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**Impact of biomineralization on the preservation of microorganisms during
fossilization: an experimental perspective**

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ABSTRACT

The biogenicity of fossil microbial biomorphs is often debated because their morphologies are poorly informative and the chemical, structural and isotopic signatures of biogenic organic molecules have been altered during their incorporation into the sediments and the geological history of the host rock. Here, we investigated the effect of encrustation by biominerals on the morphological and chemical degradation of *Escherichia coli* cells during experimental thermal treatments with the aim of simulating fossilization processes. Non-calcified *E. coli* cells and *E. coli* cells encrusted by calcium phosphates were exposed to heating under an Argon atmosphere at two different temperatures (300°C, 600°C) for different durations (2 h, 20 h, 100 h). Products were characterized down to the nm-scale using a combination of scanning electron microscopy, transmission electron microscopy, Raman microspectroscopy, electron paramagnetic resonance (EPR) spectroscopy, Fourier transform infrared (FT-IR) spectroscopy and scanning transmission X-ray microscopy. In absence of encrusting biominerals, the morphological structure of *E. coli* cells was completely lost after heating at 300 or 600°C, even after short (2h) heating experiments. *E. coli* cells heat-treated at 600°C for 20 h had an increased content of aromatic functional groups, and the amide functional groups were lost. Moreover, the EPR spectrum of these cells showed a supra-Lorentzian line shape, similar to the EPR spectrum of organic carbon detected in the Apex chert (ca. 3460 Myr) for example. In contrast, calcified *E. coli* exposed to the same conditions (300 or 600°C) showed limited morphological alteration as observed by electron microscopies and less chemical transformation as detected by FT-IR and EPR spectroscopies. These experiments evidence the strong influence of cell encrustation by minerals on their preservation potential during fossilization processes.

Keywords: Experimental taphonomy, Biomineralization, Fossilization, Exceptional preservation, EPR, FT-IR

1. Introduction

A wide diversity of microorganisms can form a variety of crystalline or amorphous biominerals via biomineralization within their cells, in their cell walls, at the cell surface, on extracellular structures and away from the cells (e.g., Lowenstam, 1981; Weiner and Dove, 2003; Konhauser and Riding, 2012). These biomineralization processes are relatively fast and can occur in a few hours or days (e.g., Miot et al., 2009; Chan et al., 2011), resulting in the formation of diverse organo-mineral complexes including biominerals and biomineral-encrusted cells/biostructures (Li J. H. et al., 2013). Recent analytical advances offer promises to characterize chemically and mineralogically these complexes down to the nm-scale (e.g., Miot et al., in press). Encrustation of microbial cells by biominerals can be seen as a first stage of fossilization (e.g., Oehler and Schopf, 1971; Westall, 1995; Toporski et al., 2002; Benning et al., 2004; Lalonde et al., 2005; Miot et al., 2009; Orange et al., 2009; Li et al., 2010; Chan et al., 2011). It has often been hypothesized that biomineral-encrusted cells/biostructures may be more resistant to diagenesis and/or metamorphism than pure organic molecules and structures, and therefore that they may be better preserved over geological timescales (see Li J. H. et al., 2013 for a review). Yet, such assumptions have not yet been evidenced experimentally.

Assessing the biogenicity of microbial-like structures in ancient rocks is challenging since the original biological information they contain can be significantly altered during fossilization processes (e.g., Gourier et al., 2004; Vandenbroucke and Largeau, 2007; Papineau et al., 2010), and these biostructures may thus be mistaken with abiotic structures (Brasier et al., 2002; García-Ruiz et al., 2003; Van Zuilen et al., 2007; Cosmidis et al., 2013). In addition, determining unambiguously the syngenecity of putative microfossils within a rock requires a precise assessment of the thermal history that they have experienced (Brasier and Wacey, 2012).

Exposing modern microorganisms in the laboratory to well-constrained temperature conditions may help improving our knowledge on the transformations of such fossils induced by burial and maturation processes (Li J. H. et al., 2013). Such experiments have been used increasingly to study the thermal maturation of biomolecules or biominerals under various pressure and temperature conditions (e.g., Stankiewicz et al., 2000; Gupta et al., 2006; Skrzypczak-Bonduelle et al. 2008; Watson et al., 2012; Bourbin et al., 2013; Li Y. L. et al., 2013). For instance, Skrzypczak-Bonduelle et al. (2008) reported the continuous evolution of electron paramagnetic resonance (EPR) line shapes gradually from a Gaussian-Lorentzian to a supra-Lorentzian profile for organic matter experiencing step-heating from room temperature up to 800°C, similarly to what is reported for field samples. Similar results were recently obtained by Bourbin et al. (2013) on bacteria heat-treated in the laboratory, thereby confirming that EPR spectroscopy provides a powerful tool to study the geochemical maturity of organic matter.

