



# Metal cation binding by the hyperthermophilic microorganism, *Archaea Methanocaldococcus Jannaschii*, and its effects on silicification

François Orange, Jean-Robert Disnar, Francès Westall, Daniel Prieur, Patrick Baillif

## ► To cite this version:

François Orange, Jean-Robert Disnar, Francès Westall, Daniel Prieur, Patrick Baillif. Metal cation binding by the hyperthermophilic microorganism, *Archaea Methanocaldococcus Jannaschii*, and its effects on silicification. *Palaeontology*, 2011, 54 (5), pp.953-964. 10.1111/j.1475-4983.2011.01066.x . insu-00616553

**HAL Id: insu-00616553**

**<https://insu.hal.science/insu-00616553>**

Submitted on 12 Oct 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

METAL CATION BINDING BY THE HYPERTHERMOPHILIC MICROORGANISM,  
ARCHAEA *METHANOCALDOCOCCUS JANNASCHII*, AND ITS EFFECTS ON  
SILICIFICATION

by FRANÇOIS ORANGE [1,2,3], JEAN-ROBERT DISNAR [2], FRANCES WESTALL  
[1,3], DANIEL PRIEUR [4] and PATRICK BAILLIF [2]

[1] Centre de Biophysique Moléculaire, CNRS, Rue Charles Sadron, 45071 Orléans Cedex 2,  
France. e-mails : francois.orange@cnrs-orleans, frances.westall@cnrs-orleans.fr

[2] Institut des Sciences de la Terre d'Orléans - UMR 6113, CNRS/Université d'Orléans, 1A  
Rue de la Férollerie, 45071 Orléans Cedex 2, France. e-mail : jean-robert.disnar@univ-  
orleans.fr

[3] Observatoire des Sciences de l'Univers en région Centre, 1A Rue de la Férollerie, 45071  
Orléans Cedex 2, France.

[4] Institut Universitaire Européen de la Mer, Technopôle Brest-Iroise, Rue Dumont d'Urville,  
29280 Plouzané, France. e-mail : daniel.prieur@univ-brest.fr

**Abstract:** A series of experiments was made to determine the capacity of an archaeal strain, *Methanocaldococcus jannaschii* to bind metals and to study the effects of metal-binding on the subsequent silicification of the microorganisms. The results showed that *M. jannaschii* can rapidly bind several metal cations ( $\text{Fe}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ). Considering the lack of silicification of this strain without metal-binding, these experiments demonstrate that Fe(III) ion binding to the cell wall components was of fundamental importance for successful silicification and, especially, for the excellent preservation of the cell wall. This study brings

new elements to the understanding of fossilisation processes, showing that the positive effect of Fe(III) on silicification, already known for Bacteria, can also apply to Archaea, and that this preliminary binding can be decisive for the subsequent fossilisation of these organisms. Knowledge of these mechanisms can be helpful for the search and the identification of microfossils in both terrestrial and extraterrestrial rocks, and in particular on Mars.

**Keywords:** Archaea, *Methanocaldococcus jannaschii*, metal binding, fossilisation, Early Archean.

THE earliest fossil traces of life in 3.5–3.3 billion years old (Ga) Early Archaean rocks comprise silicified microfossils that probably included chemotrophic and possibly anoxygenic photosynthetic microorganisms (Westall and Southam 2006). These fossils, however, most likely represent only a portion of the diversity of microorganisms living at that epoch, mainly because of the differential susceptibility of different species of microorganisms to fossilisation and preservation (Orange *et al.* 2009). Although still a matter of debate (Kasting *et al.* 2006), ocean water temperatures in the Early Archaean were estimated to be warm (< 55°C; van den Boorn *et al.* 2007) to hot (70–80 °C; Knauth and Lowe 2003), the higher temperatures being probably related to the pervasive hydrothermal activity (Hofmann and Bolhar 2007). In these conditions, early life was probably represented by mesophilic to thermophilic microorganisms, as well as hyperthermophiles in the vicinity of hydrothermal vents.

In a previous study we showed that two species of hyperthermophilic Archaea, *Methanocaldococcus jannaschii* and *Pyrococcus abyssi*, have very different reactions to

silicification (Orange *et al.* 2009). Whereas *P. abyssi* cells were able to bind silica and become silicified, *M. jannaschii* cells lysed rapidly and were not preserved (Figs. 3A-B-C).

However, if experiments with silica alone constitute a necessary preliminary step in the experimental study of the silicification of microorganisms, they do not necessarily take into account of the variety of elements that are contained in natural media, some of which might also intervene in the fossilisation process. Accordingly, it has been demonstrated that the fixation of Fe(III) on bacterial cell surfaces can inhibit early degradation of the cell walls and improve silica fixation (Ferris *et al.* 1988; Urrutia and Beveridge 1993, 1994; Fortin *et al.* 1998; Fein *et al.* 2002; Phoenix *et al.* 2003). More specifically, it has been suggested that Fe(III) ions fixed to bacterial cell walls inhibit the activity of autolytic enzymes responsible for cell decay after death of the cell, allowing more time for the fixation of silica and, thus, efficient preservation of the cellular structures (Ferris *et al.* 1988). Fein *et al.* (2002) have suggested that, in the case of a negatively charged cell wall, Fe(III) ions can form a ‘cation bridge’ and act as an intermediate between the cell wall functional groups and silica.

As part of a wide-ranging study on the preservation of the kinds of microorganisms that could have lived under the extreme environmental conditions of the early Earth and following on from a preliminary fossilisation experiment using pure silica (Orange *et al.* 2009), we undertook an investigation to determine whether Fe(III) ions and other metal cations could be taken up by microorganisms in the Archaea domain and, thus, improve their chances of fossilisation. We focused especially on delicate strains, such as *M. jannaschii*, whose cells are particularly fragile (Jones *et al.* 1983) and can quickly lyse following changes in the osmotic equilibrium of their environment (e.g. pH changes), or simply by prolonged growth. *M. jannaschii* was not fossilised after being exposed to silica alone (Orange *et al.* 2009).

For this study, five common metal cations (Fe, Ca, Zn, Cu, Pb) were tested. As previously mentioned, Fe(III) was firstly chosen for its positive effect on the fossilisation of Bacteria (Ferris *et al* 1988; Fein *et al* 2002). Fe and Ca are elements common in many environments and are concentrated by microorganisms with various processes (Beveridge and Murray 1976). Furthermore, numerous Fe-rich deposits (banded iron formations) show that the Early Archean (4.0–3.3 Ga) seawaters were rich in metal ions, especially iron (Beukes and Kelin 1992). Cu, Zn and Pb are also known to be organophilic species (Disnar 1981). Finally, Cu and Zn cations are found on the extreme points of the Irving-Williams series, which classify the stability of the complexes formed with divalent cations (Irving and Williams 1948; Schnitzer and Khan 1972).

## **MATERIAL AND METHODS**

### *Cell growth*

*Methanocaldococcus jannaschii* is a hyperthermophilic, methanogenic and autotrophic Archaea that was isolated from a hydrothermal vent on the East Pacific Rise. Cells can grow between 45 and 86°C and at pH values between 5.2 and 7.0 (Jones *et al.* 1983). Pure cultures were provided by the Laboratoire de Microbiologie des Environnements Extrêmes (Brest, France).

*M. jannaschii* was cultured in an autotrophic medium. The medium contained (per liter of distilled water) 25 g of NaCl, 3 g of MgCl<sub>2</sub>, 1 g of NH<sub>4</sub>Cl, 0.15 g of CaCl<sub>2</sub>, 0.5 g of KCl, 0.3 g of KH<sub>2</sub>PO<sub>4</sub>, and 0.001 g of resazurin. The pH was adjusted to 6.5 and the medium was sterilized by autoclaving. The medium was dispensed (20 mL) into 50-mL sterile vials.

