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RPPR Final Report
as of 08-Jul-2022

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STEM Degrees: 0 **STEM Participants:**

Major Goals: The objective of this project was to elucidate microbe-mineral interactions in rock-inhabiting microbial communities and how these interactions impact the assembly, adaptations, and activities of these communities in extreme environments.

We used a coordinated technical approach of materials science and biology to unravel the physical and chemical mechanisms that underpin the adaptations of these microorganisms. The work we accomplished can be summarized in three main areas: (1) identify key cellular networks linked to survival and sense-and-respond mechanisms of endolithic microbial communities from extreme environments, (2) determine at the physical and chemical levels key interactions between microorganisms and their rock environment for water extraction from gypsum rock, and (3) for magnetite dissolution and iron acquisition by cyanobacteria in extreme environments.

ARO's mission is to provide basic research in engineering, physical, information and life sciences, developing and exploiting innovative advances to insure the Nation's technological superiority. Our findings provide valuable insights for uncovering the evolved "design strategies" used by microorganisms to maintain their viability in the face of multiple environmental challenges. The findings may help researchers develop practical applications such as material synthesis, power generation, strategies for advanced water storage methods, and engineered living material under harsh environmental conditions, potentially enabling tactical advantages for DOD related functions

Accomplishments: 1. Identify key cellular networks linked to survival, sense-and-respond mechanisms to perturbation, and interactions between community members and with the rock substrate.

These results have been published in Ertekin et al. 2021. https://sfamjournals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.15287

2. Determine at the physical and chemical levels key interactions between microorganisms and their rock environment: water extraction from gypsum rock

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3. Determine at the physical and chemical levels key interactions between microorganisms and their rock

environment: magnetite dissolution and iron acquisition.

A manuscript is in preparation with the results presented above (Huang et al., 2022).

See pdf file for full text and figures.

Training Opportunities: Four postdoctoral fellows were trained in material sciences (Wei Huang, T. Wang) and in molecular microbiology (Emine Ertekin and Cesar Perez-Fernandez).

Results Dissemination: Huang W., Wang T., Perez-Fernandez C., DiRuggiero J., and D. Kisailus. Biogeochemical landscaping: iron acquisition and mineral modification by cyanobacteria from extreme environments. Submitted.

Ertekin E., V. Meslier, A. Browning, J. Treadgold, and J. DiRuggiero. 2021. Functional and taxonomic diversity is driven by substrate architecture in endolithic communities from extreme environments. Env. Microbiol. 23: 3937–3956 PubMed PMID: 33078515.

Huang W., E. Ertekin, T. Wang, L. Cruz, M. Dailey, J. DiRuggiero and D. Kisailus. 2020. Mechanism of water extraction from gypsum rock by desert colonizing microorganisms. Proc Natl Acad U.S.A. 117: 10681-10687 PubMed PMID: 32366642. In Faculty Opinions, 15 Sep 2020; 10.3410/f.737901485.793578113.

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Authors: Huang W., E. Ertekin, T. Wang, L. Cruz, M. Dailey, J. DiRuggiero and D. Kisailus
Keywords: microorganisms | water extraction | anhydrite | gypsum | phase transformation
Abstract: Endolithic (rock-dwelling) microbial communities are ubiquitous in hyper-arid deserts around the world and the last resort for life under extreme aridity. These communities are excellent models to explore biotic and abiotic drivers of diversity because they are of low complexity. Here, we investigated how water availability and substrate architecture modulate the taxonomic and functional composition of gypsum endolithic communities in the Atacama Desert, Chile. We found that communities inhabiting gypsum rocks with a more fragmented substrate architecture had higher taxonomic and functional diversity, despite having less water available. This effect was tightly linked with community connectedness and likely the result of niche differentiation. Gypsum communities were functionally similar, yet adapted to their unique micro-habitats by modulating their carbon and energy acquisition strategies and their growth modalities. Reconstructed population genome
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Authors: Emine Ertekin, Victoria Meslier, Alyssa Browning, John Treadgold, and Jocelyne DiRuggiero
Keywords: extremophiles, endolithics, microbial communities, genome-resolved metagenomics, gypsum, Atacama Desert
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Partners

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Final Technical Progress Report for ARO grant # W911NF-18-1-0253

Adaptive mechanisms and substrate interactions of microbial communities in dry extremes

J. DiRuggiero (PI), D. Kisailus (co-I)

The objective of this project was to elucidate microbe-mineral interactions in rock-inhabiting microbial communities and how these interactions impact the assembly, adaptations, and activities of these communities in extreme environments.

We used a coordinated technical approach of materials science and biology to unravel the physical and chemical mechanisms that underpin the adaptations of these microorganisms. The work we accomplished can be summarized in three main areas: (1) identify key cellular networks linked to survival and sense-and-respond mechanisms of endolithic microbial communities from extreme environments, (2) determine at the physical and chemical levels key interactions between microorganisms and their rock environment for water extraction from gypsum rock, and (3) for magnetite dissolution and iron acquisition by cyanobacteria in extreme environments.

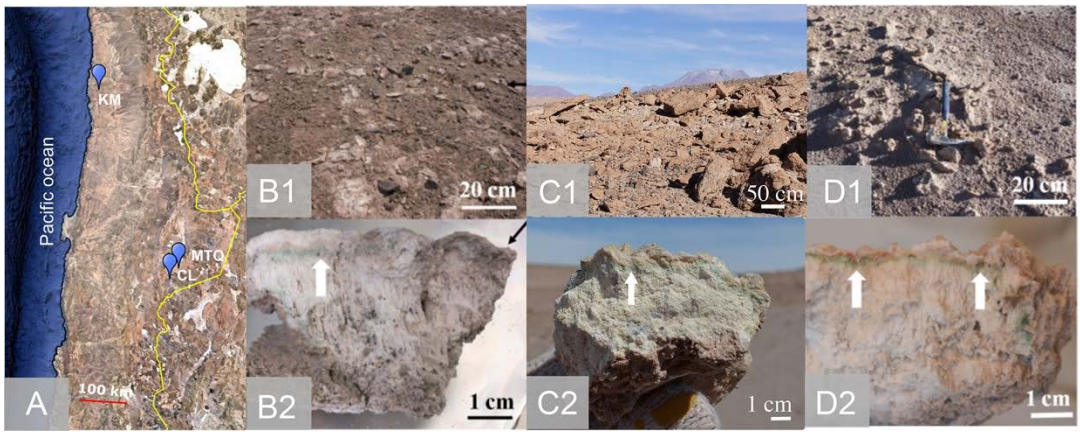

Figure 1. Sampling locations in the Atacama Desert and cross sections of gypsum samples. (A) Map of the Atacama Desert in Chile with the three sampling locations marked in blue: KM (KM37), CL (Cordon de Lila) and MTQ (Monturaqui). Field view of sampling sites at KM (B1), CL (C1) and MTQ (D1). Cross sections of gypsum samples from KM (B2) CL (C2) and MTQ (D2). White arrows show orange and green cryptoendolithic colonization zones.