However, comparing extant microbes or biominerals with putative ancient microfossils or fossil biominerals is still a challenge as the impact of mineral encrustation on the preservation of microbial fossils has not yet been tested in detail (Li J. H. et al., 2013). Here, we report results of thermal degradation experiments performed on *Escherichia coli* cells with or without Ca-phosphate encrusting their cell walls at 300 and 600°C under argon atmosphere for various durations (up to 100 h). Thermally degraded cells were characterized using scanning electron microscopy (SEM), transmission electron microscopy (TEM), Raman microspectroscopy, EPR spectroscopy, Fourier-Transform infrared spectroscopy (FT-IR) and scanning transmission X-ray microscopy (STXM) coupled with X-ray absorption near edge structure (XANES) spectroscopy. These techniques are highly complementary. While Raman spectroscopy is sensitive to the organization of the aromatic skeleton in carbonaceous material (e.g., Beyssac et al., 2003; Lahfid et al., 2010; Beyssac and Lazari, 2012), EPR spectroscopy detects the electron spin of organic radicals in carbonaceous matter with high sensitivity (e.g., Mrozowski, 1988; Bourbin et al., 2013), C-XANES spectroscopy provides information on organic carbon speciation (e.g., Bluhm et al., 2006; Solomon et al., 2009), and FT-IR spectroscopy allows identifying the different chemical bonds within organic molecules (e.g., Yule et al., 2000).

2. Materials and methods

2.1 Sample preparation

Calcium-phosphate encrusted (i.e., calcified) and non-encrusted (i.e., non-calcified) *E. coli* cell samples used in the present study were derived from the same *E. coli* Apm1 strain. Strain Apm1 was obtained by transformation of *E. coli* BL 21 cells by the pET gene expression system (Studier and Moffatt, 1986). This plasmid contained (i) a *phoA* gene coding for an alkaline phosphatase linked to a promoter activated by isopropyl β -D-thiogalactoside (IPTG), and (ii) an ampicillin resistance marker. The addition of IPTG to the culture medium induced the transcription of the *phoA* gene, resulting in the over-expression of alkaline phosphatase enzymes (PHO A). Encrustation of the cells by Ca-phosphates was achieved by culturing cells over-expressing PHO A in a calcification medium.

To obtain cells encrusted by Ca-phosphates, cells from one single colony grown on LB agar medium (Sigma-Aldrich Co.), were inoculated within 5 ml of autoclaved liquid LB medium (Sigma-Aldrich Co.) and grown overnight at 37°C and 180 rpm. This 5-ml culture was transferred into 500 ml of liquid LB for bacterial amplification culture. This culture was also carried out at 37°C and 180 rpm; the optical density at 600 nm (OD₆₀₀) was monitored during the growth. Once the OD₆₀₀ reached a value between 0.6 and 0.8 after ~4-6 h incubation (corresponding to the exponential growth phase of *E. coli* cells), 1 ml of IPTG (0.4 mM) was added to induce the overexpression of the *phoA* gene, leading to the production by the cells of high amounts of PHO A. After 4-hours of incubation under these conditions, cells were harvested by

centrifugation at 6500 rpm for 15 min and rinsed three times with distilled water. Cells were then transferred to 2 liters of a calcification medium and incubated at 37°C with shaking at 120 rpm. The calcification medium, specifically designed to favor encrustation by Ca-phosphates, was prepared by dissolving calcium-glycerophosphate (glycerol phosphate calcium salt, Sigma-Aldrich Co.) in a HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) pH-buffer (20 mM). HCl (1M) was used to adjust the pH to 7.5 and the medium was sterilized by filtration through a 0.22- μ m filter. After a week, the incubated cells, completely encrusted by Ca-phosphates were centrifuged at 8000 rpm for 15 min and rinsed three times in distilled water.

To obtain the non-encrusted *E. coli* cells, 2-ml suspension of precultured Apm1 were transferred into 1 liter of liquid LB medium and allowed to grow overnight, and then harvested by centrifugation and rinsed with distilled water using the same protocol as for the calcified *E. coli*.

All media contained a final concentration of ampicillin of 50 μ g·ml⁻¹ to prevent growth of potential contaminants.