Anaerobiosis was obtained by first applying vacuum to the vial and saturating it with N<sub>2</sub> (Balch and Wolfe 1976), and then replacing the N<sub>2</sub> by a H<sub>2</sub> / CO<sub>2</sub> atmosphere (80:20; 300 kPa). The medium was finally reduced by adding 0.2 mL of a sterile ten per cent (wt/vol) solution of Na<sub>2</sub>S.9H<sub>2</sub>O to each vial. The medium was inoculated to a final concentration of one per cent and was incubated at 80°C with shaking. Microbial growth was monitored by a phase-contrast microscope (Olympus CX 40). The end of the exponential growth phase was usually obtained after 24 to 36 hours.

### *Metal binding experiment*

The experimental setup used for the metal-binding experiment was simplified from Beveridge and Murray (1976) and Beveridge and Koval (1981).

Metal ion solutions at an approximate concentration of ten mM were prepared by dissolving the following salts in distilled water: FeCl<sub>3</sub>.6H<sub>2</sub>O, CuCl<sub>2</sub>.2H<sub>2</sub>O, ZnCl<sub>2</sub>, Pb(NO<sub>3</sub>)<sub>2</sub> and CaCl<sub>2</sub>.2H<sub>2</sub>O. Before use, the metal solutions were passed through a 0.2 µm filter for removal of particles in suspension.

At the end of the exponential growth phase, cells were harvested by collecting ten mL of culture that were filtered on a Millipore 0.2 µm filter and rinsed with 20 mL of ultrapure water passed through the filter. For each of the metal ions tested, the filter, acting as a support for the cells, was placed in a plastic vial and covered with ten mL of the metallic solution. The microorganisms were exposed to the metal solutions for 1 hour in an oven at 60°C. After this period, the filters with cells were rinsed with distilled water and placed in a 2.5 per cent glutaraldehyde solution for fixation. The samples were stored at 4°C until preparation for the electronic microscopy. The remaining metal solutions were filtered at 0.2 µm and kept for metal concentration measurements. For each metallic ion tested, a control sample was

prepared by exposing a blank Millipore filter to the same metal solutions for one hour at 60°C.

### *Experimental fossilisation*

To test the effect of the iron binding on silicification, *M. jannaschii* cells were successively exposed to Fe(III) cations and then to a 350 ppm Si (750 ppm SiO<sub>2</sub>) silica concentration. The silicification procedure is identical to that used by Orange *et al.* (2009), and is derived from the methods used for previous silicification experiments (Birnbaum *et al.* 1989; Toporski *et al.* 2002).

At the end of the exponential growth period, a sterilized Fe(III) solution was added to the culture medium to obtain a final approximate concentration of ten mM and the vials were placed in an oven at 60°C for one hour. Experimental silicification immediately followed. As a silicifying agent, we used a commercially available pure sodium silicate solution (Riedel de Haën) containing *c.* 27 per cent SiO<sub>2</sub> and *c.* 10 per cent NaOH (Na<sub>2</sub>Si<sub>3</sub>O<sub>7</sub>, M = 242 g/mol). This pure solution was diluted ten times to make a stock solution and filtered at 0.2 µm to remove particulate material. 0.4 mL of this stock solution was injected into vials to obtain a final approximate silica concentration of 350 ppm Si (750 ppm SiO<sub>2</sub>). The vials were incubated in an oven at 60°C until sampling. After two and nine months, samples were collected with a syringe and filtered onto a 0.2 µm Millipore filter. This filter was then placed in a 2.5 per cent glutaraldehyde solution and stored at 4°C until preparation for TEM analysis. To test the effect of Fe(III) on the lysis rate of *M. jannaschii* cells, two cultures (both at the end of the exponential growth phase) were used as control samples. The first vial was left untouched (i.e. prolonged growth) and in the second one a Fe(III) solution was added to obtain a final approximate concentration of ten mM. Vials were then incubated at 60°C for

two weeks. The number and the state of the cells were regularly checked by a phase-contrast microscope (Olympus CX 40).

### *Electron microscopy*

Transmission electronic microscopy (TEM) was used to qualitatively evaluate the metal binding capacity of archaeal cell walls. Metal cations bound to cellular structures produce an electron-scattering effect, in an identical manner to the usual metallic compounds used for staining ultrathin sections (e.g. uranyl acetate or lead citrate). Samples from the experimental fossilisation experiment were prepared for electron microscopy following the same procedure. Samples were rinsed four times for 15 minutes in a pH 7.4 sodium phosphate buffer, while being gently shaken. They then were filtered on a 0.2 µm filter that was cut into small mm-sized squares and embedded in a 20 g/L agar-agar solution. After the agar-agar cooled and hardened, the sample was dehydrated using a series of increasing ethanol / distilled water solutions in percentage steps of 10, 30, 50, 70, 93 and finally three times in absolute ethanol (15 minutes for each step), followed by an ethanol / acetone series (10, 30, 50, 70, 90 and finally three times in 100 per cent acetone, 15 minutes for each step). The samples were prepared for ultrathin sectioning by embedding in TAAB 812 Resin (TAAB Laboratories, UK), with a series of acetone / resin mixtures in proportions step 3:1, 1:1, 3:1, followed by two baths in 100 per cent resin (three hours for each step). The resin blocs were finally left to harden in a 60°C oven for three days. Ultrathin sections were made using a Diatome diamond knife mounted on a Reichert ultramicrotome and placed on carbon coated copper TEM grids. As the objective of the metal binding experiments was to obtain evidence of metal ion binding on cellular structures by observing the contrast they produced, no post fixation (e.g. with osmium tetroxide) or staining (e.g. with lead citrate or uranyl acetate) was performed on the ultrathin sections from the metal binding experiment. However, some sections of the



fossilisation experiment were treated with standard electron microscopy stains to verify if additional contrast could be obtained and to better identify the preservation of the cellular components: they were placed on drops of a four g/L lead citrate solution for ten minutes and then for 20 minutes on drops of a saturated uranyl acetate saturated solution prepared in 50 per cent ethanol. Observations and analysis were made with a Philips CM20 Transmission Electron Microscope, equipped with an EDX detector (Oxford Instruments).

#### *Atomic absorption spectroscopy*

Atomic absorption spectroscopy (AAS) was used to measure metal concentrations in solutions after exposure in order to quantify the metal uptake by *M. jannaschii* cells. Concentrations were determined with a GBC 905AA flame spectrophotometer, with the following procedure.

Each sample was diluted 100 times (500 times for Zn) and acidified and split in four parts. In three of them, a standard solution was used to add two, four and six ppm (0.2, 0.4 and 0.6 ppm for Zn) of the cation analysed (standard addition method). The concentration was then calculated from absorbance measurements in the four parts. For each sample, the maximum absorbance difference between two successive measurements did not exceed 0.0016. This value was converted into mM to obtain the error margin. Uptake values were determined as the difference in the concentrations of the remaining solutions of the experiments performed with and without microorganisms.

## **RESULTS**

#### *Metal immobilisation by *M. jannaschii* cells*

Table 1 shows the metal ion concentrations measured in the remaining solutions after one-hour exposure at 60°C in experiments made with and without *M. jannaschii* cells. Table 2 shows pH values in the initial metal stock solutions and final pH after one-hour exposure at 60°C in absence or presence of *M. jannaschii* cells.

For each metal ion, the same stock solution was used for cell exposure and for the control sample without cells. This guarantees identical initial concentrations. The comparison of the two measurements (with and without cells) allows us to determine the particular influence of the cells on the metal ion concentrations, avoiding disturbance by other factors (culture medium, glassware and plastic materials used and temperature).

In all cases, the quantity of metal in solution was always lower in the experiments with cells, compared to the experiments without cells, although the total uptake was relatively low (Fig. 1). Concentration differences varied for the different metals tested. For the  $\text{Fe}^{3+}$  experiment, a Fe(III) precipitate formed in the sample with cells during incubation (EPS), leading to a greater concentration difference and preventing determination of the real influence of *M. jannaschii* cells on the  $\text{Fe}^{3+}$  concentration. No precipitate was observed for the other metals tested. Thus, the 0.94 to 5.95 per cent decrease in the concentrations of the metal cations show that *M. jannaschii* cells exert a slight influence on the uptake of these ions.