ARO's mission is to provide basic research in engineering, physical, information and life sciences, developing and exploiting innovative advances to insure the Nation's technological superiority. Our findings provide valuable insights for uncovering the evolved "design strategies" used by microorganisms to maintain their viability in the face of multiple environmental challenges. The findings may help researchers develop practical applications such as material synthesis, power generation, strategies for advanced water storage methods, and engineered living material under harsh environmental conditions, potentially enabling tactical advantages for DOD related functions

1. Identify key cellular networks linked to survival, sense-and-respond mechanisms to perturbation, and interactions between community members and with the rock substrate

Endolithic (rock-dwelling) microbial communities are ubiquitous in hyper-arid deserts around the world and the last resort for life under extreme aridity. These communities are excellent models

to explore biotic and abiotic drivers of diversity because they are of low complexity. We investigated how water availability and substrate architecture modulate the taxonomic and functional composition of gypsum endolithic communities in the Atacama Desert, Chile. We collected gypsum samples from 3 locations (KM, CL, and MTQ) characterized by different geographies and climate regimes (KM and CL/MTQ) (Fig. 1). Using computer tomography scanning (CT-scan), we showed that the KM gypsum architecture presented large pores and spaces connected to each other and to the surface of the substrate (Fig. 2A1 and A2). When combined with higher water availability, this architecture allowed for better water retention, the mixing of soluble ions and nutrients, and increased microbial interactions; this led to high homogeneity of the micro-environment and ultimately lower microbial diversity. In contrast, the CL and MTQ gypsum architecture had small pores, often disconnected from each other and from the surface, hindering water and nutrient circulation; when combined with higher aridity, this architecture produced a more heterogeneous microenvironment composed of a multitude of micro-niches leading to more diverse communities (Fig. 2B1 and B2).

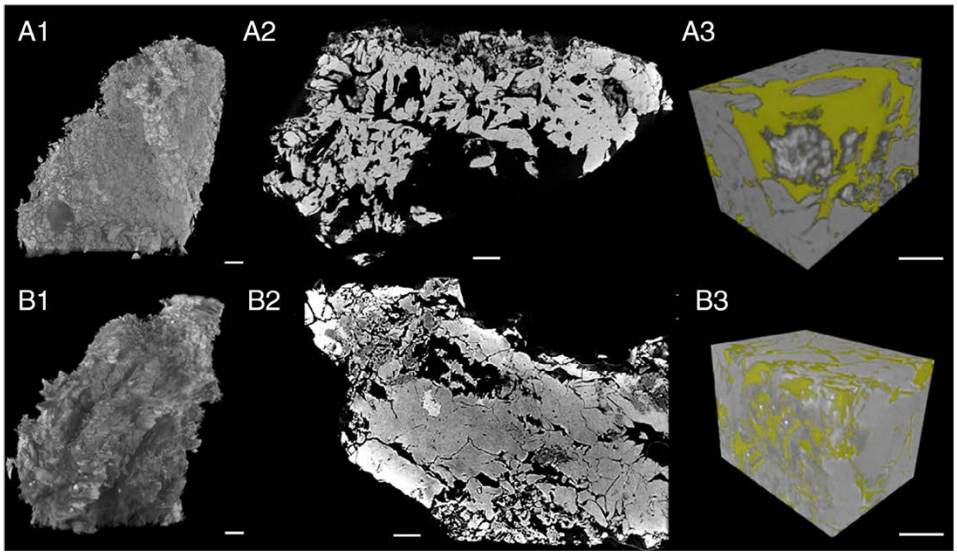

Figure 2. Computed tomography scan images of gypsum from KM and CL. Full field imaging and three-dimensional (3D) rendered volumes of gypsum from KM (A) and CL (B). 3D full field imaging of gypsum from KM obtained at 2 μm voxel resolution (A1) and gypsum from CL at 1.35 μm voxel resolution (B1). Two-dimensional reconstructed slices showing the structure of the gypsum from KM (A2) and CL (B2). Examples of 3Drendered rectangles used to calculate the available space for colonization (yellow) in gypsum from KM (A3) and from CL (B3). Scale bars indicate 100 μm.

These findings were validated using next-generation sequencing, where we found that communities inhabiting gypsum rocks with a more fragmented substrate architecture had higher taxonomic diversity, despite having less water available. This effect was tightly linked with community connectedness and was likely the result of niche differentiation. The large differences in the “habitable space” of gypsum from KM and CL-MTQ were also linked to different functional adaptations between these communities (Fig. 3A). The differentially abundant metabolic functions between KM and CL-MTQ provided insights into how the inhabiting community members adapted to their unique micro-habitats. The CL and MTQ communities were enriched in genes involved in the metabolism of saccharides, such as galactose and mannose, amino-sugars and nucleotide sugars, all compounds typically found in extracellular polymeric substances (EPSs). When enough water is available, according to the energy reserve hypothesis, EPSs are then released and potentially used as nutrient sources by heterotrophic bacteria. The presence of multiple sugar

transporters and lipopolysaccharide exporters present in the CL-MTQ communities, with high numbers of extracellular hydrolytic enzymes for the degradation of amino acids, lipids, and extracellular nucleic acids, would indeed be consistent with this hypothesis. In contrast, the KM community, and *Actinobacteria* MAGs recovered from this community, were significantly enriched in genes encoding group V [NiFe]-hydrogenases, potentially involved in hydrogen oxidation (Fig. 3B). In hyper-arid and oligotrophic ecosystems, [NiFe]-hydrogenases are thought to play an essential role in overcoming carbon and nutrient starvation. Previous studies and our findings of group V [NiFe]-hydrogenases in our metagenomes, strongly indicated that gypsum *Actinobacteria* potentially used hydrogen oxidation as a supplemental energy source.

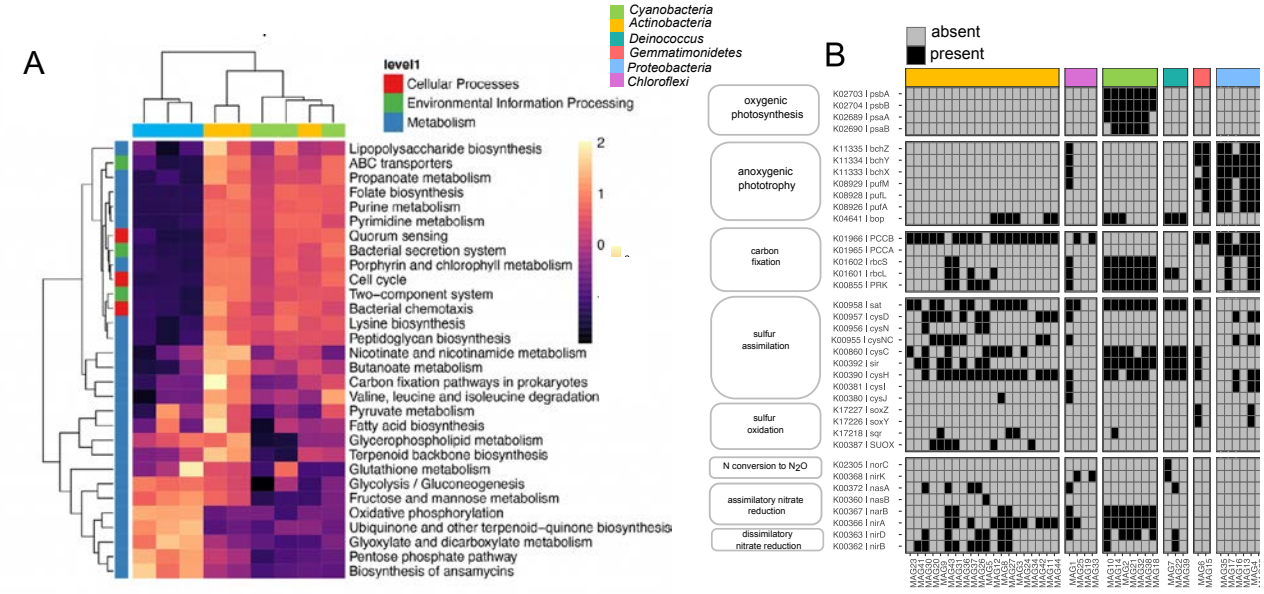

Figure 3. Functional potential of gypsum communities. (A) Hierarchical clustering of the 30 most abundant KEGG pathways (KEGG level 3) represented by the differentially abundant KOs (FDR < 0.05) using Euclidean distance and complete-linkage clustering. Color scale show pathway abundances normalized according to z-scores. Row names denote level 3 pathway annotations and color code on the left maps the pathways to their level 1 metabolic classification. (B) Presence/ absence of genes encoding key enzymes of carbon and energy pathways in MAGs. The JGI annotation pipeline and the KEGG database were used for gene annotations. MAG numbers are at the bottom and color coded phyla are at the top. The y axis show KEGG orthologs (KO) numbers and enzyme names; putative carbon and energy pathways are shown in boxes.