2.2 Thermal treatments

Calcified and non-calcified *E. coli* cells were first dehydrated at ambient temperature in a vacuum dryer for two weeks at ambient temperature (i.e. ~20°C), and then heat-treated at different temperatures (i.e., 300 and 600°C) for various durations (i.e., 2, 20 and 100 h) in an inert Argon atmosphere using a Carbolite single zone horizontal tube furnace (GHA12/300) equipped with a Quartz work-tube. For all experiments, the increase of temperature followed a constant ramp of 5°C·min⁻¹. Experimental solid products were characterized using SEM and TEM while their chemical, structural and molecular signatures were analysed by Raman, EPR, XANES and FT-IR spectroscopies.

2.3 SEM and TEM

Experimental solid products were finely ground in distilled water. Samples for SEM were deposited on cover slides (~3 mm × 3 mm) mounted on aluminum SEM stubs with high-conductivity copper tape. Samples for SEM were coated with carbon or gold. SEM observations were performed on a Zeiss Supra 55 SEM microscope at a 10 kV accelerating voltage and a working distance of 7.5 mm. SEM images were collected with both in-Lens and Angle selective Backscattered (AsB) detectors. Samples for TEM analyses were deposited on carbon coated Formvar grids. TEM experiments were carried out on a JEOL-2100F microscope operating at 200 kV, equipped with a field emission gun, an ultra high-resolution (UHR) pole piece, a Gatan energy filter GIF 200, a JEOL detector with an ultrathin window allowing detection of light elements, and a scanning TEM (STEM) device, which allows Z-contrast imaging in

high angle annular dark field (HAADF) mode. Elemental mapping was acquired by EDXS (energy dispersive X-ray spectroscopy) in the STEM mode.

2.4 Raman spectroscopy

Raman data were obtained on powders with a Renishaw INVIA microspectrometer as described in Bernard et al. (2008). Spectra were measured from 500 to 3500 cm^{-1} at constant room temperature using the 514.5 nm wavelength of a 50 mW Modulaser Argon laser (green laser) focused on the sample through a Leica DMLM microscope with a long working distance 50X objective (NA = 0.55). This configuration yielded a planar resolution of $\sim 1 \mu\text{m}$ for a laser power delivered at the sample surface set at around 250 μW to prevent irreversible thermal damage that could be due to laser-induced heating (Beyssac et al., 2003). Light was dispersed by a grating with 1800 lines/mm and the signal was analyzed with a RENCAM CCD detector. Measurements were performed with a circularly polarized laser using a $\frac{1}{4}$ wavelength plate placed before the microscope in order to limit polarization effects. The collected Raman signal was fitted using the procedure introduced by Lahfid et al. (2010). Additional Raman analyses were performed using the 785 nm wavelength of a 300mW Renishaw diode laser (near-infrared laser) with a planar resolution of $\sim 2\text{-}3 \mu\text{m}$ and low laser power. As this configuration did not improve the signal-to-fluorescence ratio, only data collected using the green laser are presented here.

2.5 EPR spectroscopy

EPR data were collected on powders at room temperature at the X-band (9.86 GHz) using a BRUKER EMX spectrometer with the following parameters: center field 3530 G, modulation frequency 100 kHz, microwave power 1 mW, and modulation amplitude 0.3 G. The spectrometer was calibrated using the diphenylpicrylhydrazyl (DPPH) standard with a known g factor ($g = 2.0037$). As the detection scheme of EPR gave spectra in the forms of a first derivative of the EPR absorption, a plot of $[A_{\text{pp}}\Delta B/(\Delta B_{\text{pp}}A)]^{1/2}$ versus $(\Delta B/\Delta B_{\text{pp}})^2$ was used to further characterize the line shape by its deviation from a pure Lorentzian line, where ΔB_{pp} and A_{pp} are the peak-to-peak line width and amplitude, respectively, ΔB is the magnetic field offset from this line center, and A is EPR amplitude values at each point of the EPR line (Skrzypczak-Bonduelle, 2008). The line shape parameter R_{10} , which quantified the deviations of the experimental points from the Lorentzian shape in this plot, was calculated following Skrzypczak-Bonduelle et al. (2008).