The pH of the solution varied slightly during the one hour of incubation but the variations did not exceed 0.5 pH unit and could not be attributed with certainty to the presence of microorganisms.

The evolution of the metal ion concentrations showed immobilisation of these ions by *M. jannaschii* cells but did not provide any information on the mechanisms of the process. TEM study of unstained ultrathin sections allowed identification of the cellular structures that bind the metal ions. The ultrathin sections were not stained in order to clearly document the

adsorption, binding and incorporation of metal ions by observing the contrast they produce by interception of the electron beam.

Figures 2A–B are taken from a study of the experimental fossilisation of Archaea (Orange *et al.* 2009) and are used here to illustrate the appearance of *M. jannaschii* cell in unstained and stained ultrathin sections. Due to the lack of additional contrast, the outline and the structures of the cell on the unstained section are hardly visible (Fig. 2A). The cytoplasm shows a slightly higher contrast than the resin, while the different layers of the cell wall appeared clearer than the resin. On the section treated with usual lead citrate and uranyl acetate stains, the cell wall is clearly visible and the stained cytoplasm exhibits a granular texture. Given the difficulty to identify an unstained cell in ultrathin section, as well as the low cell numbers in the *M. jannaschii* cultures, one the main challenges of the TEM observations was to locate and clearly distinguish cells from artefacts. This was possible for the  $\text{Fe}^{3+}$  and  $\text{Pb}^{2+}$  experiments, but unfortunately was not possible with the other metals tested.

After exposure to the  $\text{Fe}^{3+}$  solution, the *M. jannaschii* cells exhibited sufficient contrast to allow their structure to be visible in ultrathin section (Fig. 2C). A large area, including the wall and the outer part of the cytoplasm, had an electron dense appearance and the cell wall was intermittently visible. A 100 nm thick area surrounding the cell appeared clearer and less electron dense than the resin and probably represents extracellular polymeric substances (EPS) bound to the cell wall. Numerous black particles, possibly hydrous iron oxide deposits (Beveridge 1989) were visible around and inside the cells (Figs. 2C-D). As they were clearly linked to the cell, they are unlikely to be artefacts or dirt on the section. High magnification observation of the cell wall (Fig. 2D) showed a sharp, thin, electron dense structure that could be attributed to the plasma membrane (*Mb* arrow). Another structure, discontinuous and less contrasted than the membrane, lies externally adjacent to the plasma membrane and was identified as the external glycoprotein surface layer (S-layer) (*S* arrow).

Cells exposed to a  $\text{Pb}^{2+}$  solution (Fig. 2E) demonstrated a similar aspect to those stained with the usual lead-based stains used in cytological TEM studies (Fig. 2B). The cell shown in Figure 2E was found inside a deposit containing dark particles of all sizes. This deposit could possibly be EPS mixed with lead particles. The cell wall appears clearly, but the staining was diffuse. Several darker linear features running parallel to the cell wall (Fig. 2E, arrow) may be the plasma membrane or the S-layer. The cytoplasm was also stained and showed a granular texture.

Samples exposed to  $\text{Ca}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  were also prepared and observed with transmission electron microscopy, but it was impossible to distinguish cells from artefacts on the ultrathin sections.

#### *Experimental silicification after exposure to metal cations*

As previously mentioned, the experimental silicification of *M. jannaschii* without preliminary metal ion exposure was not successful (Orange *et al.* 2009). Most of the *M. jannaschii* cells naturally lysed within a week after the beginning of the experiment and silicification of the cells remains was extremely rare. Only a few highly deformed cells and cell remains were still present after one month (Figs. 3A-B) and, after one year, the very few remaining cells were heavily deformed (Fig. 3C) and had lost all recognisable features.

As for the fossilisation experiment performed with silica alone, a precipitate formed spontaneously within a few hours after injection of the silica to the Fe(III)-exposed cells. The pH of the medium, which was not adjusted after the addition of Fe nor silica, was about 2.5 and did not change during the experiment. Monitoring of the control cultures for natural lysis by light microscopy suggested that lysis was uninhibited by the presence of Fe and the low pH as only a few cells were visible after two weeks.

After two months of silicification, numerous cells (Figs. 3D-E) and some cell remains (Fig. 3F) were still clearly visible as documented in the unstained sections (compare Figs. 2A and 3D). Cells exhibited better contrast after two months than after the one hour exposure to the Fe(III) solution, possibly because of continued Fe(III) binding to the cell wall components (compare Figs. 2C and 3D). Cells were trapped in a light grey granular precipitate which included some dark particles, possibly silica or hydrous iron oxide (Fig. 3D). EDX analyses indicate that this precipitate contains both silica and iron (Fig. 4A), with traces of Cl, coming from the iron chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) used, and P, coming from the sodium phosphate buffer used for sample preparation.

Cells were in a good state of preservation (e.g. no apparent lysis, and with a still perfectly recognizable cell wall structure). Most of them appeared empty, as no major structures could be seen inside, apart from a few particles (Fig. 3D, arrow) that could be hydrous iron oxide, as in the Fe binding experiment (Fig. 2C). About ten percent were almost entirely filled with a homogeneous Fe-SiO<sub>2</sub> granular precipitate (Fig. 3E), that also coated the inside of the cell wall. This precipitate had a darker and finer texture than the one embedding the cells. No differences in composition between the two precipitates (Figs 4A-B) could be detected by the EDX analyses.

Staining of some ultrathin sections with lead citrate and uranyl acetate produced no additional contrast of the cellular structures (Fig. 3G). The stained sections showed mainly empty cells, indicating lysis, although some of them had kept some cytoplasmic material (Fig. 3G). The stained sections revealed that the dark particles that formed inside cells (cf. Fig. 3D) had precipitated on remains of the cytoplasmic material (Fig. 3G, arrow).

After nine months, unstained ultrathin sections showed no major changes and still clearly showed a high number of intact cells, with most of them appearing empty (Fig. 3I, bottom).

Some cells were filled with the Fe-SiO<sub>2</sub> precipitate (Fig. 3I, top). The Fe-SiO<sub>2</sub> precipitate surrounding the cells now appeared more dense (compare Figs. 3D and 3I).

Throughout the experiment, the cell wall structure was well preserved and the S-Layer and plasma membrane remained clearly visible (Figs. 3H-J) as a result of iron binding. However, high resolution micrographs showed that the two layers of the plasma membrane were visible only when the Fe-SiO<sub>2</sub> precipitate started to infill the cell and precipitate on the inner part of the cell wall (Fig. 3H, upper right). When this was not the case (“empty cell”), only the outer layer of the cell wall was visible (Fig. 3H, bottom), as was the case for the metal binding experiment (Fig. 2D). After nine months of silicification details of the cell wall structure were still clearly recognizable for all cells and no detectable degradation occurred (Fig. 3J).

## DISCUSSION

### *Metal uptake by M. jannaschii cells*

TEM micrographs and AAS data document uptake of dissolved metal cations by *M. jannaschii*. Owing to technical difficulties electron microscopy could only demonstrate binding to the cell wall of Fe and Pb but the AAS data, that all point to a lowering of metal concentration during the experiments, suggested that the other metals (Ca, Cu, and Zn) were also immobilized by the cells and possibly fixed to the cell walls. These two techniques were thus complementary and show that *M. jannaschii* cells are able to immobilise and bind several metal ions.

