Microbial mediated formation of Fe-carbonate minerals under extreme acidic conditions

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Discovery of Fe-carbonate precipitation in Rio Tinto, a shallow river with very acidic waters, situated in Huelva, South-western Spain, adds a new dimension to our understanding of carbonate formation. Sediment samples from this low-pH system indicate that carbonates are formed in physico-chemical conditions ranging from acid to neutral pH. Evidence for microbial mediation is observed in secondary electron images (Fig. 1), which reveal rod-shaped bacteria embedded in the surface of siderite nanocrystals. The formation of carbonates in Rio Tinto is related to the microbial reduction of ferric iron coupled to the oxidation of organic compounds. Herein, we demonstrate for the first time, that Acidiphilium sp. PM, an iron-reducing bacterium isolated from Rio Tinto, mediates the precipitation of siderite (FeCO3) under acidic conditions and at a low temperature (30°C). We describe nucleation of siderite on nanoglobules in intimate association with the bacteria cell surface. This study has major implications for understanding carbonate formation on the ancient Earth or extraterrestrial planets.

In the geologic record, evidence for the existence of microorganisms has been observed in sedimentary rocks as old as 3.45 Ga, and their influence on mineral precipitation throughout Earth’s history, particularly for carbonate deposits, has been and remains significant1,2. However, thermodynamic conditions fundamentally restrict carbonate precipitation to high pH environments (pH > 7), and, in terrestrial environments, the production of carbonates at pH < 4.5 does not occur either by abiotic or biotic mechanisms3–5. In spite of this general rule, Fe-rich carbonate minerals have been recently recognized in the subsurface of Rio Tinto in mildly acidic to neutral pH (5–7) and somewhat reducing (Eh < 0) conditions6,7. Rio Tinto is an acid-sulphate system considered as one of the potential analogues for life on early Earth and Mars8. Acidiphilium species are very abundant in Rio Tinto9, these alphaproteobacteria can grow on organic compounds under microaerobiosis and anaerobic conditions using ferric iron (Fe3+) and/or oxygen as electron acceptors10,11. In this study, we conducted culture experiments with Acidiphilium species to investigate the mediation of carbonate precipitation under low pH and temperature conditions. We present a high-resolution transmission electron microscopy (TEM), atomic force microscopy (AFM), scanning electron microscopy (SEM) and Raman spectroscopy study of the microbial carbonate precipitates (see methods section). We propose that the direct mediation of acidophilic microorganisms can overcome kinetic barriers to carbonate formation and that they may play an active role in the formation of carbonates in acidic and natural environments.

The culture experiments were designed with an iron-reducing bacterium, Acidiphilium sp. PM, and incubated at 35°C in acidic media for 45 days. To identify crystal precipitates, the cultures were observed periodically with optical microscope. We measured the pH at the end of the culture experiments. Parallel control experiments without bacteria and non-viable cells were run. Using a combination of Raman, sensitive energy dispersive X-ray Spectroscopy (EDS), TEM, SEM and AFM analyses, we have identified the mineral composition and investigated the involvement of Acidiphilium sp. PM in the nucleation of carbonates. Our research demonstrates that bacteria can create chemical microenvironments in the region directly surrounding their cell walls, and, thus, can...
effectively produce spatially restricted supersaturated conditions in which otherwise unpredicted minerals can precipitate.

**Results**

No precipitation was observed in non-viable cell and cell-free control experiments. *Acidiphilium* sp. PM reduced 94% of the Fe$^{3+}$ present in the medium. The starting concentration of Fe$^{3+}$ was 1.33 g/L and the final concentration 0.04 g/L.

AFM and TEM observations show mineral precipitates less than 200 nm in diameter (termed nanoglobules$^{11,12}$) with an irregular size and distribution (Figs. 2,3). Most of the nanoglobules are 20–100 nm in size while the remaining globules are 100–200 nm (large nanoglobules). The nanoglobules are composed of Fe-carbonate, most likely siderite based on Raman and EDX analyses (Figs. 4a,5), and occur attached to the surface of *Acidiphilium* sp. PM cells, where they are in some cases embedded in a thin organic film that surrounds the cells (Fig. 2). This organic film is produced by *Acidiphilium* sp. PM during growth and is composed of EPS as demonstrated by Raman spectroscopy analyses (Fig. 4b). In detailed view, we can observe that the surface of *Acidiphilium* sp. cells is surrounded by nanoglobules (Figs. 2b,c).