2.6 STXM-based XANES spectroscopy

Experimental solid products were finely ground and deposited on Si_3N_4 windows for STXM analyses. XANES data were collected on beamline 5.3.2.2 (STXM Polymer beamline - Kilcoyne et al.,

2003) at the Advanced Light Source (ALS) and beamline 10ID-1 (SM beamline - Kaznatcheev et al., 2007) at the Canadian Light Source (CLS). Beamline 5.3.2.2 (ALS) works in the soft X-ray energy range (250 - 800 eV) and is based on a bending magnet. The ALS storage ring was operated at 1.9 GeV and 500 mA current in a top-up mode. Beamline 10ID-1 (CLS) works in the soft X-ray energy range (130 - 2500 eV) and is based on an elliptically polarized undulator (EPU). The CLS storage ring was operated at 2.9 GeV and between 100 and 250 mA current. On both beamlines, the microscope chamber was first pumped down to 100 mTorr after sample insertion and back-filled with He.

Rationale for the use of STXM to study biominerals can be found in Cosmidis and Benzerara (2014). Energy calibration was accomplished using the well-resolved 3p Rydberg peak at 294.96 eV of gaseous CO₂ for the C K-edge. Alignment of images of stacks and extraction of XANES spectra were done using the aXis2000 software (ver2.1n). Normalization and assessment of spectral peak positions were determined using the Athena software package (Ravel and Newville, 2005). Extensive databases of reference C-XANES spectra were available from the literature (e.g., Solomon et al., 2009).

2.7 FT-IR Spectroscopy

Experimental solid products were finely ground, diluted within dried potassium bromide (KBr), and pressed under 8 tons of pressure for 1 min to be characterized by FT-IR spectroscopy. FT-IR spectra were recorded between 400 and 4000 cm⁻¹ with a resolution of 0.5 cm⁻¹ by using a Nicolet 7600 FT-IR spectrometer. Two hundred absorbance spectra were obtained and averaged for each sample.

3. Results

3.1 Electron microscopy

After dehydration, non-calcified Apm1 cells display a rod-shaped morphology typical of *E. coli* cells, with a width of ~0.5-0.8 µm and a length of ~1-3 µm (Fig. 1a). In contrast, calcified Apm1 cells appear as large aggregates of cells encrusted heavily by calcium phosphates that can be observed at the cell surface as well as within the cells and the cell walls (Fig. 1c and Fig. S1a-c). STEM-EDXS mapping, HRTEM imaging, and selected area electron diffraction (SAED) analyses reveal that these calcium phosphates are poorly crystalline with d-spacing values (e.g., 3.46 Å and 2.73 Å) consistent with those of hydroxyapatite (HA) (Cuisinier et al., 1987) (Fig. S1d-k).

Cell structures of non-calcified *E. coli* were completely disrupted after all heat-treatments, including at 300°C for 2 h (Fig. 1b). In contrast, calcified *E. coli* cells appeared well preserved morphologically after all thermal degradation experiments, including heat-treatment at 600°C for 20 h (Fig. 1d). The morphological and chemical features of calcified *E. coli* cells and their associated minerals remained relatively constant upon heating as evidenced by SEM, TEM and STEM-EDXS analyses except for the

crystallinity of calcium phosphates, which increased with temperature as shown qualitatively by SAED and HRTEM analyses (Fig. S2).

3.2 Raman and EPR spectroscopy

Raman microspectroscopy is sensitive to the organization of the aromatic skeleton in graphitic carbons at the micrometer-scale (Bernard et al., 2010), while EPR spectroscopy is a bulk technique sensitive to the physiochemical structures of aromatic radical moieties (Mrozowski, 1988). These two techniques were used to characterize the solid products obtained after various thermal degradation experiments. However, the very high fluorescence overwhelming the Raman signal prevented collecting Raman data on most of the experimental solid products except for non-calcified *E. coli* cells heated at 600°C for 20 h (Fig. 2a). The Raman spectrum of this sample exhibited two dominant broad features centered at about 1360 and 1605 cm^{-1} . These two features could be assigned to the main defect D1 band centered at 1355 cm^{-1} , and to a composite band including the graphite G and the D2 defect bands. Additionally, an intense D3 defect band centered at $\sim 1515 \text{ cm}^{-1}$ was clearly present. Such a spectrum was highly similar to the Raman spectra of partially combusted organic compounds such as soots, charcoals or organic residues of wood fires (e.g., Paris et al., 2005; Sadezky et al., 2005).