Our analysis of MAGs uncovered that these population genomes harbored a more diverse metabolic potential compared to those previously reported from desert endolithic habitats (Fig. 3B). For example, in addition to oxygenic photosynthesis by *Cyanobacteria*, several *Proteobacteria*, *Gemmatimonidetes* and *Chloroflexi* MAGs harbored genes involved in anoxygenic phototrophy (AP). Some of the *Chloroflexi* and *Proteobacteria* MAGs encoding AP genes, along with two *Actinobacteria* MAGs, also encoded type I RuBisCO and phosphoribulokinase, suggesting that these microorganisms were potentially capable of autotrophy.

We identified a diverse array of secondary metabolite clusters in gypsum communities, comprised mainly of non-ribosomal peptides and polyketides (Fig. 4). Homology analysis predicted that these biosynthetic clusters encoded for antimicrobials such as anabaenopeptins, nostopeptins, rhizomides, barbamides and hapalosins. This was highly reminiscent of previous findings in the

Atacama Desert, where ignimbrite communities colonizing the small pores of this substrate encoded a significantly higher number of genes related to antimicrobial compounds than those colonizing calcite, a substrate with large cracks and fissures . Similarly, the CL-MTQ gypsum, where secondary metabolite clusters were enriched closed to two-folds compared to the KM gypsum, might also be the stage of intense microbial warfare over the colonization of small pores.

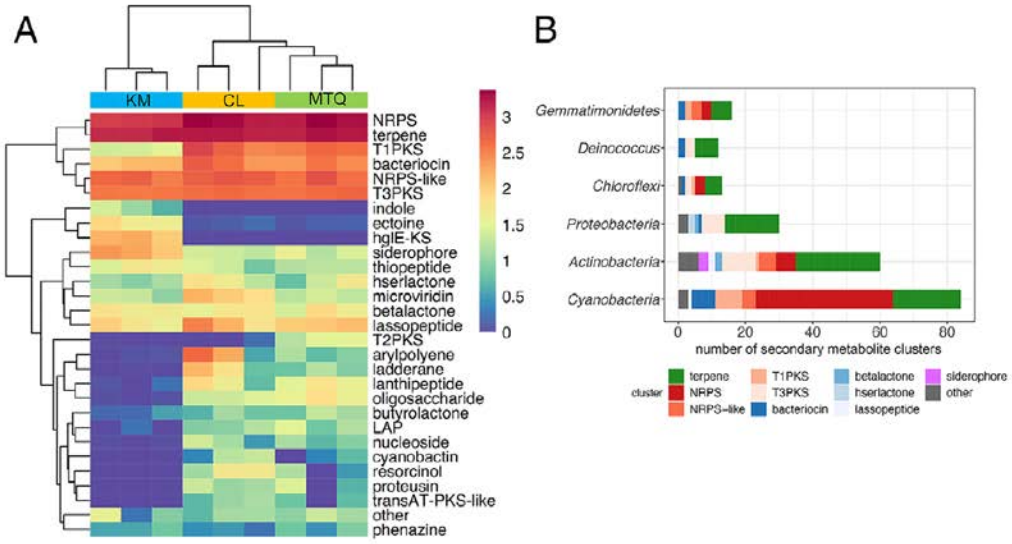

Figure 4. Biosynthetic clusters in gypsum communities identified by annotating contigs with AntiSMASH. (A) Heatmap representing hierarchical clustering of biosynthetic cluster relative abundances across samples using Euclidean distance and complete-linkage clustering. Columns are clustered by samples and rows by cluster types. Color scale indicate log10 normalized abundances. (B) Composition of the most abundant secondary metabolite clusters identified in MAGs and classified by phylum.

Our in-depth analysis of gypsum communities from the Atacama Desert revealed reconstructed population genomes that were potentially more versatile metabolically than previously reported for endolithic communities, with functional potential for anoxygenic phototrophy and atmospheric H_2 oxidation. Using 3D X-ray microscopy, we showed that a higher taxonomic and functional diversity in the community was linked to the more fragmented architecture of its substrate, despite lower water availability. This effect was tightly linked with community connectedness and was likely the result of niche differentiation. These findings emphasize the need for more characterization of the micro-environment inhabited by microorganisms, whether in rocks, soils, or any other type of substrate. While molecular tools have given us the power to investigate microbial communities in great detail, more efforts are needed to explore further the molecular level interactions between microorganisms and the substrates they inhabit.

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https://sfamjournals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.15287

2. Determine at the physical and chemical levels key interactions between microorganisms and their rock environment: water extraction from gypsum rock

Water plays many roles in organismal function: it is not only critical for metabolic processes but also acts as a structural component in materials and tissues. In the hyper-arid desert of the

Atacama, endolithic microbial communities inhabiting gypsum substrates are subjected to severe xeric stress. We use a combination of microscopy and spectroscopy to characterize gypsum samples and their inhabitants from both geological and laboratory environments, revealing the processes by which colonizing microorganisms obtain water from their substrate and the resulting effect on the rock. We report that the cyanobacteria attached onto high surface energy crystal planes ({011}) of gypsum samples generate a thin biofilm that induced mineral dissolution accompanied by water extraction. This process led to a phase transformation to an anhydrous calcium sulfate, anhydrite, which was formed via reprecipitation and subsequent attachment and alignment of nanocrystals.

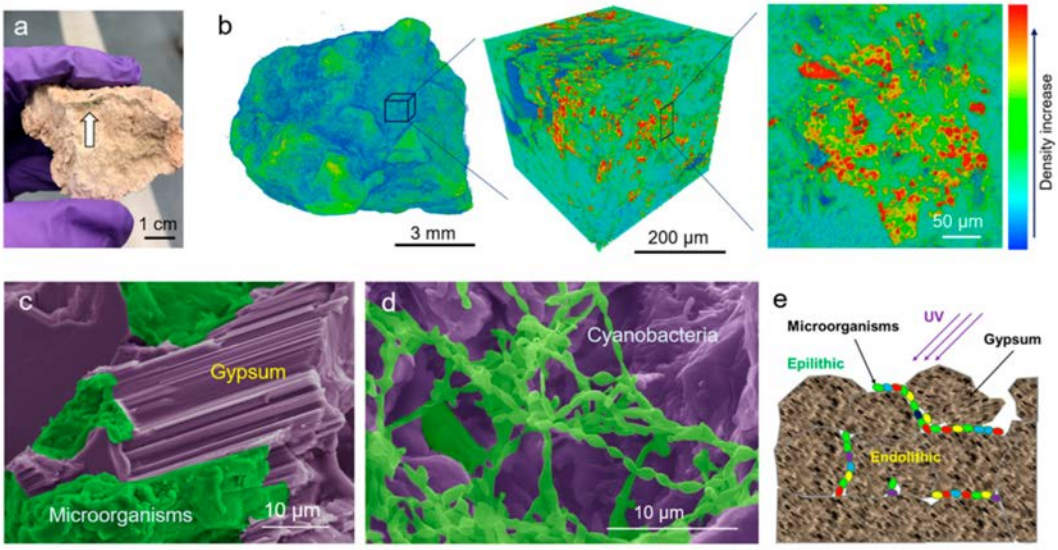

Figure 5. Microorganisms live within gypsum rocks from the Atacama Desert. (A) Photo of gypsum rock samples. The green color indicated with a white arrow shows the area colonized by microorganisms. (B) µ-CT images of gypsum rocks, highlighting the microorganisms living within. The yellow and red colors represent microorganism colonies inside the rock. (C and D) SEM images of gypsum. The extracellular matrix surrounding cyanobacteria cells in gypsum samples is indicated in green in D. (E) Diagram of the microbe colonization and their location in the gypsum rock. UV, ultraviolet.