Beveridge and Murray (1976, 1980) and Beveridge and Koval (1981), studying metal uptake by Bacteria *Bacillus subtilis* and *Escherichia coli*, identified the carboxyl and hydroxyl functional groups of peptidoglycan in the cell wall as the main cation-binding site. Phosphoryl

groups in membrane lipids are also able to bind cations (Beveridge and Fyfe 1985). Archaea exhibit a great variety in the composition of their cell walls, although they have in common membrane lipids made of isoprenoid di- and tetra-ethers and the lack of peptidoglycan (König 2001). *M. jannaschii* cell wall consist only of a plasma membrane and an external S-layer, which is the most common and the simplest archaeal cell wall structure. Despite this, TEM micrographs coupled with AAS analysis show that metallic ions can bind to several components, such as the S-layer and the plasma membrane, of the *M. jannaschii* cell wall (Fig. 2D). This demonstrates the presence of negatively charged functional groups within the cell wall, despite the low pH (Fein *et al.* 1997). Furthermore, dark aggregates were also visible inside cells suggesting that cations are able to cross the cell wall and penetrate into the cytoplasm. In the  $\text{Pb}^{2+}$  experiment, the S-layer and the plasma membrane could not be clearly distinguished on the micrographs (Fig. 2E). However, as the whole cell wall showed significant staining, it is possible that both the S-layer and the plasma membrane had bound  $\text{Pb}^{2+}$  ions. In this case also,  $\text{Pb}^{2+}$  ions were able to pass through the membrane and penetrate inside the cytoplasm. Finally, the accumulation of dark deposits around the cells (Fig. 2E) suggests  $\text{Pb}^{2+}$  fixation by the EPS.

Despite the lack of peptidoglycan, several functional groups in *M. jannaschii* cell wall and EPS are able to bind dissolved metal ions. Although bacterial and archaeal membrane lipids have different compositions, the phosphoryl groups of the polar heads of the lipid molecules are similar and can explain metal fixation by *M. jannaschii* plasma membrane. The S-layer is composed of proteins and glycoproteins whose carboxyl and hydroxyl groups can also bind metal ions (Schulze-Lam *et al.* 1996). The S-layer appeared less contrasted than the membrane, which may indicate that it could bind a smaller amount of dissolved cations than the membrane. This in turn suggests that it has fewer functional groups available for binding cations than the membrane. The EPS consist mainly of polysaccharides and proteins and the

metal ions would be able to bind to the carboxyl groups on these proteins (Schultze-Lam *et al.* 1993; Fortin *et al.* 1998; Chan *et al.* 2004; Fortin 2004).

For all the other metal ions tested ( $\text{Ca}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ), immobilisation mechanisms and binding sites are uncertain, but are likely to be similar to those involved for  $\text{Fe}^{3+}$  and  $\text{Pb}^{2+}$  cations. Thus,  $\text{Ca}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  cations may have bound on the different layers of *M. jannaschii* cell wall and EPS and could have penetrated into the cytoplasm.

TEM micrographs show that  $\text{Fe}^{3+}$  and  $\text{Pb}^{2+}$  cations are able to cross the membrane and to make their way into the cytoplasm. Grown in an autotrophic medium, *M. jannaschii* cells use several cations for their metabolism (e.g.  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^{+}$ , see the detailed growth medium composition in Materials and Methods section). Mechanisms and pathways thus exist that allow dissolved cations to enter the cell. The metallic ions were able to bind onto and cross the cell wall within an hour, which suggests that there were no protective mechanisms, either active or passive, to avoid metal adsorption and binding. This is in contrast to previous experiments, which showed that such protective mechanisms exist in the case of dissolved silica (Lalonde *et al.* 2005; Orange *et al.* 2009).

### *Experimental fossilisation*

The fossilisation and the remarkable preservation of most of *M. jannaschii* cells show that preliminary Fe(III) ions uptake was decisive for, and spectacularly improved the result of the experimental silicification. Thus, the positive effect of Fe(III) binding on silicification appears to also apply to Archaea.

Initially mineral particles precipitated rapidly on the cell wall, especially on the S-Layer (Fig. 3H), and embedded the cells within an Fe-SiO<sub>2</sub> precipitate, while hydrous iron oxide particles formed inside the cells using the remaining cytoplasmic material as a template (Figs. 3D and



3G). The inability of uranyl acetate and lead citrate stains to increase cell wall contrast shows that most of, if not all, the functional groups of the cell wall, where the strains usually bind, were occupied by Fe(III) ions.

The formation of a dense precipitate inside the cells (Fig. 3E) appeared to be the next step of the fossilisation. However, as the percentage of ‘precipitate-filled’ cells did not significantly increase between two and nine months of fossilisation, this sequence of events remains hypothetical. As this inner precipitate had a different aspect from the precipitate embedding the cell, we can assume that it formed directly inside the cells after dissolved iron and silica ions had penetrated into them. The inner layer of the plasma membrane evidently acts as a support for the formation of this precipitate, from which it grew and gradually filled the cell (Figs. 3E and 3I). The remaining cytoplasmic compounds could also have served as a support for the precipitate (Fig. 3G), which could explain its rapid growth within the cells. Note that the cell preservation was efficient even when mineralisation was limited to the outer cell wall. The loss of the cytoplasm in most of the cells indicates lysis of the *M. jannaschii* cells, probably within a few days after the start of the experiment, as a consequence of a prolonged growth. As suggested by light microscopy observations on prolonged growth cultures, with and without Fe, the presence of Fe alone does not seem to have stopped the lysis of *M. jannaschii* cells. Thus, the inhibiting effect of Fe cations on the autolytic enzymes does not seem to apply here.

As silica alone could not also prevent rapid *M. jannaschii* cell lysis (Orange *et al.* 2009), it is likely that the combination of Fe and silica binding led to good preservation of the cell remains. Preliminary Fe(III) binding appeared to have had a positive effect on the rate of silicification by serving as an intermediate between the silica particles and the organic molecules. This is demonstrated by the fact that the cell wall was completely mineralised and the inner cell began to be filled either by a few particles or by a dense Fe-SiO<sub>2</sub> precipitate

after only two months. This phenomenon was not observed in the fossilisation of the Archaea *P. abyssi* until one year of fossilisation (Orange *et al.* 2009).

Another positive effect of the preliminary Fe(III) binding was the faithful preservation of the cell wall structure during fossilisation. Although cells can be preserved by silicification, this process alone does not result in the preservation of the archaeal cell wall structure. Without prior metal fixation, the cell wall structure disappears after a few months and is hidden by the growing silica precipitate (Orange *et al.* 2009). In this study, the mineralisation of the cell wall appears to have been very delicate and mainly limited to the exterior and the interior of the cell wall. Only fine particles were bound in between the different layers of the cell wall, allowing it to be perfectly recognizable after nine months (Fig. 3J). Consequently, the morphological features that allow identification of these cells as Archaea (i.e. a simple and thin cell wall made only of an S-Layer and a plasma membrane) are still present at the end of the fossilisation.

This study was designed to obtain a first qualitative insight on the metal binding ability of Archaea and on the effect of preliminary Fe binding on silicification. pH values (2.5) during the experimental fossilisation were lower than those found normally in the natural environment, and lower than those estimated for the Early Archaean oceans (6-7; Grotzinger and Kasting 1993). They are, however, normal for acidic environments, such as hot springs (Jones and Renaut 2006), the Rio Tinto river (González-Toril *et al.* 2003; Fernández-Remolar *et al.* 2004), mine tailings (Fortin and Beveridge 1997), or some cave environments (Vlasceanu *et al.* 2000). Although the low pH does not appear to have influenced cell lysis, it did change the electric charges of the functional groups in the organic components and the dissolved species involved in the binding, and thus may have had an effect on the binding rates of Fe and silica, which are difficult to evaluate. For example, Amores and Warren (2007) suggest that low pH would allow a better silica binding on cellular structures whereas

Swedlund and Webster (1999) and Fein *et al.* (2002) concur that low pH would lower the adsorption of undersaturated silica by Fe.

### *Implications for the preservation of Archaea in the rock record*

In a previous experiment (Orange *et al.* 2009), we concluded that the fossilisation potential of Archaea species was low because of the relatively weak silica binding ability of the archaeal cell wall and because only the most robust strains were fossilised. Our present results show, on the contrary, that preliminary binding of Fe(III) ions appears to significantly increase the rate of fossilisation and allows the fossilisation of even the most fragile strains, such as *M. jannaschii*. It implies that all microbial species can theoretically be fossilised and preserved in rocks if they have been able to bind metals to their surfaces. As ideal fossilisation conditions (in our case, the presence of dissolved Fe(III) cations and silica) may not always be met, the microfossil assemblage in the rock record is unlikely to be representative of the total original microbial community, although it may represent a wider range than previously believed. The fact that the cell walls of Archaea can be well preserved also provides hope for the possible identification of fossilised Archaea in the rock record.

