toporski et al. 2002; Westall 1997; Westall et al. 1995). In particular, scanning electron microscopy (SEM) observation of siliceous sinters has revealed a general chronology of fossilization where silica particles on the cells progressively grow and merge, eventually forming a crust that completely embeds the microbial community.

Silicification permits the excellent preservation of cells and microbial communities (e.g., Jones et al. 2001, 2008). The small size of initial silica precipitates (10s of nanometres in diameter) facilitates high fidelity molding of cell shape and preservation of details, such that septa separating two cells, internal cellular components, and details of the cell wall may be conserved (Cady and Farmer 1996; Jones et al. 1998, 2003, 2005; Jones

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*Address correspondence to François Orange, Universidad de Puerto Rico, Facultad de Ciencias Naturales, Departamento de Fisica, P.O. Box 70377, San Juan, PR 00936-8377, USA; Email: francois.orange@gmail.com

and Renaut 1996; Kyle et al. 2007; Renaut et al. 1998; Schultze-Lam et al. 1995; Tobler et al. 2008). By contrast, without fossilization, microorganisms typically degrade within a few days of death, leaving unidentifiable cell remnants (Bartley 1996; Cassie and Cooper 1989; Francis et al. 1978; Oehler 1976; Oehler and Schopf 1971; Orange et al. 2009; Phoenix and Konhauser 1999; Schultze-Lam et al. 1995; Walter et al. 1976). The quality and preservation of silica cell molds appears to depend on the rapidity of silica precipitation and complete entombment of microbial structures by silica.

Fossilized microorganisms, or their molds, may also be subject to co- or post-depositional modification in shape, structure or size, depending on the conditions of fossilization and characteristics of the microorganisms. Jones et al. (2001) surveyed the complexity of the microbial fossilization process in nature, noting that microfossils from the same species may appear morphologically quite different, yet at other times, different species may appear very similar. This pattern has a number of underlying reasons.

For instance, precipitation of silica on the outer surface of the cells may increase the size of the microfossil, making it appear as though the initial cell was larger than in actuality, whereas silica filling the void space left by the microorganism can lead to the elimination of the cell contour, including septa separating two cells, unless the sheath, if present, has been mineralized. Secondary minerals such as halite may also fill void molds, possibly altering their quality. In a general way, all of these processes may potentially modify or destroy information related to initial size and morphology of the microfossils or of the mold, and thus constitute a preservational bias. Therefore, if identification of microorganism taxa from the microbial molds is possible, it should be done with extreme care, and complete knowledge of the possible preservational biases.

The present study centers on an experimental approach to better characterize such potential preservational biases. Using simple silica gelation experiments and cultured cyanobacterial biomass, our objectives were to understand how (1) molds develop during the formation of a siliceous matrix, (2) the matrix behaves when subjected to different stresses (evaporation, high temperature), and (3) these conditions impact initial and long term silica mold fidelity.

Materials and Methods

Cell Growth

We used for this study the rod-shaped cyanobacterium, *Synechococcus elongatus* PCC 7942, whose genus includes microorganisms commonly found forming surface layers of microbial mats in hot spring systems (e.g., Ferris et al. 1996; Jones et al. 1998; Walter et al. 1972). Pure cultures were grown under constant illumination of $\sim$100 μE/m^2/s in liquid BG-11 media (Rippka et al. 1979) at 30°C with shaking and filtered air bubbling. Cells were harvested by centrifugation (7500 rpm, 15 min).

Silica Gelation Experiments

Concentrated sodium silicate solution (40°–42° Bé, soda water glass, 1.38–1.42 kg/L; 29.2% SiO_2, 9.1% NaOH; Fisher Scientific) served as the silica source, diluted 10 times to a working stock. Silica gel was obtained by adding 140 μL of 12M HCl for each 5 mL of silicate stock solution. Gel formed within minutes after acid addition. For experiments with cells, 100 μL of a *S. elongatus* cell pellet was added before gelation. The final gel thus contained $\sim$2.9% SiO_2 and > 96% H_2O.

The following experiments were performed with gels containing *S. elongatus* cells: (1) Immediately after the addition of acid, and before the formation of the gel, the cell-silica solution was spread at the bottom of a Petri dish, forming a $\sim$1-mm-thick layer, and left to dry at room temperature, with frequent sampling (24 h, 2 weeks, 2 months) and weighing. (2) A fresh cell-silica gel, as well as a cell-silica gel air-dried for 2 months, were put in an oven at 200°C to induce accelerated drying and important stress, and sampled after 6 h, and then 6 days. As a control experiment, a drop of silica gel was deposited after HCl addition on a piece of Parafilm, and a TEM grid (copper, grid square size = 60 $\times$ 60 μm) was put on the drop to form a regular micrometer-scale pattern on the silica gel. The drop was either let to dry at room temperature or put in an oven at 200°C for 6 h to study the compaction of silica and morphological changes.

Electron Microscopy

Fresh gel samples, or samples at early stages of drying, were prepared for SEM using the critical point drying method after dehydration in ethanol with the procedure described in Orange et al. (2009). Other samples were observed directly after air-drying, or after rinsing in distilled water to remove any other precipitated salts (e.g., halite). Samples were placed on silver-painted SEM sample stubs and gold coated. SEM observations were performed at 5 kV using a JEOL 6301F Field Emission Gun Scanning Electron Microscope (FEG-SEM) in the Department of Earth and Atmospheric Sciences at the University of Alberta.

Results and Discussion

S. elongatus cells are characterized by various lengths (Figure 1a), but little variation in width (0.8–1.0 μm; Figures 1a, 2b). Measurements of mold and cell widths were subsequently used to characterize the evolution of gel or sinter volume through the different conditions tested.