X-band EPR spectra were collected on both calcified and non-calcified *E. coli* cells heat-treated at 600°C for 20 h (Fig. 2b). Although both samples were characterized by the same EPR center ($\sim 3535 \text{ G}$), they exhibited different EPR spectra in terms of line shape and width. The line width parameter ΔB_{pp} was 0.246 mT for non-calcified *E. coli* cells heated at 600°C for 20 h and 0.441 mT for calcified *E. coli* cells heated at 600°C for 20 h. The EPR line shape of heated calcified *E. coli* cells was a combination of Gaussian and Lorentzian profiles with a R_{10} factor of 0.82, while the heated non-calcified *E. coli* cells exhibited a supra-Lorentzian EPR line shape (close to 2-D dipolar) with a R_{10} factor of -1.85 (Fig. 2c), typical of more mature natural organic matter (Skrzypczak-Bonduelle et al., 2008; Bourbin et al., 2013).

3.3 STXM-based XANES spectroscopy

STXM-based XANES spectroscopy was used to document the evolution of carbon speciation in non-calcified *E. coli* cells heated at increasing temperatures for various durations (Fig. 3). STXM measurements could not be performed on calcified *E. coli* cells since they were opaque to X-rays because of heavy encrustation.

The C-XANES spectrum of non-heated and non-calcified *E. coli* cells was similar to the spectra measured for other bacteria by previous studies (e.g., Benzerara et al., 2004; Miot et al., 2009; Li J. H. et al., 2013), with a main peak at 288.2 eV, attributed to $1s \rightarrow \pi^*$ electronic transitions of carbon in amide groups, a peak at 285.2 eV, attributed to electronic transitions of carbon in aromatic or olefinic carbon

groups (C=C) and two additional peaks at 287.3 eV and 289.4 eV, attributed to $1s \rightarrow \pi^*$ electronic transitions of carbon in hydroxyl (C-OH) functional groups associated with phenols, and aryl ethers or hydroxylated aromatics and $C1s \rightarrow 3p/\sigma^*$ electronic transitions of carbon in hydroxylated- or ether-linked carbon species, respectively (Solomon et al., 2009).

The intensity and the width of the peak at 285.2 eV progressively increased with increasing heating duration at 300°C, while the intensity of the peak at 288.2 eV decreased and its width increased. The broadening of the peak at 288.2 eV might be related to the increase of aliphatic and carboxylic group contents, which have major absorption peaks at 287.8 eV and 288.5 eV, respectively.

With increasing temperature, the spectral evolution of heat-treated non-calcified *E. coli* cells was even more pronounced. At 600°C, the peak at 288.2 eV almost disappeared, suggesting massive loss of amide functional groups. Based on C-XANES spectra, non-calcified *E. coli* cells heat-treated at 600°C were composed of aliphatic-rich organic molecules dominated by poorly condensed aromatic structures (Solomon et al., 2009).

3.4 FT-IR spectroscopy

Non-calcified and non-heated *E. coli* cells showed a FT-IR spectrum very similar to spectra of bacteria reported in the literature (e.g., Filip et al., 2008). Three major peaks could be observed at 1655 cm^{-1} , 1540 cm^{-1} , and 1237 cm^{-1} and were attributed based on previous studies to amide I, amide II, and amide III vibrational modes, respectively (Fig. 4a). Noticeably, C-O and C=C stretchings may also contribute to these peaks. The band at 1455 cm^{-1} was assigned to C-H deformations of CH_2 or CH_3 groups in aliphatics; the band at 1393 cm^{-1} to C-H bending ($-\text{CH}_3$ stretch in fatty acids); the band at 1082 cm^{-1} to C=O asymmetric stretching (glycopeptides, ribose, aliphatic esters), and the band at 2958-2854 cm^{-1} to C-H stretching in aliphatics of cell walls (fatty acids, carbohydrates) (e.g., Filip et al., 2004 and references therein). Consistently with C-XANES data, the FT-IR spectra of heat-treated non-calcified *E. coli* cells varied with increasing temperature indicating some chemical transformation of the organic carbon composing the cells: the peaks of amide (1655 cm^{-1} , 1540 cm^{-1} , and 1237 cm^{-1}), aliphatics (2958-2854 cm^{-1} , 1455 cm^{-1} , and 1393 cm^{-1}) and carbonyl or carboxyl groups (1082 cm^{-1}) progressively disappeared when samples were heated up to 600°C. Peaks at 1628 cm^{-1} , 1702 cm^{-1} and 1265 cm^{-1} , assigned to aromatic C=C, C-O bonds of CO_2H groups and C=O bonds of COOH groups, respectively (e.g., Tseng et al., 1996; Smidt et al., 2005), could be observed in non-calcified *E. coli* cells heat-treated at 300°C for 20 h. Infrared absorption of non-calcified *E. coli* cells heated at higher temperature (600°C for 20 h) could not be measured, since this sample highly reflected the IR beam (Fig. 4a). Such high reflectivity likely traduced an important maturity level (e.g., Lis et al., 2005).