The chemical composition of gypsum rocks collected in the Atacama Desert and interactions between microorganisms and the rock environment were examined by scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy and synchrotron micro X-ray fluorescence microscopy (µXRF) (Fig. 5). Micro-computed tomography (µ-CT) images (Fig. 5B) uncovered microbial colonies inside the cracks and fissures of the rock. Further observations provided by SEM (Figs. 5C and D) showed that the microorganisms had a preferable attachment to specific crystal facets of gypsum. Raman spectroscopy and mapping and SEM confirmed that the microbial cells assembled primarily on the {011} planes of gypsum. To further investigate the microbe−substrate interface, we applied a combination of elemental and structural analyses to the colonized gypsum rocks. Our results indicated that the microorganisms are likely responsible for the transformation of gypsum to the anhydrite phase. In previous studies, it has been shown that gypsum can transform to anhydrite by losing its water of crystallization when annealing at 440 K. Thus, it is plausible that microorganisms can also drive this transformation by extracting the water they require for survival. To test this hypothesis, culture experiments were performed in a laboratory under controlled conditions.

We conducted a "reconstructed" reactor experiment with gypsum coupons (0.5 × 0.8 × 0.5 mm average size pieces of gypsum rocks) and cultures of a cyanobacterium previously isolated from gypsum rocks to test the following hypotheses: (i) microorganisms are specifically associated with the mineral sepiolite in gypsum rocks and (ii) microorganisms can obtain water from gypsum under extremely dry conditions (Fig. 6A). At a 30-d incubation period, cells on, and within, the gypsum coupons had a bright green color, indicating the presence of photosynthetic pigments (Fig. 6B). The presence and distribution of the cyanobacteria within the substrate were further validated by the coexistence of carbon and nitrogen, by EDS mapping (Fig. 6C) and SEM imaging (Fig. 6D).

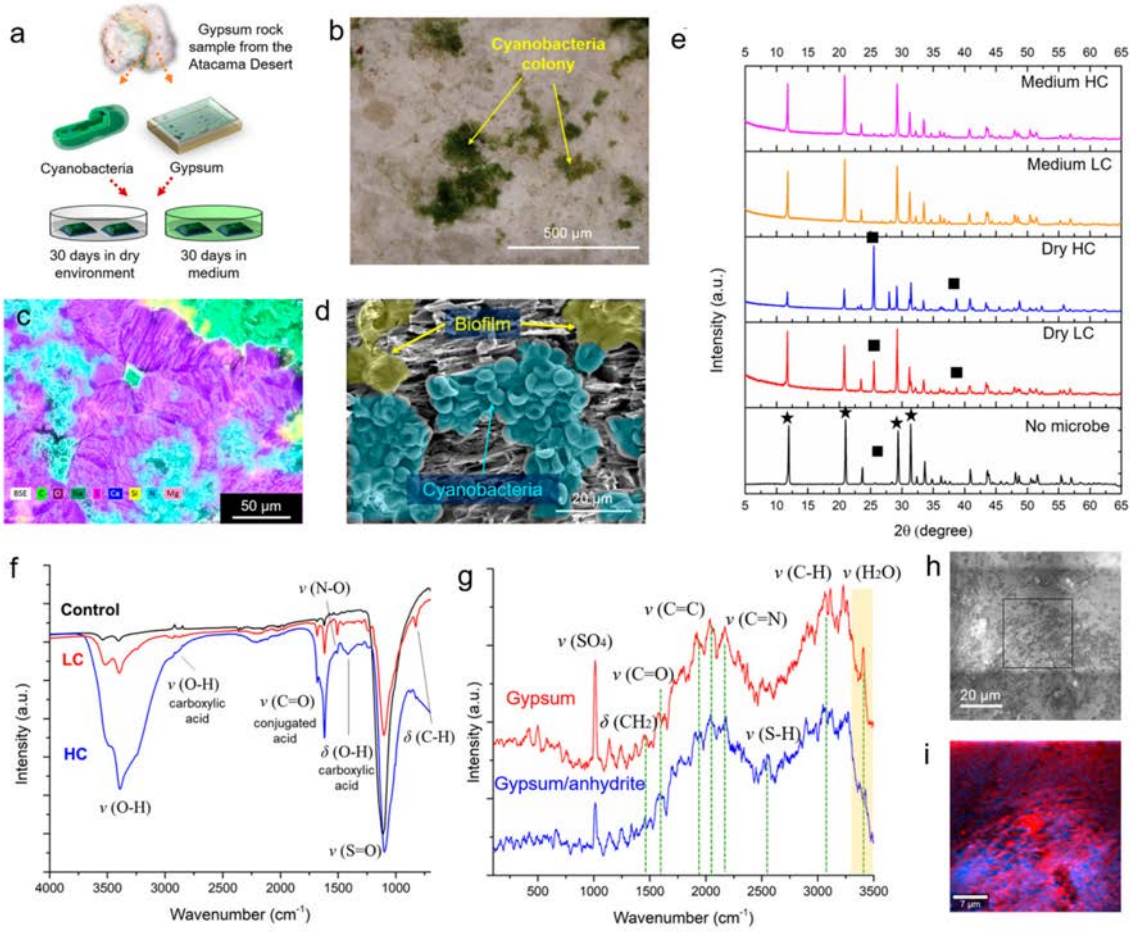

Figure 6. Cyanobacteria culture on gypsum samples. (A) Schematic of cyanobacteria cultured in dry and liquid media. (B) Optical microscopy image of gypsum samples showing colony of cyanobacteria (green color) on the gypsum after culture experiments. (C) EDS mapping of cyanobacteria cultured on the gypsum. (D) SEM images of gypsum sample after culture, showing a porous structure and attachment of cyanobacteria (green color) on the surface. Biofilm is found surrounding the cyanobacteria. (E) XRD of gypsum rock control (black curve; i.e., not exposed to microbes), samples cultured in low concentration (LC) and high concentration (HC) of cyanobacteria in both dry and liquid medium environments. Diffraction peaks labeled with black squares represent the anhydrite phase, while those labeled with stars are from gypsum. (F) FTIR of samples in the control group and the cyanobacteria cultures at low and high concentrations. Specific absorption bands, representing organic acids, are found on the surfaces of the gypsum samples with cyanobacteria cultures. (G) Raman spectroscopy of gypsum samples cultured with a high concentration of cyanobacteria. Both gypsum and anhydrite are detected on the sample surface.

Red and blue spectra represent two different areas indicated in I. Absorption from water in gypsum is marked with a yellow band. (H and I) Mapping of gypsum and anhydrite phases from Raman spectroscopy. (H) Optical micrograph shows the mapping area (black box) used in I. (I) Gypsum (red) and anhydrite (blue) phase map.

XRD reveals that anhydrite was present in gypsum coupons cultured in “dry conditions,” but was not found in gypsum without microorganisms (negative control) or those cultured under hydrated conditions (i.e., in a liquid medium; Fig. 6E). This suggests that the “dry conditions” promoted the extraction of water by cyanobacteria from the gypsum rock, leading to its transformation to anhydrite. FTIR analysis of gypsum coupons cultured with high concentrations of cyanobacteria showed significantly more intense absorption bands for C=O and O−H, indicating the existence of higher concentrations of organic material (Fig. 6F). The presence of organic acids in the biofilm surrounding the cyanobacteria cells was also confirmed by Raman spectroscopy (Fig. 6G) and Raman maps highlighted the phase transformation from gypsum to anhydrite (Fig. 6H, pure gypsum and Fig. 6I, a mixture of gypsum and anhydrite). We suggested that the organic acids in the biofilm reacted and etched the gypsum rock, releasing water in its lattice to the cyanobacteria. As the bacteria grow, they produce more organic acid and thus extract additional water that induces further transformation of gypsum.