Figure 4a shows average of three Raman spectra collected from nanoglobules formed in culture experiments and the spectra of a siderite standard taken from Somorrostro (Vizcaya, Spain). The spectra were collected with an acquisition time of 60 s each. For precise positioning, an AFM tip was put on a nanoglobule, and the laser beam of a combined AFM-confocal Raman setup (see Methods) was focused onto the tip apex. All sample spectra contain Raman band at 520 cm$^{-1}$ assigned to the Si-Si stretching vibration of the AFM tip material. The band at 1087 cm$^{-1}$ is the most prominent band in the Raman spectrum of siderite (Fig. 4a). The band at 1087 cm$^{-1}$ in both spectra (nanoglobules and reference material) is in good agreement with the symmetric stretching vibration of CO$_3^{2-}$ ($\gamma_1$) in siderite as described previously$^{13,14}$.

**Discussion**

**Bacterial nucleation and precipitation of carbonate.** Our AFM, TEM and SEM studies demonstrate that siderite crystals nucleate on baterial nanoglobules, as previously suggested for Ca and/or Mg-carbonate precipitation$^{11,12}$. The initial steps of nanoglobule formation occur in the outer side of the bacterial envelopes and in some cases within an organic film composed of exopolysaccharides (EPS) in intimate association with the bacteria cell surface (Figs. 2,4b). Because EPS have the capacity of binding metal ions, the organic film may serve as a site for globule nuclei formation$^{15,16}$ in addition to the bacterial outer membrane. These experimental findings show that metabolically active, acidophilic, Fe-reducing bacteria possess the ability to produce mineral precipitates in a spatially restricted manner that can include Fe-carbonate minerals such as siderite.
bacteria are capable of producing conditions that promote the nucleation of Fe-carbonate under acidic conditions.

The nanoglobules attached to the bacteria cell surfaces (Figs. 2, 3) and some mineralized bacteria within the siderite crystals (Figs. 1, 5) provide evidence that bacterial precipitation of siderite may begin with the accumulation of Fe in the external bacterial envelopes and/or in the organic film and be followed by precipitation as nanoglobules on the cell surface. Based on the results of our culture experiments, we propose that the presence of acidophilic bacteria can mediate carbonate precipitation under acidic conditions and overcome the kinetic barrier to its formation by creating neutral to alkaline conditions in the microenvironment surrounding bacterial cells. It is well known that bacteria are capable of changing physico-chemical conditions (e.g., pH, CO$_2$, metal ion concentration) in their surrounding microenvironment$^{2}$–$^{5}$, $^{22}$. Thus, carbonate minerals that are undersaturated in abiotic medium can precipitate because bacteria concentrate around their cells the ions of interests, in this case Fe$^{2+}$ and CO$_3^{2-}$, until supersaturation is reached$^{26}$. There is a bacteria-medium interface in which reaction mechanisms change and thermodynamic activation energies can be modified leading to the precipitation of minerals$^{2}$–$^{5}$, $^{19}$. Therefore, pure aqueous solution chemistry cannot be applied to study such bacterial precipitating microenvironments$^{29}$–$^{32}$. Abiotic mineral precipitation does not occur in natural systems or in sterile laboratory experiments unless thermodynamic conditions are favourable$^{2}$–$^{5}$, $^{32}$. However, in a biotic medium, bacteria induce mineral precipitation (1) by modifying the conditions of their surrounding environment and/or concentrating ions in the bacterial cell envelope and (2) by acting as nucleation sites$^{20}$–$^{24}$. For instance, as we show here, iron-reducing bacteria are capable of precipitating carbonates under acidic conditions by neutralizing the pH and lowering the Fe$^{3+}$/Fe$^{2+}$ ratio locally.

Table 1 shows the mineral saturation index (SI) values for the initial conditions of the culture medium assayed. These values indicate that the medium is undersaturated with respect to anhydrite, calcite, dolomite, siderite and vivianite. This geochemical modelling suggests that the physico-chemical conditions of the medium are favourable to promote the abiotic precipitation of jarosite, goethite, hematite and strengite. However, none of these mineral phases were observed in our cultures. Hence, we conclude that Acidiphilium sp. control the processes of mineral precipitation. Its metabolic activity creates a microenvironment enriched in Fe$^{2+}$, CO$_2$, NH$_3$ and PO$_4^{3-}$, i.e., suitable for the precipitation of Fe$^{2+}$-rich minerals (e.g., siderite) instead of Fe$^{3+}$ minerals (e.g., jarosite, hematite, goethite and/or magnetite).