Amide (1652 cm^{-1} , 1540 cm^{-1} , 1237 cm^{-1}) and aliphatics (1455 cm^{-1} , $2958\text{--}2854\text{ cm}^{-1}$) bands could be observed in the FT-IR spectrum of initial calcified *E. coli* cells, although this spectrum was dominated by the characteristic PO_4^{3-} absorption bands at 565 , 603 and 1035 cm^{-1} (bending and stretching modes of P-O groups and stretching and vibrational modes of OH^- groups) (Fig. 4b), indicative of the Ca-phosphate minerals encrusting the cells (e.g., Pleshko et al., 1991; Bazin et al., 2012). Additional weak features at 1455 cm^{-1} and 1415 cm^{-1} were attributed to aliphatic and aromatic carbons, respectively (e.g., Trchová and Stejskal, 2011). Interestingly, the FT-IR spectra of calcified *E. coli* cells did not change significantly during thermal treatment at 300 or 600°C in contrast to non-calcified *E. coli* cells. The FT-IR spectrum of calcified *E. coli* cells heat-treated at 600°C for 20 h appeared quite similar to the FT-IR spectrum of initial calcified *E. coli* cells, despite a slight decrease of the relative intensities of amide bands and a slight increase of the relative intensities of aliphatic (1455 cm^{-1}) and aromatic (1415 cm^{-1}) bands (Fig. 4b). Noteworthy, the peaks corresponding to amide functional groups were still detected even after thermal treatment at 600°C for 20 h and their presence interpreted as resulting from the (partial) preservation of the original molecular signatures of original biomolecules.

4. Discussion

4.1 Impact of biomineralization on the preservation of bacteria

Calcified *E. coli* cells were morphologically and chemically better preserved upon heating than non-calcified *E. coli* cells. This result was clearly evidenced by electron microscopy, EPR and FT-IR data. Calcified cells heat-treated at 600°C for 20 h exhibited a wider EPR spectrum than non-calcified cells, with ΔB_{pp} of 0.441 mT and R_{10} factor of 0.82 (Figs. 1 and 2) which is typical of low maturity organic matter (Skrzypczak-Bonduelle et al., 2008). Consistently, FT-IR spectroscopy evidenced the slight chemical transformations of calcified *E. coli* cells with increasing temperature compared to the strong transformations undergone by non-calcified *E. coli* cells (Fig. 4).

The pervasive Ca-phosphate precipitation within the cell structures can explain the better morphological preservation of the cells during heating as it may have provided a mechanical resistance. In addition to a morphological preservation, the present study also evidenced the chemical preservation of calcified *E. coli* cells upon heating. A similar chemical resistance to thermal treatments was recently evidenced in experiments performed on bio-encrusted cells of iron-oxidizing bacteria, which formed amorphous iron phosphate minerals within the cell walls (Li J. H. et al., 2013). Periplasmic encrustation by Ca-phosphates as observed here has been suggested to be a widespread mechanism (e.g., Goulhen et al., 2006; Benzerara et al., 2008; Miot et al., 2009, 2011; Cosmidis et al., 2013; Li J. H. et al., 2013) and may cause sorption and entrapment of organic molecules such as peptidoglycan proteins and polysaccharides on/within minerals (e.g., Miot et al., 2009, 2011). Such organic-mineral complexes may

facilitate the preservation of bacterial cell ultrastructural details, and potentially their chemical signatures, by shielding and protecting organic molecules from reactive solutions and/or oxidizing gas phases.

This mechanism remains hypothetical and deserves further experimental work. However, the present observations can be related to numerous previous reports that showed that even poorly resistant organic molecules such as amino acids or polysaccharides can be preserved during diagenesis if encapsulated within mineral biostructures such as coral skeletons and diatoms (e.g., Ingalls et al., 2003a, 2003b) or microbialites (e.g., Benzerara et al., 2006; Kaur et al., 2011). A major role for minerals in organic matter protection has also been recognized for soils and the occurrence of adsorptive protection of organic compounds as molecular monolayers onto clay minerals has been shown to be efficient against diagenetic degradation (e.g., Hedges and Keil, 1995; Ransom et al., 1998; Vandenbroucke and Largeau, 2007).