These results thus highlight the importance of the role of metals in the preservation of microorganisms by silicification. Habitats on the early Earth, for instance, included metal and silica-rich effluents of hot springs and hydrothermal vents (Nisbet and Sleep 2001). The association between iron (or other metals), silica and well-preserved micro-organisms in recent hydrothermal environments (Ferris *et al.* 1986; Fortin *et al.* 1998), as well as in the Proterozoic and Archean epochs (Southgate 1986; Rasmussen 2000) has been previously documented. Considering certain similarities between environmental conditions on the early Earth and early Mars, these results have important implications in the search for possible fossilised traces of life in early Martian rocks from the Noachian period, especially

considering the high amount of iron oxides associated with Martian rocks (Bibring *et al.* 2005; Poulet *et al.* 2005; Carr 2006) and the possible presence of hydrothermal systems on early Mars (Squyres *et al.* 2008).

## CONCLUSIONS

This study is a first attempt to determine the metal binding ability of an Archaea and to verify its effect on the silicification of the microorganisms. The results provide new information regarding the binding abilities of Archaea and further understanding of the fossilisation process. The association of electron microscopy observations and atomic absorption spectroscopy analyses demonstrated that an Archaea (*M. jannaschii*) with a relatively simple cell structure can rapidly immobilize several metal cations ( $\text{Fe}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ). Fe(III) and Pb ions were able to bind on the plasma membrane and the S-Layer and to pass through the cell wall to be incorporated into the cytoplasm. Although preliminary exposure of *M. jannaschii* cells to Fe(III) cations alone did not affect cell preservation, the rate of mineralisation of the cellular structures by a Fe-SiO<sub>2</sub> precipitate was faster, probably as a result of Fe(III) acting as an intermediate between the cell functional groups and the silica. This combination of Fe and silica binding and its rapidity appears to be one of the main factors responsible for the cell preservation. In contrast to silicification alone, the cell wall structural components were extremely well preserved, allowing the cells to be still identifiable as Archaea at the end of the experiment. This study thus underlines the importance of metal binding in the fossilisation of microorganisms, as it allows good preservation of even the most fragile archaeal strains, despite their low binding capacities. Archaea may thus be preserved,

and could possibly be still identified, in recent and ancient rocks and, possibly even in Martian rocks.

## **ACKNOWLEDGEMENTS**

This study was financially supported by the Centre National de la Recherche Scientifique (CNRS) and the Centre National d'Etudes Spatiales (CNES), with a support from the Observatoire des Sciences de l'Univers en Région Centre (OSUC). The authors would like to warmly thank Dominique Jalabert (Centre de Microscopie Electronique, Université d'Orléans) for the help for the electronic microscopy, and Valentin Crépeau for precious assistance in the culture experiments.

## REFERENCES

AMORES, R. and WARREN, L. A. 2007. Identifying when microbes biosilicify: the interconnected requirements of acidic pH, colloidal SiO<sub>2</sub> and exposed microbial surface. *Chemical Geology*, **240**, 298–312.

BALCH, W. E. and WOLFE, R. S. 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. *Applied and Environmental Microbiology*, **32**, 781–791.

BEUKES, N. J. and KLEIN, C. 1992. Models for iron-formation deposition. 147–151. In SCHOPF, J.W. and KLEIN, C. (eds). *The Proterozoic biosphere*. Cambridge University Press, Cambridge, xxiv + 1348 pp.

BEVERIDGE, T. J. and FYFE, W. S. 1985. Metal fixation by bacterial cell walls. *Canadian Journal of Earth Sciences*, **22**, 1893–1898.

—— and KOVAL, S. F. 1981. Binding metals to cell envelopes of *Escherichia coli*. *Applied and Environmental Microbiology*, **42**, 325–335.

—— and MURRAY, R. G. E. 1976. Uptake and retention of metals by cell walls of *Bacillus subtilis*. *Journal of Bacteriology*, **127**, 1502–1518.

——— 1980. Sites of metal deposition in the cell wall of *Bacillus subtilis*. *Journal of Bacteriology*, **141**, 876–887.

——— 1989. Role of cellular design in bacterial metal accumulation and mineralization. *Annual Review of Microbiology*, **43**, 147–171.

BIBRING, J. P., LANGEVIN, Y., GENDRIN, A., GONDET, B., POULET, F., BERTHE, M., SOUFFLOT, A., ARVIDSON, R., MANGOLD, N., MUSTARD, J., DROSSART, P. and the OMEGA team 2005. Mars surface diversity as revealed by the OMEGA /Mars Express Observations. *Science*, **307**, 1576–1581.

BIRNBAUM, S. J., WIREMAN, J. W. and BOROWSKI, R. 1989. Silica precipitation by the anaerobic sulphate reducing bacterium *Desulfovibrio desulfuricans* : effects upon cell morphology and implications for preservation. 507–516. In CRICK, R. E. (ed.). *Origin, evolution, and modern aspects of biomineralization in plants and animals*. Plenum Press, New York, 548 pp.

CARR, M. H. 2006. *The Surface of Mars*. Cambridge University Press, Cambridge, 322 pp.

CHAN, C. S., DE STASIO, G., WELCH, S. A., GIRASOLE, M., FRAZER, B. H., NESTEROVA, M. V., FAKRA, S. and BANFIELD, J. F. 2004. Microbial polysaccharides template assembly of nanocrystal fibers. *Science*, **303**, 1656–1658.

DISNAR, J. R. 1981. Etude expérimentale de la fixation de métaux par un matériau sédimentaire actuel d'origine algale — II. Fixation 'in vitro' de  $\text{UO}^{2+}_2$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ ,

$\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ , ainsi que de  $\text{VO}_3^-$ ,  $\text{MoO}_4^{2-}$  et  $\text{GeO}_3^{2-}$ . *Geochimica et Cosmochimica Acta*, **45**, 363–379.

FEIN, J. B., DAUGHNEY, C. J., YEE, N. and DAVIS, T. 1997. A chemical equilibrium model for metal adsorption onto bacterial surfaces. *Geochimica and Cosmochimica Acta*, **61**, 3319–3328.

—— SCOTT, S. and RIVERA, N. 2002. The effect of Fe on Si adsorption by *Bacillus subtilis* cell walls : insights into non-metabolic bacterial precipitation of silicate minerals. *Chemical Geology*, **182**, 265–273.

FERNÁNDEZ-REMOLAR, D., GÓMEZ-ELVIRA, J., GÓMEZ, F., SEBASTIAN, E., MARTÍN, J., MANFREDI, J. A., TORRES, C., GONZÁLEZ KESLER, C. and AMILS, R. 2004. The Tinto River, an extreme acidic environment under control of iron, as an analog of the Terra Meridiani hematite site of Mars. *Planetary and Space Science*, **52**, 239–248.

FERRIS, F. G., FYFE, W. S. and BEVERIDGE, T. J. 1988. Metallic ion binding by *Bacillus subtilis*; implications for the fossilization of microorganisms. *Geology*, **16**, 149–152.

—— ——— 1986. Iron-silica crystallite nucleation by bacteria in a geothermal sediment. *Nature*, **320**, 609–611.

FORTIN, D. and BEVERIDGE, T. J. 1997. Role of the bacterium *Thiobacillus* in the formation of silicates in acidic mine tailings. *Chemical Geology*, **141**, 235–250.



—— FERRIS, F. G. and SCOTT, S. D. 1998. Formation of Fe-silicates and Fe-oxides on bacterial surfaces in samples collected near hydrothermal vents on the Southern Explorer Ridge in the Northeast Pacific Ocean. *American Mineralogist*, **83**, 1399–1408.

—— 2004. What biogenic minerals tell us. *Science*, **303**, 1618-1619.