Formation of the Silica Gel and Microbial Cell Molds

Silica gel formed within minutes after HCl addition due to pH-induced oversaturation and rapid polymerization of silica colloids (20–30 nm in diameter; Figure 1b). During its formation the silica gel immediately immobilized the *S. elongatus* cells and enveloped them as a mold that preserved their size and morphology (Table 1; Figure 2b) with a sharp outline

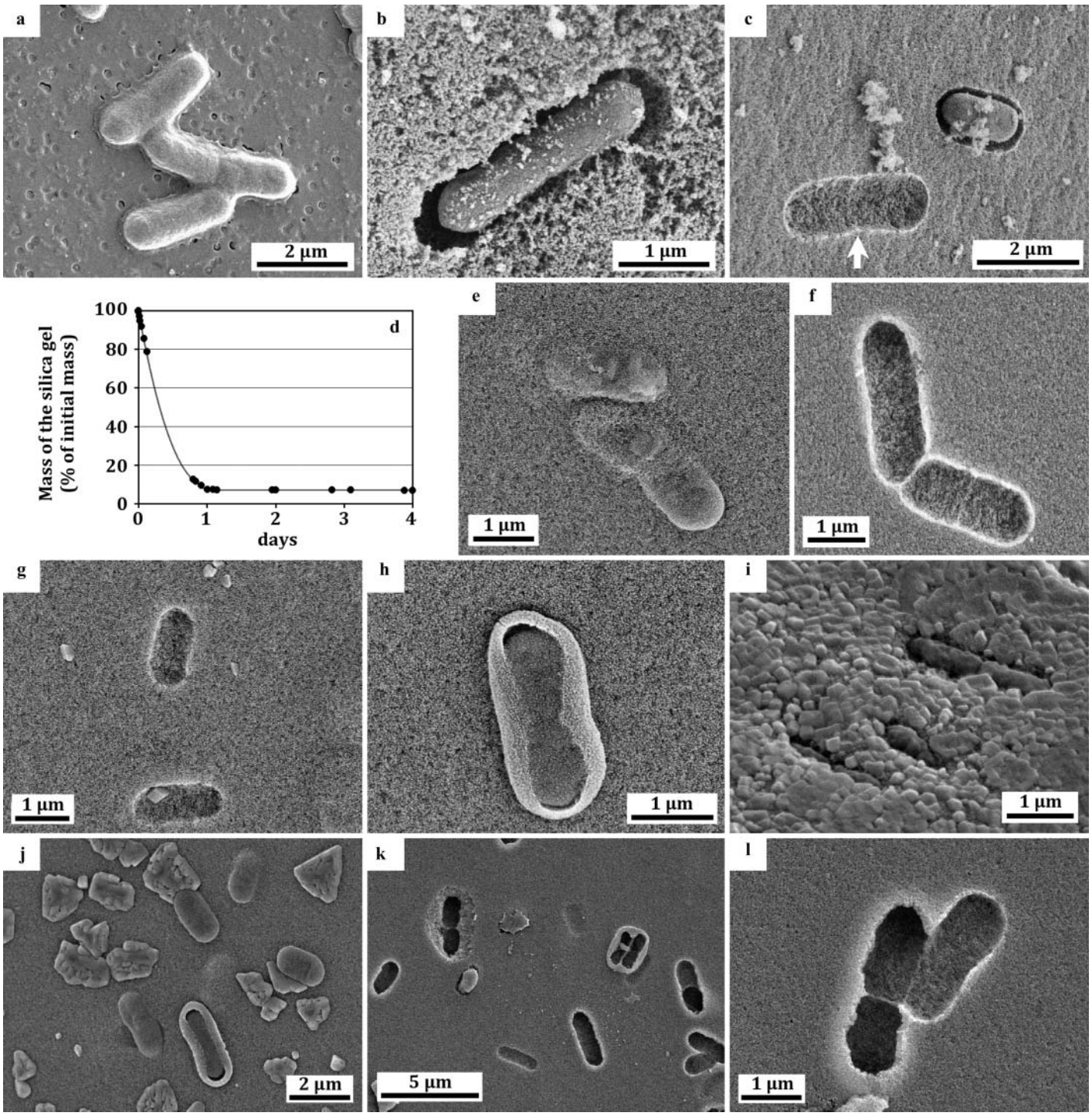

Fig. 1. Scanning electron microscope (SEM) micrographs showing the formation and air-drying of a silica gel mixed with *Synechococcus elongatus* cells. (a) fresh *S. elongatus* cells; (b) *S. elongatus* cell in a fresh silica gel, prepared with the critical point drying method; (c) *S. elongatus* cell and void mold in a silica gel air-dried for 24 h, prepared with the critical point drying method; (d) weight loss of the silica gel during air-drying; (e-h) *S. elongatus* cells (e, h) and void molds (f, g) in a silica gel air-dried for 2 weeks, prepared with the critical point drying method. Note the granular crust surrounding the cell in plate h; (i-j) *S. elongatus* cells in a silica gel covered by salt crystals air-dried for two months; and (k-l) void molds in a silica gel air-dried for 2 months, rinsed with distilled water. All micrographs were captured at 5 kV.

Table 1. Experimental conditions and mold width parameters

	Samples	Number of objects measured	Range (μm)	Mean value (μm)	Standard deviation σ (μm)
TEM grid experiments	TEM grid square	11	58.30–61.51	59.61	1.18
	Silica gel–Air-dried (6 days)	33	27.58–30.96	29.09	0.83
	Silica gel–Dried at 200°C (6 h)	30	18.81–28.76	23.83	2.70
Wet gel experiments	*Synechococcus elongatus* cells	18	0.77–0.99	0.87	0.07
	Fresh wet silica gel	13	0.77–1.02	0.87	0.06
Air-drying experiments	Silica gel–Air-dried (24 h)	12	0.86–1.12	0.95	0.11
	Silica gel–Air-dried (2 weeks)	31	0.42–1.13	0.81	0.18
	Silica gel–Air-dried (2 months)	28	0.69–1.23	0.91	0.10
High-temperature drying experiments	Silica gel–200°C (6 h)	20	0.41–0.83	0.61	0.10
	Silica gel–200°C (6 days)	21	0.38–1.08	0.68	0.21
	Air-dried gel (2 months)–200°C (6 h)	21	0.71–1.05	0.87	0.10
	Air-dried gel (2 months)–200°C (6 days)	35	0.47–0.99	0.84	0.12