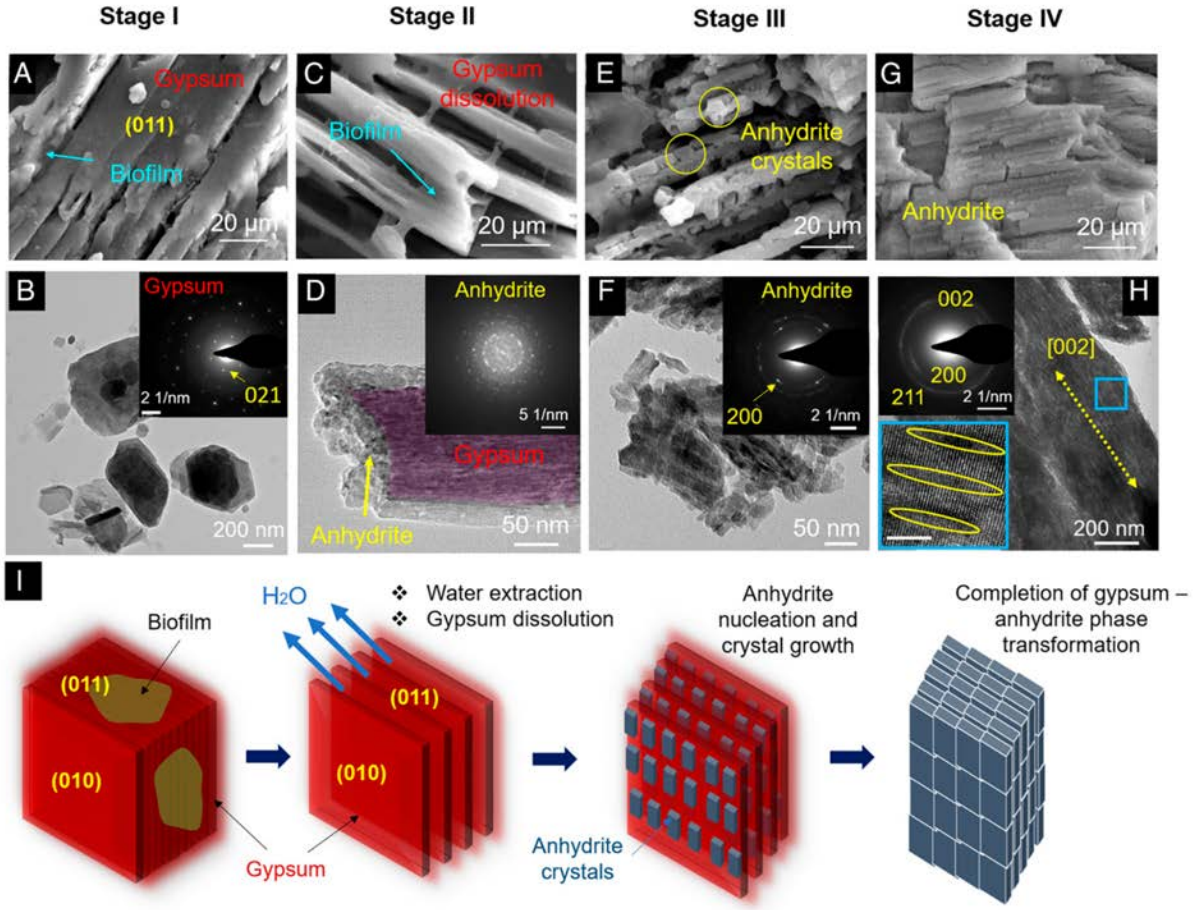

Figure 7. Mechanisms of gypsum−anhydrite phase transformation. The process is described in four stages. Stage I: microorganisms attach on the gypsum crystals and form a biofilm. (A and B) SEM and TEM images highlight the gypsum crystals. Confirmation of single crystalline gypsum provided through TEM and SAED (Inset) is shown in B. Stage II: (C and D) gypsum dissolution and water extraction with subsequent anhydrite

nanocrystal precipitation. (C) A porous structure is observed at the periphery of the gypsum particles, indicating their dissolution. (D) Anhydrite nucleation on the surface of gypsum crystals. SAED analysis (Inset) provides evidence for the random distribution of the anhydrite nanocrystals. Stage III: (E and F) Anhydrite crystal growth. (E) SEM image shows large, faceted, anhydrite particles on the surface of gypsum. (F) Bright-field TEM demonstrates the short-range alignment of anhydrite nanocrystals, suggesting particle attachment. The SAED pattern (Inset) indicates alignment of the nanocrystals during the attachment process. Stage IV: (G and H) Completion of the gypsum-anhydrite phase transformation. (G) SEM image of anhydrite particles, highlighting surface faceting. (H) Bright-field TEM image and SAED indicate a preferential alignment along the [002] direction. The blue box (Inset) shows the interfaces between nanocrystals observed through HRTEM, indicated with yellow circles. (Scale bar, 5 nm.) (I) Summary and schematic of microorganism induced gypsum–anhydrite phase transformation. Microorganisms attach and form biofilms on the {011} planes of gypsum crystals; gypsum dissolves, and water extraction occurs. Based on the crystal structure of gypsum, the water of crystallization layer is exposed to the {011} planes, but not to the {010} planes. As the single crystalline gypsum dissolves and loses water of crystallization, it transforms via precipitation of nanocrystalline anhydrite. These anhydrite nanocrystals precipitate on the surfaces of gypsum crystals. Shortrange alignment of the anhydrite nanocrystals is observed. Large micrometer-sized anhydrite crystals are formed via particle attachment and alignment.

Based on our observations, we describe the gypsum–anhydrite phase transformation in four sequential stages (Fig. 7). During stage I, microorganisms attach and form a biofilm onto {011} planes of gypsum particles (Fig. 7A). The biofilm that is coating the gypsum (Figs. 7A and C) contains organic acids that induce mineral dissolution, enabling the extraction of water that can be acquired by the microorganisms. High-resolution TEM (HRTEM) imaging and selected area electron diffraction (SAED) (Figs. 7D and H, Inset, respectively) showed anhydrite nanocrystals precipitated randomly near the surface of the dissolving gypsum, which suggests that a gypsum−anhydrite phase transformation is occurring. Upon losing the water of crystallization, the monoclinic gypsum crystals become unstable and transform to orthorhombic anhydrite crystals. The relatively insoluble anhydrite (i.e., under acidic conditions) subsequently precipitates as anhydrite nanocrystals near the surfaces of gypsum. As additional anhydrite is formed, these “primary” nanocrystals attach, via short-range alignment, to form hierarchically assembled mesocrystals (Figs. E and F). Additional assembly (Figs. 7 G and H) of these primary particles yields larger anhydrite particles. A long-range alignment of these nanocrystals along the [002] direction is observed (Fig. 7H). This growth mechanism is different from classical crystal growth pathways, which typically occur via monomer-by-monomer addition. This oriented attachment of primary particles provides a means to reduce the free energy of the system without Ostwald ripening, yielding larger crystals. The surfaces of the final anhydrite crystals are rough, highlighted by numerous interfaces from the nanocrystals.

The findings in this study shed light on how microorganisms can obtain water under severe xeric conditions, but also provide insights into potential life in even more extreme environments, such as Mars, as well as offer strategies for advanced water storage methods.

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3. Determine at the physical and chemical levels key interactions between microorganisms and their rock environment: magnetite dissolution and iron acquisition.