GONZÁLEZ-TORIL, E., LLOBET-BROSSA, E., CASAMAYOR, O., AMANN, R. and AMILS, R. 2003. Microbial Ecology of an Extreme Acidic Environment, the Tinto River. *Applied and Environmental Microbiology*, **60**, 4853–4865.

GROTZINGER, J. P. and KASTING, J. 1993. New constraints on Precambrian ocean composition. *Journal of Geology*, **101**, 235–243.

HOFMANN, A. and BOHLAR, R. 2007. Carbonaceous Cherts in the Barberton Greenstone Belt and Their Significance for the Study of Early Life in the Archaean Record. *Astrobiology*, **7**, 355–388.

IRVING, H. and WILLIAMS, R. J. P. 1948. Order of stability of metal complexes. *Nature*, **162**, 746–747.

JONES, B. and RENAUT, R. W. 2006. Selective mineralization of microbes in Fe-rich precipitates (jarosite, hydrous ferric oxides) from acid hot springs in the Waiotapu geothermal area, North Island, New Zealand. *Sedimentary Geology*, **194**, 77–98.

JONES, W. J., LEIGH, J.A., MAYER, F., WOESE, C. R. and WOLFE, R. S. 1983 *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. *Archives of Microbiology*, **136**, 254–261.

KASTING, J. F. and ONO, S. 2006. Palaeoclimates: the first two billion years. *Philosophical Transactions of the Royal Society B*, **361**, 917–929.

KNAUTH, L. P. and LOWE, D. R. 2003. High Archean climatic temperature inferred from oxygen isotope geochemistry of cherts in the 3.5 Ga Swaziland Supergroup, South Africa. *Geological Society of America Bulletin*, **115**, 566–580.

KÖNIG, H. 2001. Archaeal cell walls. 161–168. In: *Encyclopedia of Life Science*. Nature Publishing Group, London. Vol. 2, 696 pp. <http://www.els.net>

LALONDE, S. V., KOHNAUSER, K. O., REYSENBACH, A. L. and FERRIS, F. G. 2005. The experimental silicification of Aquificales and their role in hot spring formation. *Geobiology*, **3**, 41–52.

NISBET, E. G. and SLEEP, N. H. 2001. The habitat and nature of early life. *Nature*, **409**, 1083–1091.

ORANGE, F., WESTALL, F., DISNAR, J. R., PRIEUR, D., BIENVENU, N., LE ROMANCER, M. and 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.

PHOENIX, V. R., KONHAUSER, K. O. and FERRIS, F. G. 2003. Experimental study of iron and silica immobilization by bacteria in mixed Fe-Si systems: Implications for microbial silicification in hot-springs. *Canadian Journal of Earth Sciences*, **40**, 1669–1678.

POULET, F., BIBRING, J. P., MUSTARD, J. F., GENDRIN, A., MANGOLD, N., LANGEVIN, Y., ARVIDSON, R. E., GONDET, B. and GOMEZ, C. 2005. Phyllosilicates on Mars and implications for early martian climate. *Nature*, **438**, 623–627.

RASMUSSEN, B. 2000. Filamentous microfossils in a 3,235-millionyear-old volcanogenic massive sulphide deposit. *Nature*, **405**, 676–679.

SCHULTZE-LAM, S., THOMPSON, J. B. and BEVERIDGE, T. J. 1993. Metal ion immobilization by bacterial surfaces in freshwater environments. *Water Pollution Research Journal of Canada*, **28**, 51–81.

—— FORTIN, D., DAVIS, B. and BEVERIDGE, T. J. 1996. Mineralization of bacterial surfaces. *Chemical Geology*, **132**, 171–181.

SCHNITZER, M. and KHAN, S. U. 1972. *Humic Substances in the Environment*. Marcel Dekker, New York, 327 pp.

SOUTHGATE, P. N. 1986. Depositional environment and mechanism of preservation of microfossils, upper Proterozoic Bitter Springs Formation, Australia. *Geology*, **14**, 683–686.

SQUYRES, S. W., ARVIDSON, R. E., RUFF, S., GELLERT, R., MORIS, R. V., MING, D. W., CRUMPLER, L., FARMER, J. D., DES MARAIS, D. J., YEN, A., McLENNAN, S. M., CALVIN, W., BELL, J. F. III, CLARK, B. C., WANG, A., McCOY, T. J., SCHMIDT, M. E. and DE SOUZA, P. A. Jr 2008. Detection of silica-rich deposits on Mars. *Science*, **320**, 1063–1067.

SWEDLUND, P. and WEBSTER, J. 1999. Adsorption and polymerisation of silicic acid on ferrihydrite, and its effect on arsenic adsorption. *Water Research*, **33**, 3413–3422.

TOPORSKI, J. K. W., STEELE, A., WESTALL, F., THOMAS-KEPRTA, K. L. and McKAY, D. S. 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.

URRUTIA, M. M. and BEVERDIGE, T. J. 1993. Mechanism of silicate binding to the bacterial cell wall in *Bacillus subtilis*. *Journal of Bacteriology*, **175**, 1936–1945.

—— ——— 1994. Formation of fine-grained silicate minerals and metal precipitates by a bacterial surface (*Bacillus subtilis*). *Chemical Geology*, **116**, 261–280.

VAN DEN BOORN, S. H. J. M., VAN BERGEN, M. J., NIJMAN, W. and VROON, P. Z. 2007. Dual role of seawater and hydrothermal fluids in Early Archean chert formation: Evidence from silicon isotopes. *Geology*, **35**, 939–942.

VLASCEANU, L., SARBU, S. M., ENGEL, A. S. and KINKLE, B. K. 2000. Acidic Cave-Wall Biofilms Located in the Frasassi Gorge, Italy. *Geomicrobiology Journal*, **17**, 125–139.

WESTALL, F. 2009. Life on an anaerobic planet. *Science*, **232**, 471–472.

—— and FOLK, R. L. 2003. Exogenous carbonaceous microstructures in Early Archaean cherts and BIFs from the Isua greenstone belt: Implications for the search for life in ancient rocks. *Precambrian Research*, **126**, 313–330.

—— DE VRIES, S. T., NIJMAN, W., ROUCHON, V., ORBERGER, B., PEARSON, V., WATSON, J., VERCHOVSKY, A., WRIGHT, I., ROUZAUD, I., ROUZAUD, J. N., MARCHESINI, D. and ANNE, S. 2006a. The 3.466 Ga “Kitty’s Gap Chert”, an early Archean microbial ecosystem. *Geological Society of America special paper*, **405**, 105–131.

—— DE RONDE, C. E. J., SOUTHAM, G., GRASSINEAU, N., COLAS, M., COCKELL, C. and LAMMER, H. 2006b. Implication of a 3.472-3.333 Gyr-old subaerial microbial mat from the Barberton greenstone belt, South Africa for the UV environmental conditions on the early Earth. *Philosophical Transactions of the Royal Society B*, **361**, 1857–1875.

## **Figures captions**

**FIG. 1.** Comparison of metal ion concentrations measured by atomic absorption spectroscopy after a one hour incubation at 60°C in the metal solution, with and without *M. jannaschii* cells. In the Fe<sup>3+</sup> experiment with cells, the formation of a precipitate led to a greater concentration difference.

**FIG. 2. Metal binding experiments.** **A–B**, Transmission Electron Microscopy (TEM) micrographs showing examples of *M. jannaschii* cells (C) on an unstained (**A**) and stained (**B**) section, obtained from an experimental silicification experiment (Orange *et al.* 2009). Arrows indicate the outline of the cell on the unstained section. Dark particles are silica crystallites. **C**, Unstained TEM micrograph showing a *M. jannaschii* cell exposed to a Fe(III) solution during 1 hour at 60°C. Note the contrasted cell wall (arrow) and the dark particles, possibly hydrous iron oxide, around and inside the cell. **D**, High magnification view of the cell wall, showing the contrasted plasma membrane (Mb arrow) and the slightly contrasted S-layer (S arrow). **E**, Unstained TEM micrograph showing a *M. jannaschii* cell exposed to a Pb solution during 1 hour at 60°C inside a dark precipitate. Note the contrasted cell wall (arrow) and the contrasted granular cytoplasm. All TEM micrographs were made at 200 kV.