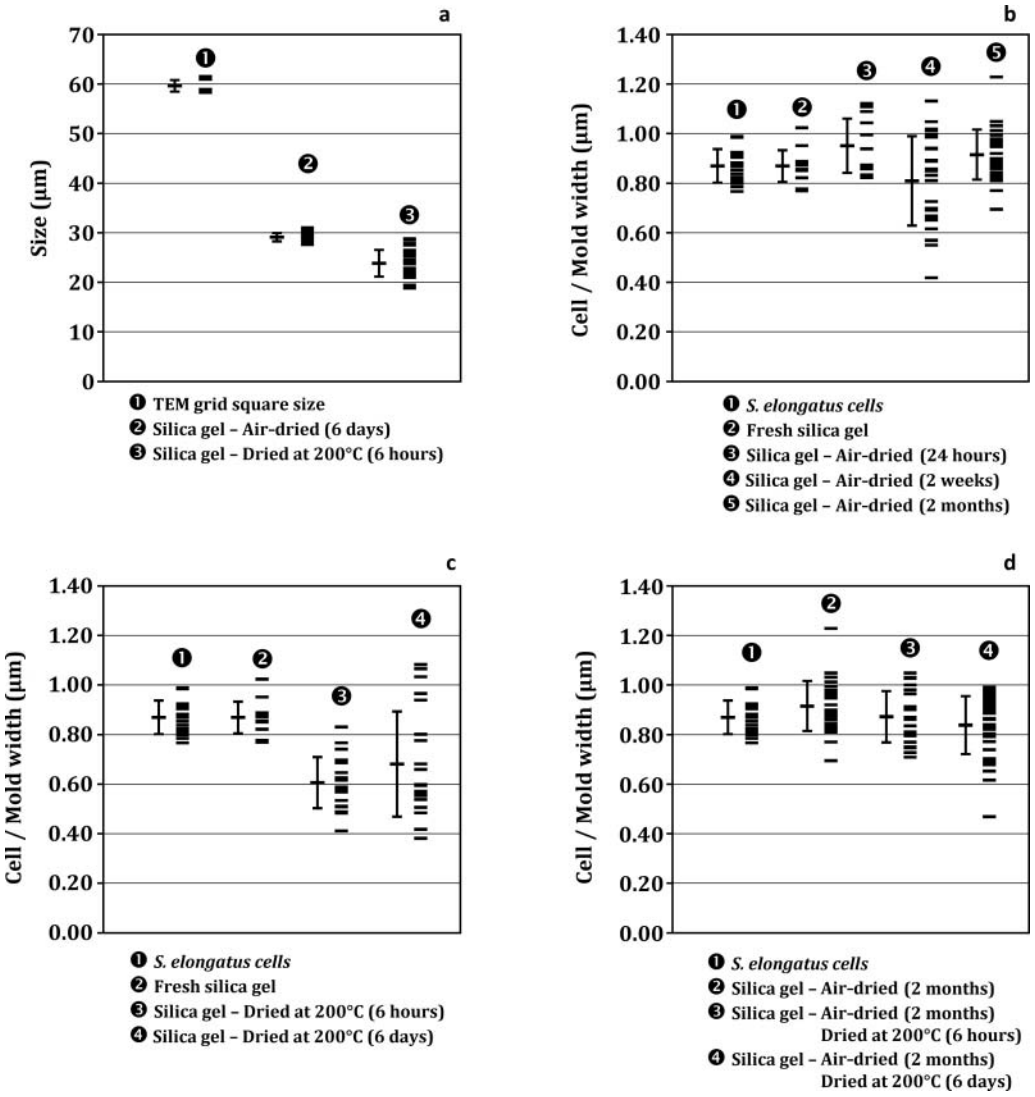

Fig. 2. Changes in size of *Synechococcus elongatus* cell molds in the silica gel. (a) Control; size of the square pattern drawn by a TEM grid in silica gel after drying at room temperature and at 200°C; (b) width of *S. elongatus* cell molds in fresh silica gel dried at room temperature for varying lengths of time; (c) width of *S. elongatus* cell molds in fresh silica gel dried at 200°C for varying lengths of time; and (d) width of *S. elongatus* cell molds in silica gel air-dried for 2 months and then dried at 200°C for 6 h or 6 days. Mean values and standard deviations are shown (side bars). Measurements were made directly from SEM micrographs.

(Figure 1b). Clearly, the molds were formed by direct casting of the cells by the silica gel. Gaps observed between the gel and cells (Figures 1b, 1c) are a consequence of the critical point drying used for SEM preparation, which reduced the size of the cells without affecting the silica gel. A repulsion of silica by cells can also produce such a gap, but would result in molds with irregular outlines (Meunier et al. 2010; Rooke et al. 2008), which was not observed here. The silica particles seen on cells (Figure 1b) may be the result of initial nucleation of silica directly on the cell wall, but may also be silica particles sticking to the cell surface after having being remobilized during sample preparation.

Air-Drying of the Silica Gel

After exposure to air, the silica gels quickly dried and fragmented, yielding pieces of dried silica that were coloured green by the cells. The drying silica gel lost more than 90% of its weight within a day of the dehydration process. This weight remained stable thereafter, with only 6.5% of its initial mass remaining, and thus having lost almost all of its water (initial content >96%; Figure 1d). Drying was also accompanied by compaction of the gel, which resulted in a ~50% size decrease, as measured from TEM grid mold dimensions (from regular 60 μm squares to 28–31 μm after air-drying; Figures 2a, 3a, 3b, Table 1). This compaction was the result of the evaporation of most of the water contained in the gel and was accompanied by the aggregation of the silica particles.