Iron is one of the most critical trace elements for the survival of many life forms, including cyanobacteria. Although iron is in relatively high abundance, its accessibility for microorganisms is low because it is found primarily as insoluble ferric iron (Fe^{3+}) in oxide minerals such as hematite, magnetite, and siderite. Pathways by which ferric iron is extracted from minerals at neutral pH in the presence of bacteria and the interactions between bacteria and iron-based mineral surfaces remain unclear. In this study, we identified magnetite and hematite phases in gypsum rocks collected in the Atacama Desert. Using synthesized magnetite nanoparticles, we showed that in iron-depleted conditions, cyanobacteria isolated from gypsum rocks were able to extract iron from the magnetite solid phase. Dissolution of the solid magnetite phase was associated with magnetite to hematite phase transformation.

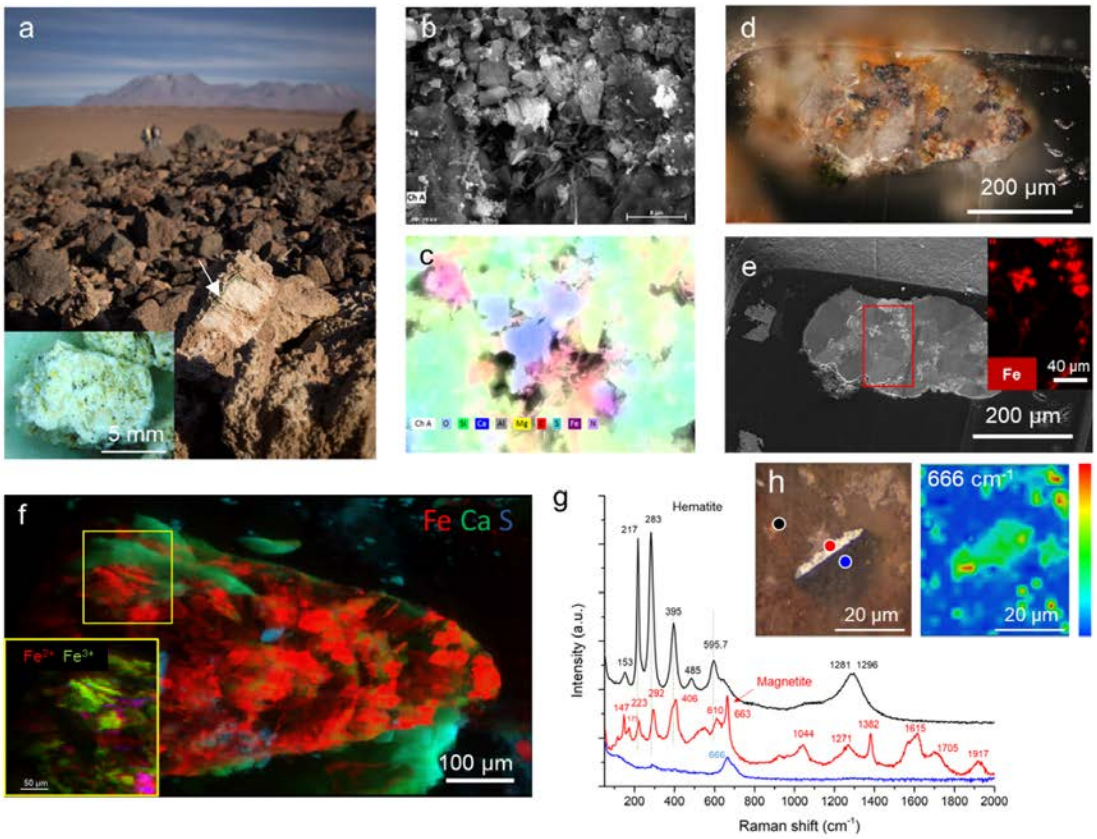

Figure 8. Iron minerals in gypsum rocks collected from the Atacama Desert. (a) Field photo of the Atacama Desert with the cross-section of one rock showing the colonization zone (white arrow). Inset photo: rocks collected from the desert. (b, c) SEM micrograph of particles in (a). The EDS mapping showed the distribution of iron and organic microorganisms. (d) Optical microscopy image of black and yellow particles found in gypsum rock. (e) SEM micrograph of the polished surface and EDS mapping of iron. (f) Synchrotron micro X-ray fluorescence microscopy (µXRF) of gypsum rock. The distribution of iron, calcium, and sulfur are indicated. The inset yellow box shows the distribution of iron with different valences. Both ferric and ferrous iron were observed. (g, h) Raman spectroscopy and mapping of the mineral sample, indicating the existence of magnetite and hematite phases.

A green colonization zone in gypsum rocks collected from the Atacama Desert was observed within ~ 1.5 mm from the surface of the rock. Black and orange-colored particles were also observed within these rocks, indicating the presence of iron minerals (inset, Fig. 8a). SEM revealed bacterial colonies within the colonization zone of the rocky substrate (Fig. 8b) and EDX mapping (Fig. 1c) of the same area provided the elemental distribution of the rock samples in

which the organic content of the bacterial colonies was observed by the C, S, N, and iron (Fe) content. The presence of iron in the black particles (Fig. 8d) was further confirmed via SEM/EDX mapping (Fig. 8e). Based on the color and presence of iron, the observed black particles within the gypsum rocks were likely magnetite. We confirmed the presence of magnetite by synchrotron X-ray fluorescence microscopy (μXRF) and X-ray absorption near edge structure (XANES) analyses of the element distribution and phases of minerals at the microscale (Fig. 8f). Additional analyses of these minerals by powder and wide-angle X-ray diffraction highlighted both magnetite and hematite phases. The occurrence of the magnetite and hematite phases was confirmed by Raman spectroscopy patterns (Fig. 8g) and microscopic mapping (Fig. 8h). These results indicated the presence of microorganisms, such as cyanobacteria, around iron-based minerals with magnetite and hematite phases within the gypsum substrate.