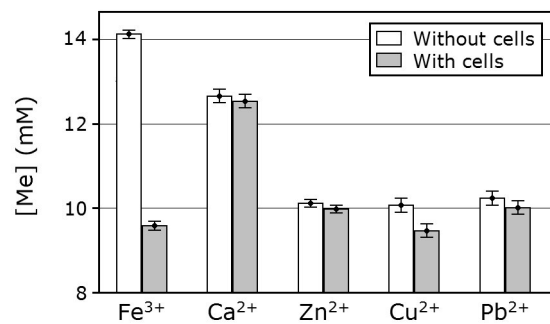
**FIG. 3. Experimental silicification experiment.**

**A–C**, TEM micrographs showing examples of the few remaining *Methanocaldococcus jannaschii* cells exposed to a 350 ppm Si silica-solution for various length of times on thin sections stained with lead citrate and uranyl acetate (from Orange *et al.*, 2009). **A–B**, 1 month, intact and deformed cells (C). **C**, 1 year, heavily deformed cell (C).

**D–J**, TEM micrographs showing *M. jannaschii* cells exposed to a *c.* 10 mM Fe(III) solution during 1 hour at 60°C, then to a 350 ppm Si silica-solution for various lengths of time. Note that the thin sections were not stained with lead citrate or uranyl acetate, unless otherwise stated. 2 months of silicification: **D**, ‘empty’ cell (C) within a Fe-SiO<sub>2</sub> precipitate; note the fine particles inside the cell (arrow). **E**, Cell (C) filled with a Fe-SiO<sub>2</sub> precipitate; note the different texture of this precipitate and its binding on the inner side of the cell wall. **F**, cell remains within the Fe-SiO<sub>2</sub> precipitate. **G**, cell (C) observed on a thin section stained with lead citrate and uranyl acetate, note the remaining cytoplasmic material on which fine particles are attached (arrow). **H**, high magnification view of the walls of two cells showing the plasma membranes (Mb arrows) and the S-Layers (S arrows) for each cell; note that the inner layer of the membrane is only visible when the Fe-SiO<sub>2</sub> has formed inside the cell (upper right), and is invisible if not (bottom). 9 months of silicification: **I**, ‘empty’ and ‘filled’ cells (C) within the Fe-SiO<sub>2</sub> precipitate; note the more dense aspect of the precipitate. **J**, high magnification view of the preserved wall structure of both cells. All TEM micrographs were made at 200 kV.

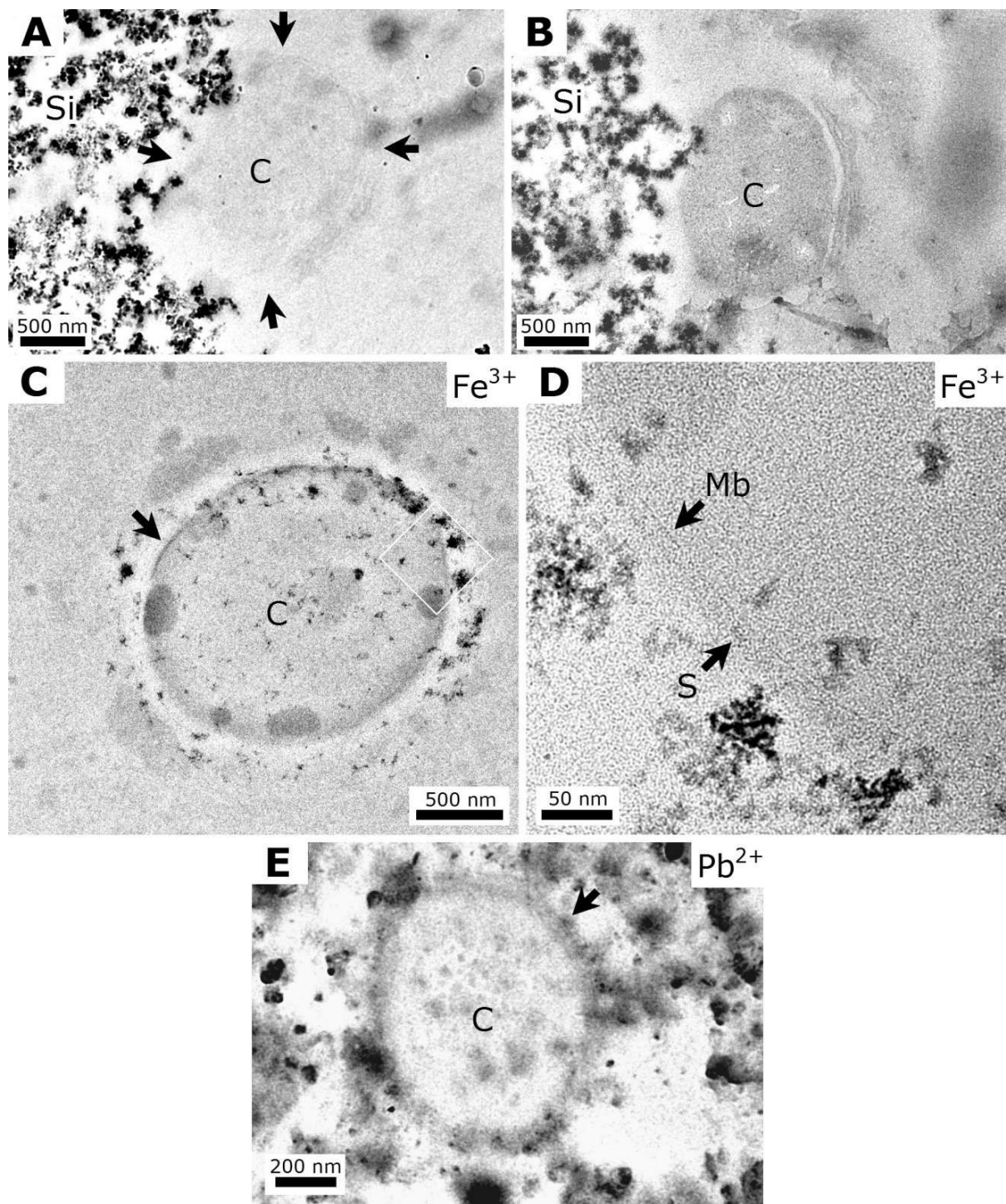
#### **FIG. 4. Experimental silicification experiment.**

EDX spectra obtained on TEM ultrathin sections of *M. jannaschii* cells exposed to a *c.* 10 mM Fe(III) solution during 1 hour at 60°C, then to a 350 ppm Si silica-solution for 9 months (Fig. 3I). **A**, spectrum made on the Fe-SiO<sub>2</sub> precipitate surrounding the cells. **B**, spectrum made on the Fe-SiO<sub>2</sub> precipitate filling the cells. Note the similar compositions. All EDX spectra were made at 200 kV.