SEM observations reveal that the dried silica gel exhibits a very compact and homogeneous granular matrix, composed of closely packed silica particles (compare Figure 1b and Figure 1c). The size of these particles remained unchanged (20–30 nm) throughout drying, indicating that no further silica precipitation occurred after the formation of the gel. Dehydration also led to the formation of salt crystals (mainly NaCl, formed of Na from the sodium silicate solution and Cl from the acid), which precipitated after the silica and partially covered the dried silica gel at the last stages of evaporation (Figures 1i, 1j). These salts crystals could be removed by rinsing, which showed that they had no damaging effects to the silica or the cells (Figures 1c, 1f, 1l).

During the entire drying experiment, *S. elongatus* cells remained well preserved, showing only a slight wrinkling of the cell surface due to dehydration and cell lysis (Figure 1i). They also remained in direct contact with the silica (Figure 1e). The compaction led to finer and more detailed molds of cell shapes in the silica matrix, with very fine structures perfectly molded by the silica (such as the septum between two dividing cells; Figure 1c, arrow; Figure 1l). The range in width of the molds increased during the drying (from 0.8–1.0 μm to 0.4–1.1 μm after 2 weeks; Figure 2b), but the mean value remained close to the initial value (Table 1), indicating no major size changes in the molds.

However, some void molds became significantly smaller (width 0.4–0.7 μm) (Figures 1g, 2b). As shown by salt crystals grown in these void molds, the cells of these molds had likely been removed early during the drying and compaction processes, which led to a reduced mold size. Some individual or grouped cells were surrounded by a granular envelope that appeared to have formed at the cell/silica interface (Figures 1h, 1j, 1k). Its nature and origin remains unclear, but it may be the result of the formation of finer silica particles on the extracellular polymeric substances (EPS) covering, or secreted, by the cells.

Table 2. Sample mass change (in percent) after air-drying or heat treatments of varying lengths of time

	Percentage of mass of sample remaining after heat treatment	
Samples	200°C–6 h	200°C–6 days
Fresh silica gel without cells	5.8%	n.d.
Fresh silica gel with *Synechococcus elongatus* cells	5.8%	5.7%
Silica gel air-dried during 2 months	90.8%	91.8%

Each value is the result of at least two separate measurements.

High-Temperature Drying of the Silica Gel

The high temperature experiments had two objectives. First, when applied to a fresh silica gel, it was to observe the effects of accelerated drying. Second, when applied to an already dried gel, it was to evaluate the effects of such an important stress on already well-formed molds.

After removal from the oven, both air-dried and wet silica gels had a yellowish colour that presumably was due to the degradation of the photosynthetic pigments of the cyanobacteria. A slightly higher weight loss of the wet gel was observed when exposed to high temperature as compared to air-drying (~95% vs. ~93.5%; Table 2, Figure 1d). Similarly, the gel experienced a higher degree of compaction compared to the control experiment (~60% vs. ~50%; Figures 2a, 3c; Table 1). Even gel that was first air-dried for two months lost ~10% of its mass after heat treatment (Table 2), likely from water remaining in the gel. At 200°C, the majority of *S. elongatus* cells were still present and in a good state of preservation after only 6 h (Figures 4a, 4i), however all cells were either completely destroyed (Figures 4k, 4n) or significantly degraded (Figure 4d) after 6 days at this high temperature.

A consequence of the exposure of fresh silica gel to high temperatures and corresponding accelerated drying was a very rapid compaction of the gel, which was complete after 6 hours. High temperature did not affect the size of the silica particles (20–30nm), but caused them merge, forming a matrix which had retained void spaces (Figure 4e) as opposed to homogeneously compact matrix of the control experiment (Figure 3c).

Due to these voids, the quality of the cells molds was typically poorer than the air-drying experiments (Figures 4b, 4d, 4f). Although cells were minimally affected through the compaction of the silica gel during air-drying (Figure 1e), it appears that this was not the case during the fast drying of wet gel. Some cells displayed obvious signs of degradation

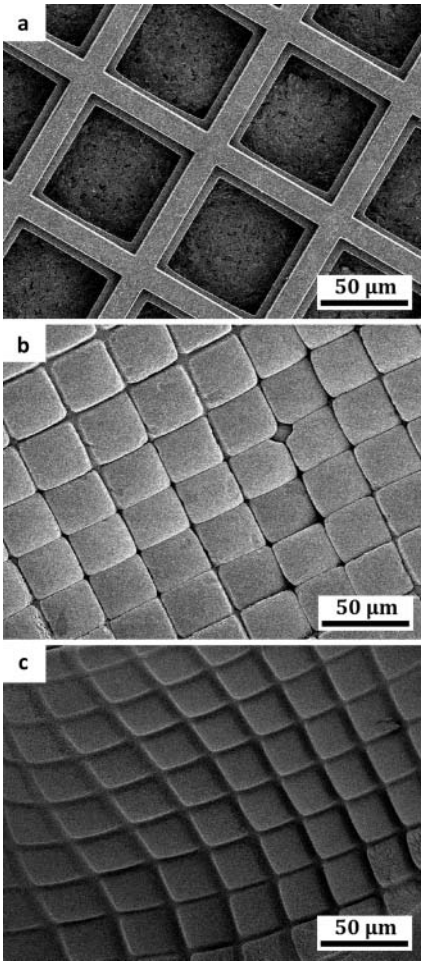

Fig. 3. SEM micrographs showing changes in the size of the pattern drawn in silica gel by a TEM grid. (a) TEM grid; (b) pattern in the silica gel after 6 days of air-drying; and (c) pattern in the silica gel after 6 h at 200°C. All micrographs are of the same magnification and were captured at 5 kV.

and crushing as a result of the quick compaction and high temperature exposure (Figure 4c). This resulted in a significant decrease in the mean width of the molds, from 0.87 μm to 0.6–0.7 μm (Table 1; Figure 2c).