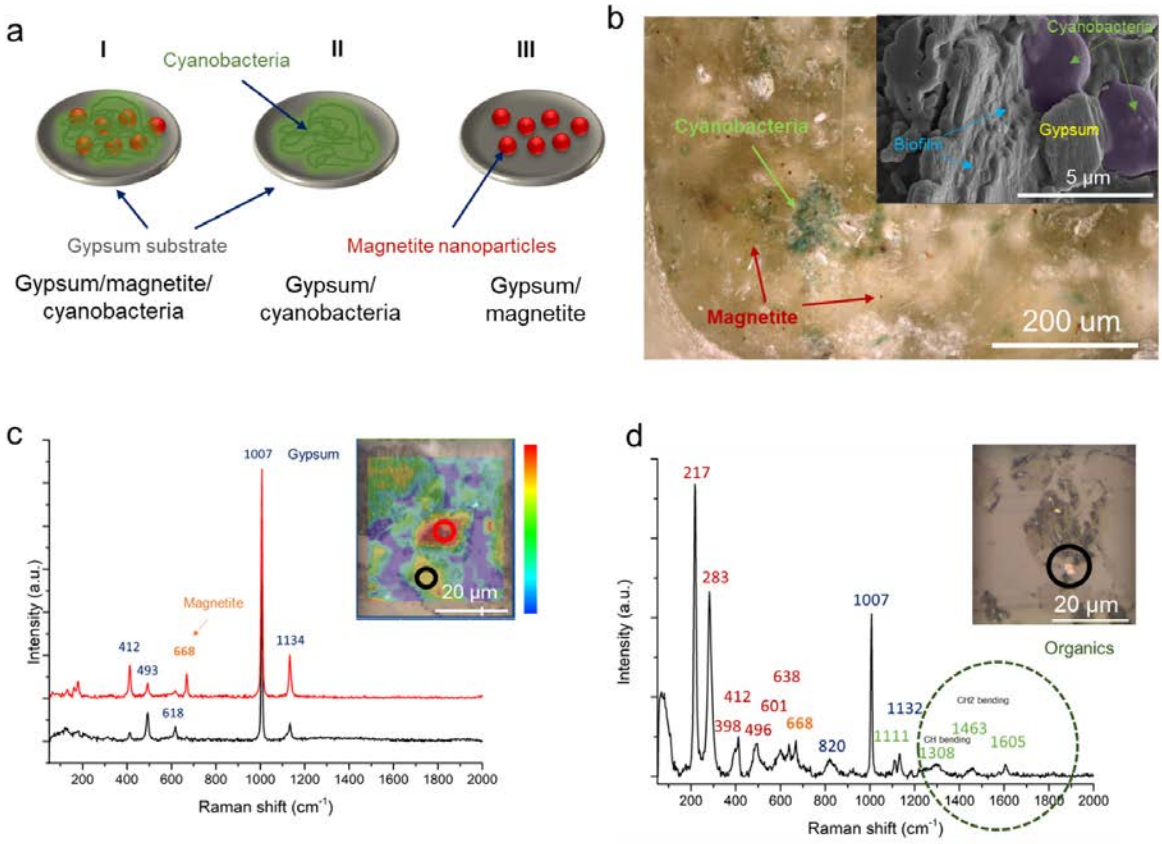

Figure 9. Experiments of cyanobacteria cultured on gypsum substrate with magnetite nanoparticles. (a) The 3 experimental groups were: I) gypsum substrate with embedded magnetite nanoparticles and with cyanobacteria cultures, II) gypsum substrate without magnetite nanoparticles and with cyanobacteria cultures, and III) control samples of gypsum/magnetite substrate without cyanobacteria. (b) Optical micrograph showed the cyanobacteria and magnetite nanoparticles in the substrate. Inset: SEM image shows the cyanobacteria attached to the gypsum substrate within a biofilm. (c) Raman spectroscopy of sample (III). The inset image shows the distribution of magnetite. (d) Raman spectroscopy of sample (I). Hematite and magnetite phases are observed. Organics were also observed.

To understand the interactions between cyanobacteria and iron minerals, cyanobacteria were cultured with gypsum substrate, with and without synthesized magnetite nanoparticles embedded, in three experimental groups (Fig. 9a). Examination of the surface of sample I indicated the presence of green cyanobacteria colonies within the biofilm attached to the substrate after 21 days of culture (Fig. 9b). Raman spectroscopy and mapping showed hematite phases in sample I while pure magnetite was found in the control sample III (Figs. 9c, d). The magnetite phase in sample III was confirmed with high-resolution transmission electron microscopy (HRTEM) imaging (Figs. 10a-d). The general size of the nanoparticles in sample III was ~15.8 ± 3.1 nm, while nanoparticles in sample I were significantly smaller in size (~ 7.1 ± 1.1 nm).

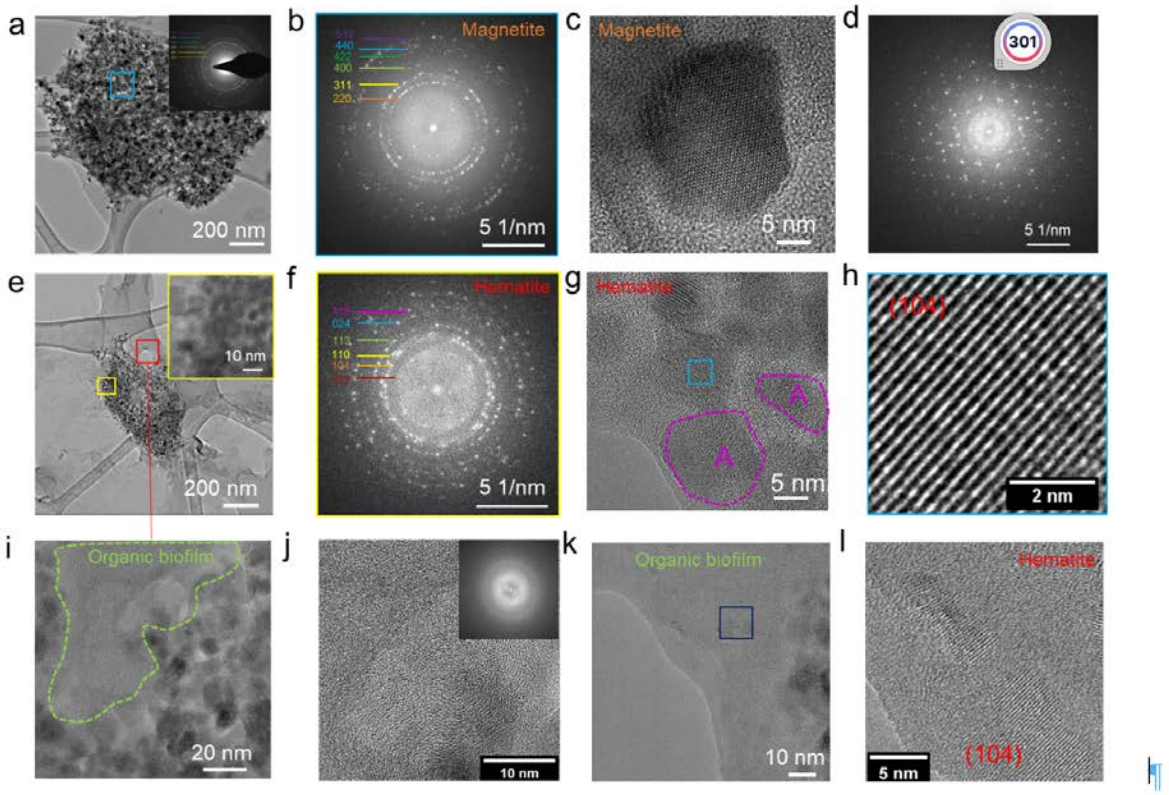

Figure 10. HRTEM of magnetite nanoparticles after cyanobacteria culture. (a) Magnetite nanoparticle aggregates in the substrates without cyanobacteria culture. Inset SAED pattern indicates magnetite phase. (b) FFT of sample from the blue box in (a). (c, d) HRTEM image of a single magnetite nanoparticle in (a). FFT of the nanoparticle shown in (d). (e) Magnetite nanoparticle aggregates in the substrates with cyanobacteria culture. (f) FFT of sample from the yellow box in (e), indicating the existence of hematite phases. (g, h) HRTEM of the hematite nanoparticles in (e). (I, j) HRTEM of the areas surrounding the nanoparticle aggregates in (a, red box). Amorphous organic phases are observed. (k, l) Hematite nanocrystals are noticed in the organic phases.

Fast Fourier Transformation (FFT) of the nanoparticles confirmed the presence of the hematite phase after the culturing of cyanobacteria (Fig. 10f). HRTEM images of the hematite nanoparticles showed both crystalline and amorphous phases (Figs. 10g and h). HRTEM images and FFT pattern (Figs. 10i and j) showed the amorphous organics surrounding the nanoparticles. In the biofilms, small hematite nanocrystals were found, indicating the dissolution of iron mineral nanoparticles within the observed organic biofilms (Figs. 10k and l).

Microbial growth and siderophore production were also measured in liquid media supplemented with magnetite nanoparticles, providing enough material for accurate measurements in contrast to gypsum coupons. Different groups of experiments were performed as described in Table 1. Chlorophyll *a* concentration in the cultures, as a proxy for cellular abundance, revealed that the samples cultured in a medium with magnetite nanoparticles had a larger amount of cyanobacteria than samples without magnetite (Table 1). The production of siderophores was also higher in the iron-depleted media compared to the iron-containing medium. Thus, it is reasonable to conclude that cyanobacteria in the surrounding biofilms were able to dissolve the solid magnetite minerals, producing siderophores that could chelate ferric ions and transport iron within the cells. The remaining magnetite nanoparticles subsequently transformed to hematite phases.