4.2 Comparison with field sample spectroscopic signatures

Extrapolating laboratory results to natural processes remains difficult, notably because geological durations cannot be replicated in the laboratory. Temperatures and heating rates considerably higher than those encountered in nature have traditionally been applied to laboratory samples to compensate for long geological time periods (e.g., Vandenbroucke and Largeau, 2007).

Here, non-calcified *E. coli* cells experimentally heat-treated at 600°C for 20 h exhibited a Raman spectrum which appeared markedly different from spectra of organic carbons having experienced diagenetic burial in natural settings, but pretty similar to spectra from soots or charcoals, i.e. organic residues having experienced only a short (on geological timescale) thermal treatment in nature. Furthermore, the high reflectivity of this sample as highlighted by FT-IR spectroscopy indicated a relatively high maturity (e.g., Lis et al., 2005). In addition, this sample exhibited a supra-Lorentzian EPR line shape (close to 2-D dipolar) with a R_{10} factor of -1.85, similar to the EPR lineshape shown by natural organic matter detected in the Apex cherts (ca. 3460 Myr) from Pilbara craton in Australia (e.g., the R_{10} factor is -1.98), which is thought to have experienced metamorphic conditions in the Prehnite-Pumpellyite facies to lower Greenschist facies, i.e. temperature conditions of about 200-300°C (Skrzypczak-Bonduelle et al., 2008). Last, the absence in the C-XANES spectrum of *E. coli* cells heat-treated at 600°C for 20 h of a sharp $1s \rightarrow \sigma^*$ exciton at 291.7 eV, related to the presence of extensive planar domains of highly conjugated aromatic layers (Ahuja et al., 1996; Bernard et al., 2010), suggested that the aromatic cluster size of these residues did not significantly increase even after having experienced such temperature conditions. Overall, this C-XANES spectrum appeared similar to C-XANES spectra of overmature kerogens (Bernard et al., 2012), i.e., kerogens having experienced a thermal history with a maximum temperature of about 200-250°C.

Altogether, Raman, FT-IR, EPR and C-XANES data obtained on non-calcified *E. coli* cells heat-treated at 600°C for 20 hours provide contrasted views when compared to natural carbons: they looked like natural samples that have experienced short thermal pulse for Raman; less than 200-250°C for EPR and XANES. Difficulties to relate directly the products from laboratory experiments with field samples partly come from the heating rate and pressure conditions at which the present experiments were conducted. Heating rate and pressure have indeed been shown to be key factors impacting the kerogen decomposition rate (Beyssac et al., 2003; Vandenbroucke and Largeau, 2007). In addition, organic maturation processes in natural low-temperature systems may also be strongly affected by the presence of water (Seewald et al., 2000; Lewan and Roy, 2011), which was rapidly lost in the present experiments. Therefore, it will be important in the future to document further whether the differential evolution between calcified and non-calcified *E. coli* cells detected here may also take place when these additional parameters (e.g., heating rates, pressure and water) are involved.

Concluding remarks

In the present study, we investigated experimentally the impact of early encrustation by biominerals on the morphological and chemical transformations of *E. coli* cells upon heating. While in the absence of biominerals, *E. coli* cells were almost totally degraded, calcified *E. coli* were better preserved, morphologically and chemically, during thermal treatment at 300 or 600°C, for durations as long as 100 h. Such results clearly evidence the influence of bio-encrustation on bacteria preservation potential during fossilization processes. The resistance of such organic-mineral complexes to diagenesis clearly illustrates why self-encrusting microorganisms should be considered as pertinent targets for the search for evidence of ancient life in the fossil record.

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Figure captions

Figure 1. Morphological alteration of non-calcified *E. coli* cells (a and b) and calcified *E. coli* cells (c and d) after heating treatments. (a) TEM image of *E. coli* cells dehydrated at room-temperature for two weeks. (b) TEM image of *E. coli* cells heated at 300°C for 2 h. The morphological structure of *E. coli* cells was completely lost after heating at 300 or 600°C, even in the 2-h long experiments. (c) SEM and TEM (inset) image of calcified *E. coli* cells dehydrated at room-temperature. (d) SEM and TEM (inset) image of calcified *E. coli* heated at 600°C for 20 h. SEM and TEM analyses show that the *E. coli* cells were heavily aggregated and encrusted by whisker-shaped minerals, and that the morphological structures including encrusted cells (indicated by single-ended arrow with a solid line) and cell aggregates were well preserved after all heating experiments.