In the gel previously air-dried, the quality of the cells molds were mostly unaffected by exposure to high temperature, and they retained very fine details (Figures 4j, 4k). The mean mold width was slightly smaller (Figure 2d), showing that high temperatures induced an additional compaction of the gel. Deformed (Figure 4l) and small (Figure 4m) molds were observed, but there was no evidence that these deformations were a consequence of the high temperature. Higher temperatures also led to the formation of well defined salts crystals on the surface of the gels (Figures 4a, 4g, 4h, 4k, 4l), filling empty cell molds (Figures 4h, 4k), and partially covering the cells (Figure 4g). As previously observed during drying at room temperature, salts were completely removed by rinsing, and had no damaging effects on cells or molds (Figures 4i, 4j). The preservation of the outer cell envelope after 6 days (Figure 4m) confirmed the siliceous nature of these structures, as they did not degrade as a result of the long exposure to heat nor dissolved by rinsing.

Mechanisms of Mold Formation and Preservation

The formation of well preserved molds was the result of two distinct phases: (1) formation of the mold, where the silica gel cast the shape of a template (i.e., TEM grid mesh or *S. elongatus* cells), and (2) subsequent improvement of the quality and robustness of these molds. The first phase was very short, as the polymerization of the silica solution occurred within a few minutes after the addition of HCl. Molds in the wet gel state were still fragile and only represented a coarse outline of the cell shape due to the voids present in the gel (Figure 1b).

The silica gel only became robust and compact as a consequence of the drying, both at room temperature or at 200°C. In either case, the quality and robustness of the molds were greatly improved. Firstly, the aggregation of the silica particles allowed the mold to include more fine details of the cell shape. The level of the details of the molds is theoretically the size of the silica particles (i.e., 20–30 nm). In the case of some very well formed and preserved molds (Figures 1f, 1l), the sharpness of the details of the molds was close to this theoretical value, as shown by the molding of very fine details. These molds are remarkably similar to structures documented in various geothermal environments (e.g., Jones et al. 1998, 2001, 2003, 2005, 2008; Kyle et al. 2007).

Incomplete compaction did not allow for the preservation of such fine details, and this was one of the reasons accounting for the poorer quality of the cell molds observed after drying wet silica gel at 200°C (Figures 4d, 4f); the other reason being the degradation of the cells during the compaction phase. Second, drying and particle aggregation gave more robustness to the silica matrix, and facilitated the preservation of the molds, such that further treatment (such as high temperature, rinsing for removing the salts or sample preparation for electron microscopy) did not affect the quality of the molds and the fidelity of the details preserved (Figures 4k, 4l).

The different treatments applied to the gels also modified their size (Figures 2b, 2c). Drying led to a weight loss of 90% (Figure 1d) and size compaction of ~50% (Figure 2a), but a ~50% size reduction of the cells molds was only observed when the cells had disappeared (for various reasons) early in the compaction process (Figures 1g, 2, 4f). When cells remained in their casts, variations in size were limited. Despite their soft nature, *S. elongatus* cells were consistently able to resist progressive compaction (i.e., during room temperature drying) (Figure 1e), but not fast compaction (i.e., high temperature drying) (Figure 4c).

Implications for the Identification of Hot Springs Microfossils

This study provides new insights related to understanding the formation and preservation of microfossils in geothermal environments where siliceous sinters commonly form. Our intent was not to faithfully replicate siliceous sinter formation, nor the fossilization of microorganisms in these environments; in our experiments, the silica concentration was higher, the