Table 1. Chlorophyll *a* content and siderophore production in G-MTQ-3P2 cyanobacteria cultures in different media after 21 day-incubation.

Experiment group	Chlorophyll a content (µg/ml)	Siderophore content (µM DFOM equivalents)
(I) Cyanobacteria + iron depleted medium	1.62 ± 0.13	9.4 ± 1.1
(II) Cyanobacteria + iron depleted medium + nanoparticles	3.65 ± 0.20	10.4 ± 1.3
(III) Iron depleted medium	na	na
(IV) Cyanobacteria + iron-containing medium	2.01 ± 0.33	4.8 ± 1.3

To further investigate the mineral dissolution in presence of cyanobacteria, we embedded bulk magnetite crystals in epoxy and polished them to expose surfaces. Raman spectroscopy and mapping confirmed that pure magnetite was exposed to the sample surfaces. These coupons were then subjected to cyanobacteria cultures in iron-depleted and in control media (Fig. 11a).

Raman spectroscopy and mapping revealed large areas of hematite phase developing during the culture experiments (Fig. 11, sample II), while the hematite phase was barely visible in the control sample (Fig. 11, sample I). In sample II, the SEM images showed cyanobacteria colonies attached to the surface of magnetite crystals with groves and cracks indicating the dissolution of the magnetite. In contrast, in cultures with sufficient iron (control medium) almost no hematite phase was observed (Fig. 11, samples III and IV).

Based on our experimental results, we proposed that, under iron-depleted conditions, cyanobacteria were able to acquire iron ions by dissolving the magnetite crystals and uptalking the ions via siderophore chelation. We observed the dissolution of nanoparticles and large magnetite crystal surfaces in the presence of cyanobacteria biofilms, possibly as the result of local low pH within the biofilm (Figs. 10 and 11). The dissolution of magnetite in acidic environment has previously been investigated and can be described as:

$$[Fe_2^{3+}Fe^{2+}]O_{4\,(Magnetite)} + 8H^+ \rightarrow Fe^{2+} + 2Fe^{3+} + 4H_2O$$

The consumption of ferric iron (Fe^{3+}) by the cyanobacteria during photosynthesis could drive the reaction kinetics, leading to the progress of dissolution reactions. Our results also indicated that

the magnetite to hematite phase transformation only occurred with the dissolution of magnetite. During photosynthesis, oxygen is released and can be used in the following reaction:

$$3[Fe_2^{3+}Fe^{2+}]O_{4\,(Magnetite)} + 2H^+ + 1/2O_2 \rightarrow Fe^{2+} + 4[Fe_2^{3+}]O_{3\,(hematite)} + H_2O$$

The oxidation of magnetite to hematite phases was previously only observed under high temperature (≥200 ℃) and hydrothermal conditions (≥120 ℃). Our experiments indicated that in the presence of metabolically active cyanobacteria, this process could be realized under ambient temperature.

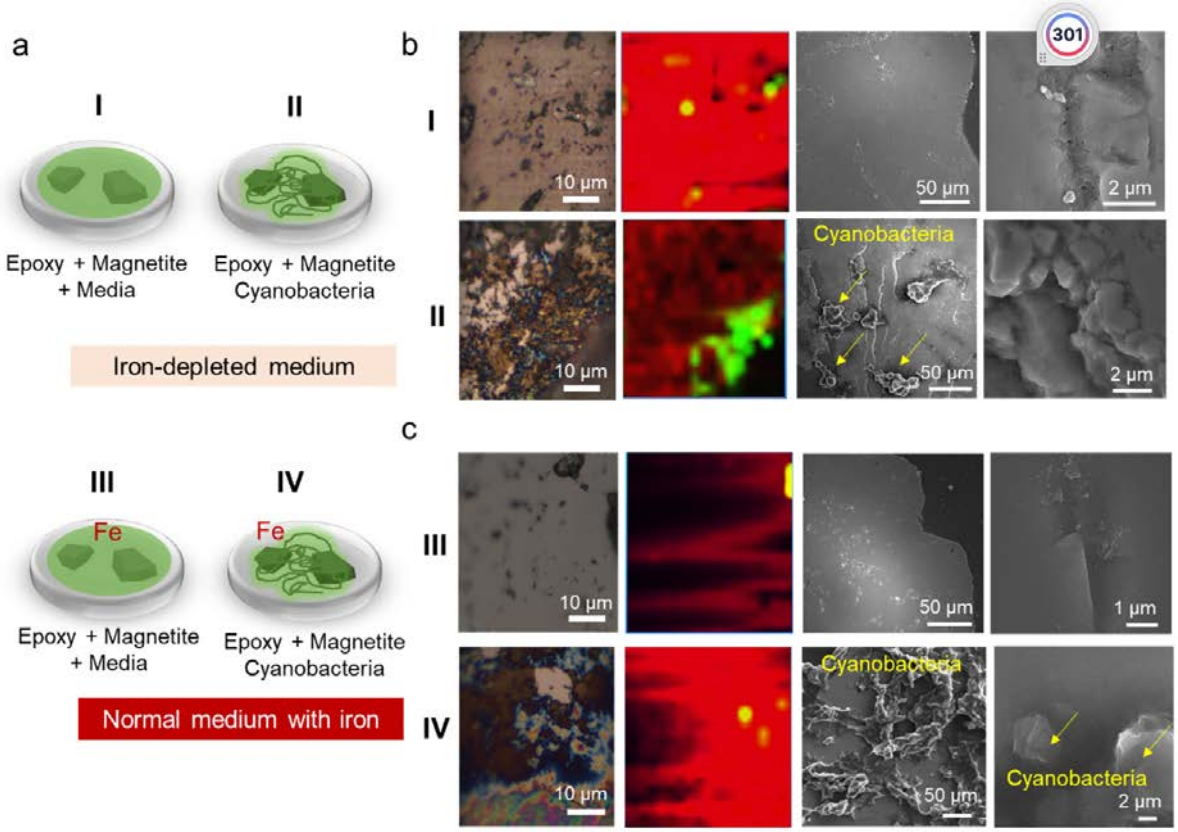

Figure 11. Interactions between cyanobacteria and magnetite bulk crystals. (a) Schematics of experimental setups. (b) Raman mapping and SEM images of samples cultured in iron-depleted media. (c) Raman mapping and SEM images of samples cultured in media with sufficient iron ions. In the Raman maps, red color indicates magnetite phase (Raman shift 666 cm^{-1}), green color indicates hematite phase (Raman shift 217 cm^{-1}). A large area of hematite phase was observed in sample II. SEM of sample II and IV showed cyanobacteria attaching to the substrate surfaces. Groves and dissolution of magnetite were observed in the SEM images of sample II.

In this study, we identified magnetite and hematite phases in gypsum rocks collected in the Atacama Desert. Microorganisms were observed associated with iron oxide minerals indicating these inorganic minerals might serve as iron sources. Cyanobacteria, isolated from gypsum rocks, were cultured with synthesized magnetite nanoparticles. Significant shrinking of nanoparticle size and emerging of amorphous phases in the nanocrystals indicated magnetite dissolution within the cyanobacterial biofilms. The increase siderophore production when cyanobacteria were cultured with magnetite nanoparticles under iron-depleted conditions further confirmed that cyanobacteria

were able to extract iron from the magnetite solid phase. Dissolution of the solid magnetite phase was further verified in large bulk magnetite crystals and was associated with magnetite to hematite phase transformation.

This work is significant because it provides potential design strategies for engineered living materials. Engineered living material is a category of smart materials that incorporate living cells within matrices (scaffolds), thus providing active responses to environmental stimuli. Applications of living materials in the biomedical field include wound healing and biosensing, while environmentally friendly building composites in have been developed by researchers in civil engineering.

A manuscript is in preparation with the results presented above (Huang et al., 2022).