**Mechanism of precipitation of carbonate.** The formation of iron carbonates is an equilibrium or kinetic process controlled by the concentration of Fe$^{2+}$ and CO$_3^{2-}$ in solution$^{25}$. Previous studies suggested$^{25}$ that the production of large amount of siderite requires high concentration of Fe$^{2+}$, which can be obtained by bacterial reduction of Fe$^{3+}$. Therefore, the occurrence of siderite in Earth’s surface sedimentary environments could be favoured by iron reducing bacteria. On the other hand, mineralization can take place on the wall or on the surface of the bacterial cells that are presumably sheathed by extracellular polysaccharides$^{23}$–$^{26}$. In this study, the surface of Acidiphilium sp. PM provides sites for nucleation of Fe-phosphate after adsorbing Fe$^{2+}$ into cellular envelopes and its EPS. Nucleation would start by binding Fe$^{2+}$ to the phosphate groups of the outer membrane (mainly phospholipids and lipopolysaccharides). This promotes the formation of transient amorphous ferrous phosphate (AFP) phases (Fig. 3).

Microbes promote the precipitation of carbonate minerals metabically either by increasing the pH and the alkalinity of their surrounding microenvironment or by the secretion of substances that facilitate the mineral nucleation$^{1}$–$^{12}$, $^{25}$–$^{28}$, $^{29}$–$^{32}$. In the Precambrian, heterogeneous nucleation on bacterial cell surfaces, apparently, exerted

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**Figure 3** TEM of siderite nanoglobules formed in *Acidiphilium* sp. PM cultures. (a) TEM overview showing *Acidiphilium* sp. PM cells covered by nanoglobules. (b), (c) Detail of *Acidiphilium* sp. PM cell in intimate association with large and small nanoglobules. (d) EDX spectrum of lighter mineralized areas composed of Fe-carbonate with some P. (e) EDX spectrum nanoglobules (dark areas) which are composed of Fe-carbonates.
a powerful control on carbonate formation\textsuperscript{26,29}. Nucleation of carbonate minerals on organic surfaces (i.e., heterogeneous nucleation) can occur when negatively charged surface groups such as carboxylate, phosphate, and sulphate complexes bind divalent metal cations (e.g., Ca, Mg, Fe, Mn). Furthermore, this nucleation can increase the number of crystal nuclei even when the bulk medium is supersaturated with the mineral carbonate phase by more than an order of magnitude\textsuperscript{26}. Heterogeneous nucleation can take place on the microbial cell surfaces and envelopes, or within the exopolymeric matrix surrounding the cells.

Nucleation on bacterial cells depends on the surface charge, which is controlled both by structural features of cell wall and by bacterial metabolism\textsuperscript{23,24,26,27,30}. Our data confirm that the formation of siderite is controlled both by structural features of cell wall and by bacterial metabolism around the cells. This scenario allows us to explain the observed increase of pH from 3.5 to 6–7 at the bacterial-medium interface (site of mineral precipitation). We propose the following mechanism of precipitation. Besides CO\textsubscript{2} and NH\textsubscript{3} close to the cells, bacterial oxidation of the organic compounds also supplies abundant PO\textsubscript{4}\textsuperscript{3–} ions that concentrate first around bacterial cells\textsuperscript{27}. Phosphate ions and particularly ammonia are hydrolysed in the same way as CO\textsubscript{2}\textsuperscript{2–} with the subsequent increase of the pH in the microenvironment around cells according to the following reactions:

\[
\text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + \text{OH}^- (3)
\]

\[
\text{PO}_3^{4–} + \text{H}_2\text{O} \rightarrow \text{HPO}_4^{2–} + \text{OH}^- (4)
\]

\[
\text{HPO}_4^{2–} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{PO}_4^- + \text{OH}^- (5)
\]