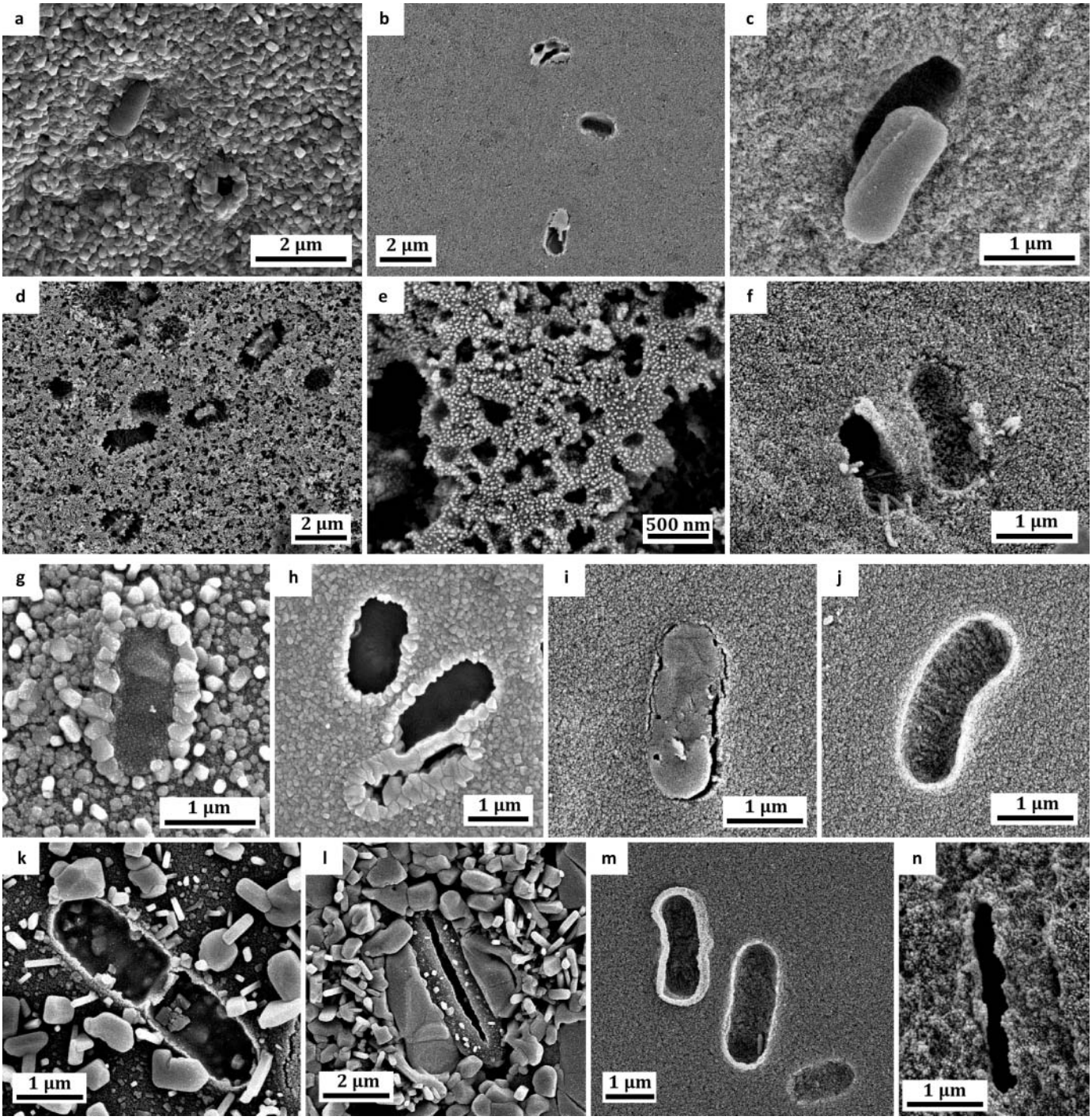

Fig. 4. SEM micrographs showing the results of drying experiments at 200°C for silica gels (fresh and previously air-dried for 2 months) mixed with *Synechococcus elongatus* cells. Fresh silica gel: (a) silica gel covered with salts crystals (200°C, 6 h); (b) *S. elongatus* cell molds, with cellular remains (200°C, 6 h, rinsed with distilled water); (c) *S. elongatus* cell with marks of crushing due to compaction and its mold (200°C, 6 h, rinsed with distilled water); (d) silica gel with *S. elongatus* cell molds (6 days, 200°C); (e) close-up on the silica matrix (6 days, 200°C); and (f) void *S. elongatus* cell molds (6 days, 200°C, rinsed with distilled water). Previously air-dried silica gel: (g-h) *S. elongatus* cell and molds in the dried silica gel, partly covered or filled with salts crystals (6 h, 200°C); (i-j) *S. elongatus* cell and mold (6 h, 200°C, rinsed with distilled water); (k-l) void molds, partly covered with salts crystals (6 h, 200°C); and (m-n) void molds (6 days, 200°C, rinsed with distilled water). All micrographs were captured at 5 kV.

polymerization and encasing of the cells was faster and no nucleation of silica on microbial surfaces occurred.

Actually, our experimental setup was more similar to methods of encapsulation, where microorganisms are encased in a silica gel for biomedical applications (e.g., Ferrer et al. 2006; Livage and Coradin 2006; Meunier et al. 2010; Roggendorf et al. 2001). Nonetheless, mold formation as simulated here does bear some similarities with the natural process, in that, following the nucleation of silica on microbial surfaces, the silica particles aggregate and eventually merge, leading to a more robust matrix, water expulsion, and a more accurate molding of the cellular structures.

Importantly, despite not being a fossilization analogue, our results show a physical role for cells in their own preservation by silica encrustation. The final quality of the molds is not only linked to compaction of the silica matrix, but also to whether cells had been potentially degraded by compaction (e.g. Figure 4c) or heat (e.g., Figure 4b). Air-dried cell mold experiments showed minimal signs of cell degradation, along with molds preserved with the best quality (Figures 1e, 1f, 1i, 1l). This appears to be similarly reflected in natural hot spring systems where numerous studies have demonstrated that very precise molds of cells in siliceous sinters are the result of a silica crust developing on either live or minimally degraded cells (Cady and Farmer 1996; Jones and Renaut 1996; Konhauser et al. 2001; Jones et al. 1998, 2003, 2005, 2008; Kyle et al. 2007; Parenteau and Cady 2010; Renaut et al. 1998; Schultze-Lam et al. 1995; Tobler et al. 2008).

Any degradation of the cells during silica particle nucleation, growth, and aggregation could prevent the formation of the molds or lead to the formation of low-quality molds of the cells. Such conditions lead to only a vague outline of the cell morphology in the resulting molds (e.g., Figure 4d). In addition, every treatment applied to the silica (i.e., drying, temperature) led to the modification of the size of the fossil traces, and ultimately, loss of information about the original size of the microorganisms. The bright 150–300 nm mineral crust that formed around some of the cells (Figures 1h, 1k, 4m) may also be identified as a remnant of a cellular structure, thereby leading to inaccurate interpretations of the size and the structure of the original microorganism. All of these factors may contribute to preservational biases observed during siliceous sinter formation and fossilization processes (see Jones et al. 2001 for an extensive listing), and as such, they should be considered to avoid being misled during the identification of microfossils.

Only the already well formed and solid molds ultimately resisted additional treatments, exemplified by the good preservation of molds formed in an air-dried gel and then exposed to a 200°C temperature (Figure 4k). In natural siliceous sinters, such molds would be subsequently exposed to various degrading processes during sinter growth and early diagenesis, such as recrystallization, erosion, deformation, secondary mineral deposition, and later generations of silica precipitation. However, we demonstrate here that most crucial steps of the feature preservation process would have already occurred; early desiccation of the silica matrix appears important for both the initial preservation of details and subsequent long-term resistance to further alteration. Traces of microorganisms entombed under such conditions appear most likely to be preserved.

Conclusions

This study provides new insight into the processes that lead to the preservation of very detailed molds of microorganisms in natural hot spring siliceous sinters through silica gelation experiments simulating rapid cell entombment and early diagenesis. We show that preservation is the result of two phases: (1) formation of the siliceous molds, as the result of the silica polymerization and precipitation, and (2) a phase of strengthening of the silica matrix and improvement of the quality of the molds, a consequence of silica particle aggregation and matrix condensation associated with desiccation. These two phases are both essential for obtaining high fidelity, robust molds. Original morphology and size of the microorganisms may be preserved in the mold, but issues such as incomplete silica particle aggregation or the rapid degradation of cells at high temperature have negative consequences on the quality of the molds. Our experiments demonstrate that the transition from a wet silica matrix to dry silica mass results in modification of the information preserved about the original size of the microorganisms, and provides the first quantitative constraints on post-depositional size modification. As has been previously observed in natural settings, this study confirms that preservation biases are unavoidable during microfossils formation, and accordingly, should be taken into account when considering the identification of microfossils.