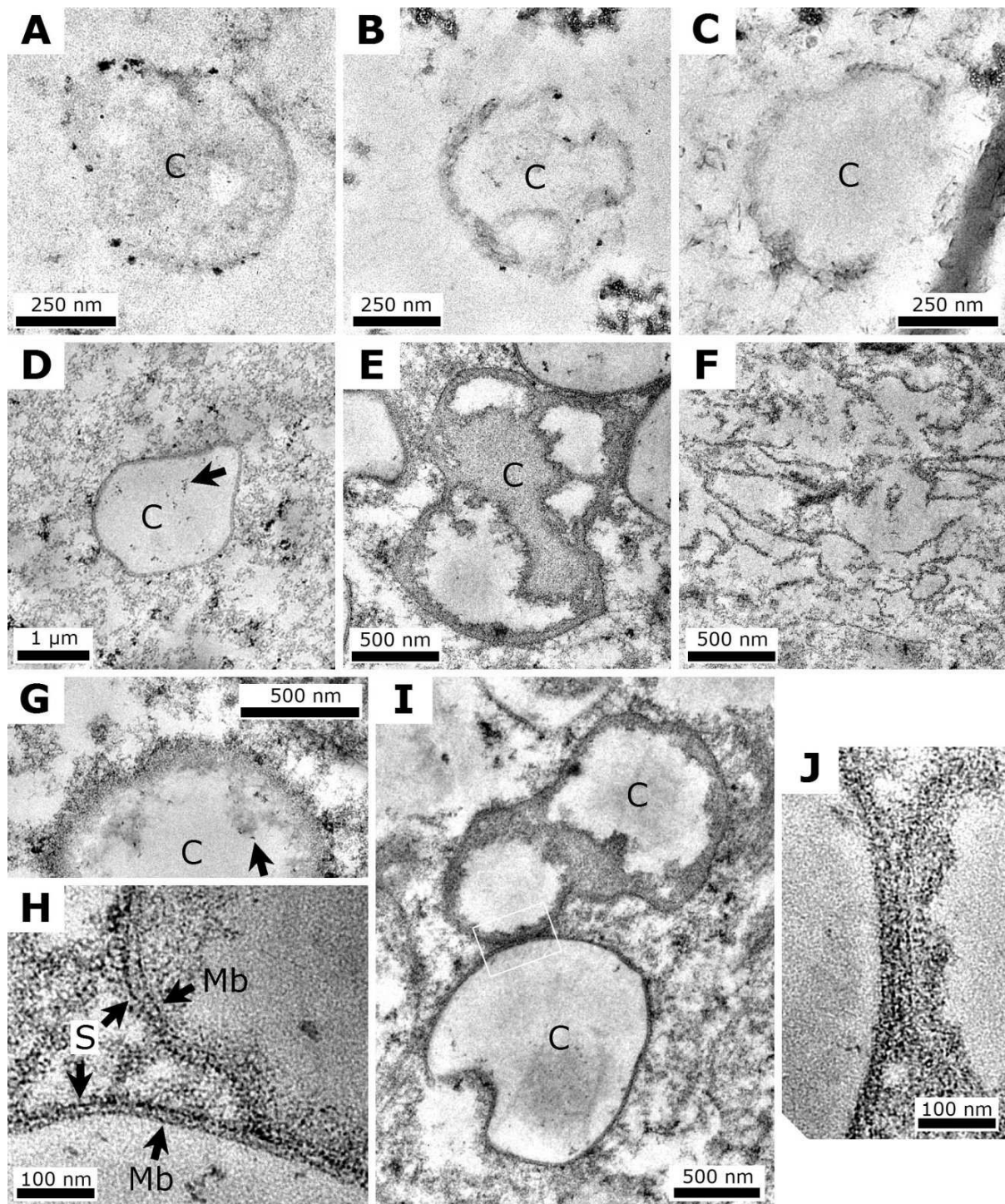


**FIG. 1.** Comparison of metal ion concentrations measured by atomic absorption spectroscopy after a one hour incubation at 60°C in the metal solution, with and without *M. jannaschii* cells. In the Fe<sup>3+</sup> experiment with cells, the formation of a precipitate led to a greater concentration difference.





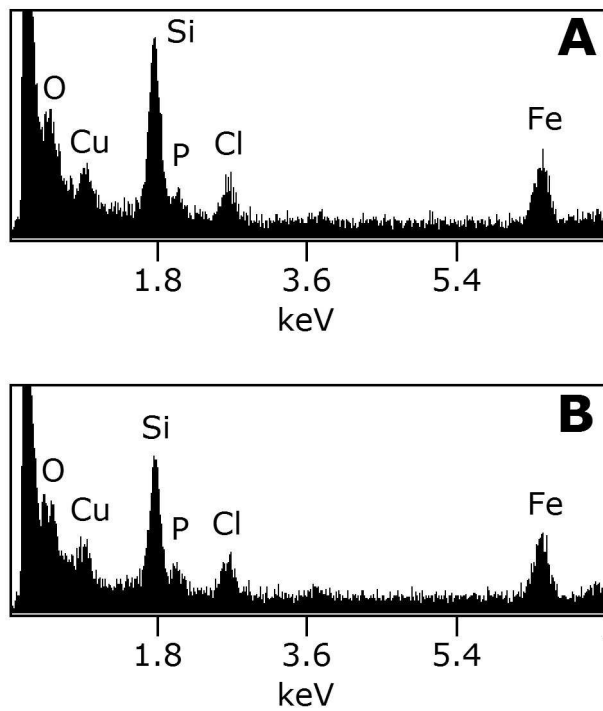
**FIG. 2. Metal binding experiments.** A–B, Transmission Electron Microscopy (TEM) micrographs showing examples of *M. jannaschii* cells (C) on an unstained (A) and stained (B) section, obtained from an experimental silicification experiment (Orange *et al.* 2009). Arrows indicate the outline of the cell on the unstained section. Dark particles are silica crystallites. C, Unstained TEM micrograph showing a *M. jannaschii* cell exposed to a  $\text{Fe}(\text{III})$  solution during 1 hour at  $60^\circ\text{C}$ . Note the contrasted cell wall (arrow) and the dark particles, possibly hydrous iron oxide, around and inside the cell. D, High magnification view of the cell wall, showing the contrasted plasma membrane (Mb arrow) and the slightly contrasted S-layer (S arrow). E, Unstained TEM micrograph showing a *M. jannaschii* cell exposed to a Pb solution during 1 hour at  $60^\circ\text{C}$  inside a dark precipitate. Note the contrasted cell wall (arrow) and the contrasted granular cytoplasm. All TEM micrographs were made at 200 kV.



**FIG. 3. Experimental silicification experiment.**

A–C, TEM micrographs showing examples of the few remaining *Methanocaldococcus jannaschii* cells exposed to a 350 ppm Si silica-solution for various length of times on thin sections stained with lead citrate and uranyl acetate (from Orange *et al.*, 2009). A–B, 1 month, intact and deformed cells (C). C, 1 year, heavily deformed cell (C).

D–J, TEM micrographs showing *M. jannaschii* cells exposed to a *c.* 10 mM Fe(III) solution during 1 hour at 60°C, then to a 350 ppm Si silica-solution for various lengths of time. Note that the thin sections were not stained with lead citrate or uranyl acetate, unless otherwise stated. 2 months of silicification: D, ‘empty’ cell (C) within a Fe-SiO<sub>2</sub> precipitate; note the fine particles inside the cell (arrow). E, Cell (C) filled with a Fe-SiO<sub>2</sub> precipitate; note the different texture of this precipitate and its binding on the inner side of the cell wall. F, cell remains within the Fe-SiO<sub>2</sub> precipitate. G, cell (C) observed on a thin section stained with lead citrate an uranyl acetate, note the remaining cytoplasmic material on which fine particles are attached (arrow). H, high magnification view of the walls of two cells showing the plasma membranes (Mb arrows) and the S-Layers (S arrows) for each cell; note that the inner layer of the membrane is only visible when the Fe-SiO<sub>2</sub> has formed inside the cell (upper right), and is invisible if not (bottom). 9 months of silicification: I, ‘empty’ and ‘filled’ cells (C) within the Fe-SiO<sub>2</sub> precipitate; note the more dense aspect of the precipitate. J, high magnification view of the preserved wall structure of both cells. All TEM micrographs were made at 200 kV.



**FIG. 4. Experimental silicification experiment.**

EDX spectra obtained on TEM ultrathin sections of *M. jannaschii* cells exposed to a *c.* 10 mM Fe(III) solution during 1 hour at 60°C, then to a 350 ppm Si silica-solution for 9 months (Fig. 3I). **A**, spectrum made on the Fe-SiO<sub>2</sub> precipitate surrounding the cells. **B**, spectrum made on the Fe-SiO<sub>2</sub> precipitate filling the cells. Note the similar compositions. All EDX spectra were made at 200 kV.