As the growth medium contains more nitrogen than phosphorous, the role of reaction (3) in the neutralization and alkalinisation is more significant than that of reactions (4) and (5). Excess of PO\textsubscript{4}^{3–} around bacterial cells provides binding sites for Fe\textsuperscript{2+}, apart from those available from phospholipids of the outer bacterial membranes, allowing amorphous Fe phosphate supersaturation and its kinetically-favoured precipitation close to bacterial surfaces\textsuperscript{27}. The involvement of phosphate precursors in biominalization processes is well known\textsuperscript{32–35}. It should be noticed that CO\textsubscript{3}^{2–} ions hinder the precipitation of phosphate minerals\textsuperscript{36,37}, while PO\textsubscript{4}^{3–} ions hinder the precipitation of Fe-carbonate. The alkalinisation necessary for FeCO\textsubscript{3} precipitation is promoted by the presence of chemical species close to the cell boundary that could act as proton sinks. As the medium contains amino acids, the alkalinisation could be induced due to NH\textsubscript{3} production in the microenvironment around cells. This scenario allows us to explain the observed increase of pH from 3.5 to 6–7 at the bacterial-medium interface (site of mineral precipitation). We propose the following mechanism of precipitation. Besides CO\textsubscript{2} and NH\textsubscript{3} close to the cells, bacterial oxidation of the organic compounds also supplies abundant PO\textsubscript{4}^{3–} ions that concentrate first around bacterial cells\textsuperscript{27}. Phosphate ions and particularly ammonia are hydrolysed in the same way as CO\textsubscript{2}\textsuperscript{2–} with the subsequent increase of the pH in the microenvironment around cells according to the following reactions:

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**Figure 4** | (a) Raman spectra of both nanoglobules formed in Acidiphilium sp. PM culture experiments and siderite standard (reference material). (b) Raman spectrum of the extracellular organic film (EPS). See main text and methods for band assignments.

**Figure 5** | TEM (a), (b) and SEM (c), (d) images of siderite precipitates formed in Acidiphilium sp. PM cultures. (a), (b) siderite dumbbells and spherulite covered by bacteria (Acidiphilium sp. PM). (c) overall view of siderite nanoglobules. (d) detail of the surface of a microspherulite of siderite with mineralized bacteria resting on nanoglobular crystals. These microspherulites were formed after 45 days of incubation. The insert of the EDX analysis of the microspherulite demonstrate that it is 100% Fe-carbonate.

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+ \leftrightarrow 2\text{H}^+ + \text{CO}_3^{2–} \quad (1)
\]

Partitioning of HCO\textsubscript{3}– and CO\textsubscript{3}^{2–} in Fe-rich environments responsible for Fe-carbonate supersaturation is explained by the reaction:

\[
\text{CO}_2 + \text{H}_2\text{O} + \text{FeCO}_3^{(solid)} \rightarrow 2\text{HCO}_3^- + \text{Fe}^{2+} \quad (2)
\]
Table 1 | Saturation index values (SI) in all media assayed. Results from geochemical computer program PHREEQC. These SI values are for initial conditions.

| Mineral phase | SI |
|---------------|----|
| Anhydrite, CaSO₄ | 0.21 |
| Gypsum, CaSO₄ | 0.03 |
| Aragonite, CaCO₃ | 0.00 |
| Calcite, CaCO₃ | 0.00 |
| Dolomite, CaMg(CO₃)₂ | 0.00 |
| Siderite, FeCO₃ | 0.00 |
| Jarosite, KFe₇[SO₄]₂(OH)₆ | 0.00 |
| Lepidocrocite, FeOOH | 0.00 |
| Goethite, FeOOH | 0.00 |
| Magnetite, Fe₃O₄ | 0.00 |
| Hematite, Fe₂O₃ | 0.00 |
| Strongeite, FePO₄ × 2 H₂O | 0.00 |
| Vivianite, Fe₃[PO₄]₂ × 8H₂O | 0.00 |

Methods

Microorganism. Acidiphilium sp. strain DSM 24941 was isolated from the acidic, heavy metal-rich waters of Rio Tinto. As other members of this genus, it is a strict acidophile and a facultative aerobe. It grows on organic matter using O₂ or Fe³⁺ as electron acceptors. This Fe³⁺-reducing bacteria may play an important role in Fe and C biogeochemistry in natural environments. Finally, these culture experiments provide valuable insight into understanding the origin of Archean Fe-carbonates.

Culture medium. Acidiphilium sp. was grown in a 1:15 medium, with the following composition, (%, wt/vol): 0.2% (NH₄)₂SO₄; 0.01% KCl; 0.03% KH₂PO₄ × 3H₂O; 0.03% MgSO₄ × 7H₂O; 0.002% Ca(NO₃)₂ × 4H₂O; 0.1% yeast extract; 0.05% proteose peptone; 0.1% glucose, 0.2% NaHCO₃ and 0.5% Fe₂(SO₄)₃ × 9H₂O. To obtain a solid medium, 2% Bacto-Agar was added. pH was adjusted to 3.5 with 0.1 M H₂SO₄ and the solution was sterilized at 121 °C for 20 minutes.