References

Bartley JK. 1996. Actualistic taphonomy of cyanobacteria: implications for the Precambrian fossil record. Palaios 11:571–586.

Benning LG, Phoenix VR, Yee N, Konhauser KO. 2004b. The dynamics of cyanobacterial silicification: an infrared micro-spectroscopic investigation. Geochim Cosmochim Acta 68:743–757.

Benning LG, Phoenix VR, Yee N, Tobin MJ. 2004a. Molecular characterization of cyanobacterial silicification using synchrotron infrared micro-spectroscopy. Geochim Cosmochim Acta 68:729–741.

Birnbaum SJ, Wireman JW, Borowski R. 1989. Silica precipitation by the anaerobic sulphate reducing bacterium *Desulfovibrio desulfuricans*: Effects upon cell morphology and implications for preservation. In: Crick RE, editor. Origin, evolution, and modern aspects of biomineralization in plants and animals. Plenum Press, New York. p507–516.

Cady SL, Farmer JD. 1996. Fossilization processes in siliceous thermal springs: trends in preservation along thermal gradients. In: Bock GR, Goode JA, editors. Evolution of Hydrothermal Ecosystems on Earth (and Mars?). John Wiley and Sons, New York. p150–173.

Cady SL, Farmer JD, Des Marais DJ, Blake DF. 1995. Columnar and spicular geyserites from Yellowstone National Park, WY; scanning and transmission electron microscopy evidence for biogenicity (abstract). Geol Soc Am Abstracts Prog 27:A305.

Cassie V, Cooper RC. 1989. Algae of New Zealand thermal areas. Bibliotheca Phycolog 78:1–159.

Ferrer ML, Garcia-Carvajal ZY, Yuste L, Rojo F, del Monte F. 2006. Bacteria viability in sol-gel materials revisited: cryo-SEM as a suitable tool to study the structural integrity of encapsulated Bacteria. Chem Mater 18:1458–1463.

Ferris FG, Fyfe WS, Beveridge TJ. 1988. Metallic ion binding by *Bacillus subtilis*: Implications for the fossilization of microorganisms. Geology 16:149–152.

Ferris MJ, Ruff-Roberts AL, Kopczynski ED, Bateson MM, Ward DM. 1996. Enrichment culture and microscopy conceal diverse thermophilic *Synechococcus* populations in a single hot spring microbial mat habitat. Appl Environ Microbiol 62:1045–1050.

Fournier RO. 1985. The behavior of silica in hydrothermal solutions. In: Berger BR, Bethke PM, editors. Geology and Geochemistry of Epithermal Systems: Society of Economic Geologists, Reviews in Economic Geology, Vol. 2, Littleton, CO: Society of Economic Geologists, p45–61.

Francis S, Margulis L, Barghoorn ES. 1978. On the experimental silicification of microorganisms. II. On the time of appearance of eukaryotic organisms in the fossil record. Precambrian Res 6:65–100.

Handley KM, Campbell KA, Mountain BW, Browne PRL. 2005. Abiotic-biotic controls on the origin and development of spicular sinter: in situ growth experiments. Champagne Pool, Waiotapu, New Zealand. Geobiology 3:93–114.

Handley KM, Turner SJ, Campbell KA, Mountain BW. 2008. Silicifying biofilm exopolymers on a hot-spring microstromatolite: Templating nanometer-thick laminae. Astrobiology 8:747–770.

Jones B, de Ronde CEJ, Renaut RW. 2008. Mineralized microbes from Giggenbach submarine volcano. J Geophys Res 113:B08S05.

Jones B, Konhauser KO, Renaut RW, Wheeler R. 2004. Microbial silicification in Iodine Pool, Waimangu Geothermal area, North Island, New Zealand: Implications for recognition and identification of ancient silicified microbes. J Geol Soc London 161:983–993.

Jones B, Renaut RW. 1996. Influence of thermophilic bacteria on calcite and silica precipitation in hot springs with water temperatures above 90°C: Evidence from Kenya and New Zealand. Can J Earth Sci 33:72–83.

Jones B, Renaut RW, Konhauser KO. 2005. Genesis of large siliceous stromatolites at Frying Pan Lake, Waimangu geothermal field, North Island, New Zealand. Sedimentology 52:1229–1252.

Jones B, Renaut RW, Rosen MR. 1998. Microbial biofacies in hot-spring sinters: A model based on Ohaaki Pool, North Island, New Zealand. J Sediment Res 68:413–434.

Jones B, Renaut RW, Rosen MR. 2001. Taphonomy of silicified filamentous microbes–Implications for identification. Palaios 16:580–592.

Jones B, Renaut RW, Rosen MR. 2003. Silicified microbes in a geyser mound: the enigma of low-temperature cyanobacteria in a high temperature setting. Palaios 18:87–109.

Konhauser KO, Ferris FG. 1996. Diversity of iron and silica precipitation by microbial mats in hydrothermal waters, Iceland: implications for Precambrian iron formations. Geology 24:323–326.

Konhauser KO, Jones B, Phoenix VR, Ferris G, Renaut RW. 2004. The microbial role in hot spring silicification. Ambio 33:552–558.

Konhauser KO, Phoenix VR, Bottrell SH, Adams DG, Head IM. 2001. Microbial–silica interactions in Icelandic hot spring sinter: possible analogues for some Precambrian siliceous stromatolites. Sedimentology 48:415–433.

Kyle JE, Schroeder PA, Wiegel J. 2007. Microbial Silicification in Sinters from Two Terrestrial Hot Springs in the Uzon Caldera, Kamchatka, Russia. Geomicrobiol J, 24:627–641.

Lalonde SV, Konhauser KO, Reysenbach AL, Ferris FG. 2005. The experimental silicification of Aquificales and their role in hot spring formation. Geobiology 3:41–52.