Figure 2. (a) Raman spectrum of non-calcified *E. coli* cells heat at 600°C for 20 h. The spectrum was deconvoluted with a four-band fitting (*G*, *DI*, *D2* and *D3*) using a Voigt profile, as described in Beyssac et al. (2002). (b) EPR spectra of non-calcified and calcified *E. coli* cells heat-treated at 600°C for 20 h. (c) Description of the EPR line shape of the two samples. ΔB_{pp} and A_{pp} indicate the peak-to-peak line width and amplitude, respectively, ΔB is the magnetic field offset from the line center, and A is EPR amplitude values at each point of the EPR line. The line shape parameter R_{10} , which was calculated following Skrzypczak-Bonduelle et al. (2008), is used to quantify the deviations of the experimental points from the Lorentzian shape in this plot.

Figure 3. C K-edge XANES spectra of heat-treated *E. coli* cells. The peaks at 285.2 eV, 287.3 eV, 287.8 eV, 288.2 eV, 288.5 eV, and 289.4 eV correspond to electronic transitions in aromatic (C=C), alcohol (C-O), aliphatics (C-C), amide (O=C-NH_x), carboxyl (O=C-O), and alcohol (C-O) functional groups, respectively. The peaks at 297.3 eV and 300 eV correspond to the potassium *L*_{2,3}-edges indicating the presence of K most likely precipitated from the culture medium.

Figure 4. Infrared spectra of non-heated and thermally treated samples of non-calcified (a) and calcified (b) *E. coli* cells. The bands at 2958-2854 cm⁻¹ correspond to C-H stretching in aliphatics. The peak 1702 cm⁻¹ corresponds to C-O stretch of CO₂H, 1655/1652 cm⁻¹ to amide I, 1628 cm⁻¹ to aromatic band C=C, 1540 cm⁻¹ to amide II, 1455 cm⁻¹ to C-H deformations of CH₂ or CH₃ groups in aliphatics, 1393 cm⁻¹ to C-H bending, 1265 cm⁻¹ to C=O stretch of -COOH, 1237 cm⁻¹ to amide III, 1082 cm⁻¹ to C=O stretching. The peaks at 565 cm⁻¹ and 603 cm⁻¹ correspond to P-O-P bending, and 1035 cm⁻¹ to P-O stretching of PO₄

585 groups, or to the vibrational modes of OH⁻ groups. The small peaks at 1093 cm⁻¹ and 962 cm⁻¹ correspond
586 to P-O-P bending.
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Supplementary Figure Captions

Figure S1. Morphological and chemical characterization of non-heated calcified *E. coli* cells. (a) SEM image of heavily encrusted and aggregated calcified *E. coli* cells. (b) STEM image showing minerals located within the cells, within the cell walls and at the cell surfaces. (c) TEM image showing the high amount of minerals at the cell surface. (d)-(i) STEM-XEDS maps showing the spatial distribution of C, O, P and Ca. (j) SAED pattern of the Ca-phosphate minerals. The d-spacing values calculated from two major electron diffraction rings are 3.44 and 2.75 Å, which can be indexed as the (002) and (121) lattice planes of hydroxyapatite, respectively. (k) HRTEM image of hydroxyapatite crystals.

Figure S2 Morphological and chemical characterization of calcified *E. coli* cells after heated at 600°C for 20 h. (a) SEM image. (b) STEM image. (c) TEM image. (d)-(i) STEM-XEDS maps of C, O, P and Ca. (i) SAED pattern showing the Ca-phosphate minerals are still hydroxyapatite. (k) HRTEM image of the hydroxyapatite minerals. SEM and TEM analyses altogether reveal a good morphological preservation of calcified *E. coli* cells after heating treatments. SAED and HRTEM lattice imaging also show an increase of crystallinity of hydroxyapatite crystals after heating treatments, e.g., sharper and narrower electron diffraction rings recorded from the 600°C-20h-heated sample compared to the unheated one.

- (1) The biogenicity of microfossils is often debated because of morphological and chemical alteration during geological history.
- (2) We experimentally studied the effect of bacterial biomineralization on fossilization during thermal treatments.
- (3) Calcified *E. coli* were better preserved morphologically and chemically during heating up to 600°C and 100 h.
- (4) These evidence the influence of bio-encrustation on bacteria preservation potential during fossilization processes.
- (5) Self-encrusting microorganisms should be considered as pertinent targets for the search for ancient life in fossil record.

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