Study of crystal formation. Acidiphilium sp. PM was surface-inoculated in Petri dishes incubated aerobically at 35 °C. Up to 45 days after inoculation, the cultures were examined periodically for the presence of minerals using a light microscopy (20X). The experiments were carried out in triplicate. Controls consisting of uninoculated culture media and media inoculated with autoclaved (non-viable) cells were included in all experiments. pH measurements were performed at the end of the growth and mineral formation experiments. pH-indicator paper (Merck Spezial-Indikatorpapier) was directly applied on the semi-solid surface. In situ fluorescence hybridization using a specific probe designed for Acidiphilium sp. (LEP636) was used to ensure the purity of the cultures.

To collect the precipitate, the colonies were scraped from the agar surface after an incubation period of 45 days. The collected material was washed several times with distilled water to remove the nutritive solution and any retained agar or cellular debris. The precipitates were then dried at 37 °C and microscopic examination showed that this treatment did not alter the crystal morphology.

Scanning electron microscopy (SEM); transmission electron microscopy (TEM); atomic force microscopy (AFM) and Raman spectroscopy analyses. The microbial precipitates from culture experiments were analyzed with a field emission SEM equipped with an electron dispersive detector (EDS) (Leo 1330, 143 eV resolution, LEO Electron Microscopy LTD, Germany).

Microbial precipitates were analyzed by TEM. AFM and Raman microscopy after 20 and 45 days of incubation, respectively, when the crystal precipitates were visible by optical microscopy. Sub-samples were prepared by scraping a small quantity of the bacteria off of the culture plates with a sterile loop and transferring it into a centrifuge tube containing nanopure water. After centrifugation at 3000 g for 10 minutes the supernatant was discarded and cells were resuspended in fresh nanopure water. This washing procedure was repeated three times.

For TEM analyses, a drop of resuspended subsample was pipetted onto colloidal/ carbon-coated mesh copper grids and air-dried for 1–2 hours. The samples were analyzed using a Philips CM 20, 200 KV, equipped with an EDX model EDAX system (EDAX Inc., Mahwah, NY, USA) for microanalysis. Mica disks were cleaned several times with air in a flow of dry nitrogen and a stream of He to remove the thin individual disks. A drop of subsample was placed on mica disk and was allowed to stand for 30 minutes, and air dry before imaging in air. Analytical electron microscopy microanalyses in scanning transmission electron microscopy mode were performed using a 5 nm beam diameter and a scanning area of 1000 × 20 nm.

Samples for AFM and Raman microspectroscopy were prepared by scraping off a small amount from the surface of the culture plate and resuspending aggregates consisting of bacteria, EPS and nanoglobules in DI water. The suspension was drop-coated onto a glass slide and dried under a stream of N₂ gas. For both, AFM and Raman analyses an NTEGRA Spectra Upright system from NT-MDT (Zelenograd/ Moscow, Russia) was employed. The system is fiber-optically coupled with a 532 nm diode-pumped solid-state (DPSS) laser and equipped with a white-light microscope module with CCD camera for sample observation, a photomultiplier tube (PMT) detector for registration of laser light that was back scattered by the sample and a spectrometer with four interchangeable gratings and a CCD detector (Newton, Andor, Belfast, Northern Ireland/UK) for collection of Raman spectra. All optical and spectroscopic experiments are performed by using a 100×/NA = 0.7 objective with approx. 10 mm working distance for both, focusing light onto the sample and collecting light backscattered from the sample. Within the working distance of the objective, modules for atomic force (AFM) or scanning-tunnelling microscopy (STM) can be placed between sample and objective lens. The system allows both simultaneous spectroscopy for AFM/STM and spectroscopic analyses as well as laser scanning for spectroscopic measurements. All measurements were controlled and data were analysed using NT-MDT’s Nova software. A detailed description of the system is available.