Lalonde SV, Smith DS, Owttrim GW, Konhauser KO. 2008a. Acid-base properties of cyanobacterial surfaces I: Influences of growth phase and nitrogen metabolism on surface reactivity. Geochim Cosmochim Acta 72:1257–1268.

Lalonde SV, Smith DS, Owttrim GW, Konhauser KO. 2008b. Acid-base properties of cyanobacterial surfaces II: Silica as a chemical stressor influencing cell surface reactivity. Geochim Cosmochim Acta 72:1269–1280.

Livage J, Coradin T. 2006. Living cells in oxide glasses. Rev Mineral Geochem 64:315–332.

Meunier CF, Dandoy P, Su BL. 2010. Encapsulation of cells within silica matrixes: towards a new advance in the conception of living hybrid materials. J Coll Interf Sci 342:211–224.

Oehler JH. 1976. Experimental studies in Precambrian paleontology: structural and chemical changes in blue-green algae during simulated fossilization in synthetic chert. Geol Soc Am Bull 87:117–129.

Oehler JH, Schopf JW. 1971. Artificial microfossils: experimental studies of permineralization of blue-green algae in silica. Science 174:1229–1231.

Orange F, Disnar JR, Westall F, Prieur D, Baillif F. 2011. Metal binding by the early Earth analogue microorganism, Archaea *Methanocaldococcus jannaschii* and its effects on silicification. Palaeontology 54:953–964.

Orange F, Westall F, Disnar JR, Prieur D, Bienvenu N, Le Romancer M, Défarge Ch. 2009. Experimental silicification of the extremophilic Archaea *Pyrococcus abyssi* and *Methanocaldococcus jannaschii*. Applications in the search for evidence of life in early Earth and extraterrestrial rocks. Geobiology 7:403–418.

Parenteau MN, Cady SL. 2010. Microbial biosignatures in iron-mineralized phototrophic mats at Chocolate Posts hot springs, Yellowstone National Park, United States. Palaios 25:97–111.

Phoenix VR, Konhauser KO. 1999. Photosynthetic controls on the silicification of cyanobacteria. In: Armannsson H, editor. Geochemistry of the Earth's Surface. A.A. Balkema, Rotterdam, Netherlands. p275–278.

Phoenix VR, Konhauser KO, Ferris FG. 2003. Experimental study of iron and silica immobilization by bacteria in mixed Fe-Si systems: Implications for microbial silicification in hot-springs. Can J Earth Sci 40:1669–1678.

Renaut RW, Jones B, Tiercelin JJ. 1998. Rapid in situ silicification of microbes at Loburu hot springs, Lake Bogoria, Kenya Rift Valley. Sedimentology 45:1083–1103.

Renaut RW, Jones B, Tiercelin JJ, Tarits C. 2002. Sublacustrine precipitation of hydrothermal silica in rift lakes: Evidence from Lake Baringo, central Kenya Rift Valley. Sediment Geol 148:235–257.

Rimstidt JD, Cole DR. 1983. Geothermal mineralization I: The mechanism of formation of the Beowawe, Nevada, siliceous sinter deposit. Am J Sci 283:861–875.

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61.

Roggendorf H, Böschel D, Templer J. 2001. Structural evolution of sodium silicate solutions dried to amorphous solids. J Non-Cryst Solids 293–295:752–757.

Rooke JC, Léonard A, Sarmento H, Descyb JP, Si BL. 2008. Photosynthesis within porous silica gel: Viability and activity of encapsulated cyanobacteria. J Mater Chem 18:2833–2841.

Schultze-Lam S, Ferris FG, Konhauser KO, Wiese RG. 1995. In situ silicification of an Icelandic microbial mat: implications for microfossil formation. Can J Earth Sci 32:2021–2026.

Tobler DJ, Stefánsson A, Benning LG. 2008. In-situ grown silica sinters in Icelandic geothermal areas. Geobiology 6:481–502.

Toporski JKW, Steele A, Westall F, Thomas-Keprta KL, McKay DS. 2002. The simulated silicification of bacteria—New clues to the modes and timing of bacterial preservation and implications for the search for extraterrestrial microfossils. Astrobiology 2:1–26.

Walter MR. 1976a. Hot-springs sediments in Yellowstone National Park. In: Walter MR, editor. Stromatolites: Developments in Sedimentology 20. Elsevier, Amsterdam. p489–98.

Walter MR. 1976b. Geyserites of Yellowstone National Park: an example of abiogenic "stromatolites." In: Walter MR, editor. Stromatolites: Developments in Sedimentology, Vol. 20. Elsevier, Amsterdam. p87–112.

Walter MR, Bauld J, Brock TD. 1972. Siliceous algal and bacterial stromatolites in hot springs and geyser effluents of Yellowstone National Park. Science 178:402–405.

Walter MR, Bauld J, Brock TD. 1976. Microbiology and morphogenesis of columnar stromatolites (Conophyton, Vacerrilla) from hot

springs in Yellowstone National Park. In: Walter MR, editor. Stromatolites: Developments in Sedimentology, Vol. 20. Elsevier, Amsterdam. p273–310.

Westall F. 1997. The influence of cell wall composition on the fossilization of bacteria and the implications for the search for early life forms. In: Cosmovici C, Bowyer S, Werthimer D, editors. Astronomical and Biochemical Origins and the Search for Life in the Universe. Editori Compositrici, Bologna, Italy. p491–504.

Westall F, Boni L, Guerzoni E. 1995. The experimental silicification of microorganisms. Palaeontology 38:495–528.

White DE, Brannock WW, Murata KJ. 1956. Silica in hot-spring waters. Geochim Cosmochim Acta 10:27–59.

Yee N, Phoenix VR, Konhauser KO, Benning LG, Ferris FG. 2003. The effect of cyanobacteria on silica precipitation at neutral pH: Implications for bacterial silicification in geothermal hot springs. Chem Geol 199:83–90.