AFM images with 256 × 256 pixels on sample areas down to 500 × 500 nm² were performed in semi-contact mode using specially shaped ‘nose-type’ silicon cantilever tips. (AdvancedTEC, Nanosensors, Neuchatol, Switzerland) allowing for focusing laser light onto the tip end from top with the AFM probe in close proximity to the sample. For collecting Raman spectra on dedicated spots of these highly heterogeneous samples, the tip was placed on the sample after collection of an AFM image and the laser spot was aligned to the tip apex by using the laser-scanning mode. The tip apex was identified on both PMT images revealing the shape of the tip end and Raman images collected in laser-scanning mode by searching for the strongest Raman signal at 520 cm⁻¹. This band is assigned to the main lattice vibration of silicon, the material the AFM tip is composed of. This procedure allows collecting Raman spectra from sample structures (e.g. dense aggregates of nanoglobules) that have been identified on AFM images. Accordingly, all sample spectra presented here contain the silicon band at 520 cm⁻¹. In order to reduce its intensity, in some measurements the laser spot was moved up to 1 μm away from the tip along the tip axis, still allowing for placing the measurement spot onto dedicated structures identified in the AFM images. Raman spectra were collected with a laser power of approx. 1 mW at the sample (λ = 532 nm) with a collection time of 60 s (sum of 6 acquisitions) with a 50× objective.

For identification of siderite in these heterogeneous samples, reference spectra of siderite were collected with the same instrument using the same measurement parameters. The spectrometer calibration was checked on each measurement day by collecting spectra of a silicon wafer and diamond particles on a grinding paper. Small deviations from the ideal spectrometer calibration were corrected by moving the central pixel of the spectrum in the way that the main silicon band appeared at 520 cm⁻¹ and the most prominent diamond band at 1330 cm⁻¹. Reference spectra were collected from siderite powder taken from Somorrostro (Vicaya, Spain) (532 nm, approx. 1 mW, 60 s). The exact same position of the most prominent band
Figure 6 | Growth curve of Acidiphilium sp. PM in S-1 medium.

for siderite and a band present in all sample spectra at 1087 cm⁻¹ (Fig. 2d) confirms the presence of siderite in the bacteria samples.

In addition to the Raman bands at 530 cm⁻¹ (silicon) and 1087 cm⁻¹ (siderite) the sample spectra are dominated by a strong and spectrally broad fluorescence background and contain several additional sharp Raman bands. These features can be generally assigned to organic material (bacteria, EPS or remaining agar from the culture plates), see Figure 4b. Also, in many different organic/biological systems, a broad signal in the range of 1250–1350 cm⁻¹ can be found, which is a superposition of C-H bending and CH₂ twisting vibrations with contributions from the amide III mode⁵⁴–⁵⁶ and is represented in our case by the signal at 1289 cm⁻¹.

The vibrations of minerals is possible. The band at 1039 cm⁻¹ is probably the C-C single bond vibration corresponding to the 1577 cm⁻¹ vibration of conjugated double bond systems⁵⁵. For the sharp bands at 1125 and 1039 cm⁻¹ an assignment to both, C-C of organic material and carbonate stretching vibrations of minerals is possible. The band at 1039 cm⁻¹ represents probably an unknown/amorphous form of a carbonate mineral or represents C-C stretching vibrations of organic material. Typical bands present in the spectra of many organic molecules are, for example, various C-H bending modes adding up to a broad band at approx. 1450 cm⁻¹ (band labelled in Fig. 4b). The broad bands at 1350 cm⁻¹ and 1580 cm⁻¹ can also be due to organic material or be explained by the presence of amorphous carbon in the samples.

Geochemical modelling. The activity of dissolved species and the degree of saturation for specific minerals (saturation index, SI) in the culture medium were determined using the geochemical computer program PHREEQC version 2nd. SI is defined by SI = log (IAP/Ksp), where IAP is the ion activity product of the dissolved mineral constituents and Ksp is the solubility product of the mineral. Thus, SI < 0 implies oversaturation with respect to the mineral, whereas SI > 0 implies undersaturation.

Growth curve and Fe²⁺ measurements. Liquid cultures were carried out in 500-ml Erlemeyer flasks containing 200 ml of S-1 medium. Bacterial growth was evaluated by following changes in the optical density (OD) at a wavelength of 600 nm (Fig. 6) using a Spectronic 20 Genesys spectrophotometer. The Fe²⁺ was measured using a RQflex 10 Merck reflectoquant.
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Author contributions
M.S.R. designed the culture experiments and performed most of the laboratory tasks, carried out the culture experiments and wrote the first draft of the manuscript. P.S.M. assisted with the design and performance of the culture experiments. A.S.N. and N.R. contributed with SEM and TEM analyses. T.S. performed the AFM and Raman analyses. D.F.R., R.A., C.V. and J.A.M. assisted with the figures and text, and all authors assisted in preparing the manuscript and read and approved the final version.

Additional information
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