Use of bacteria to stabilize archaeological iron

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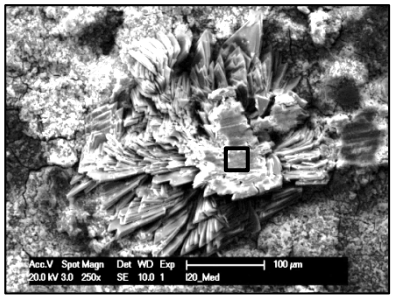
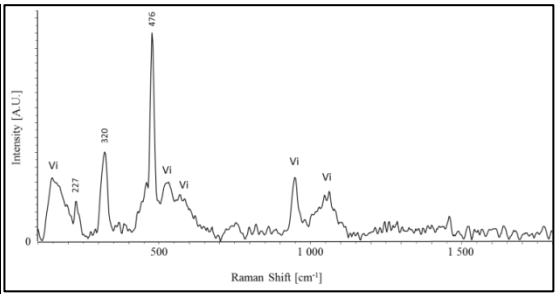
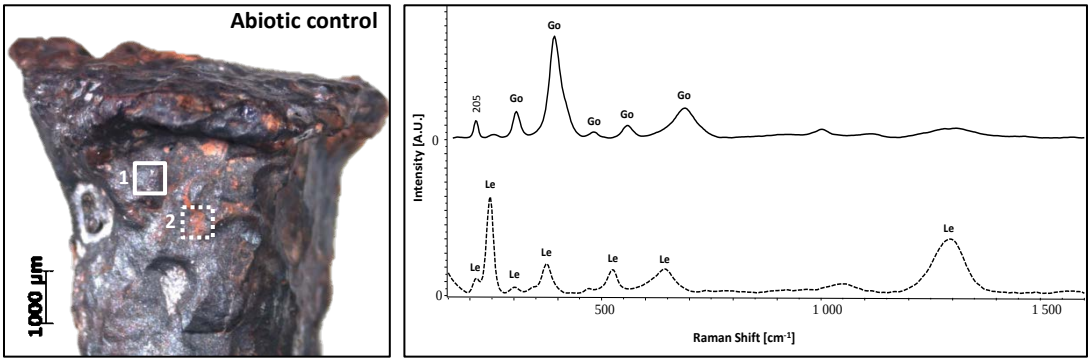
Supplementary material



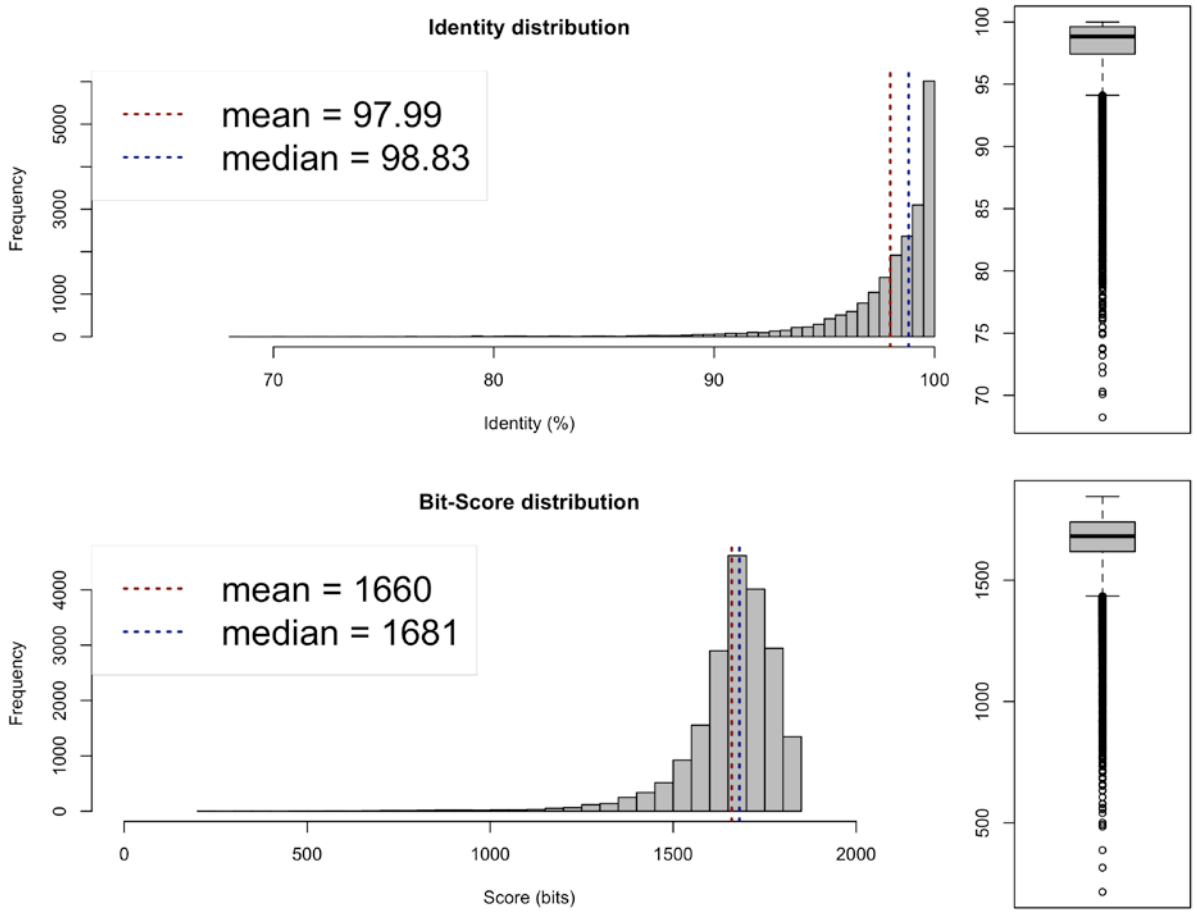
Elemental abiotic control coupons	
Main elements (>10%)	**Fe, O, P**
Secondary elements (between 10 and 1%)	**C, Ca, S, Mg, Si**
Trace elements (<1%)	**Na, Al, K**



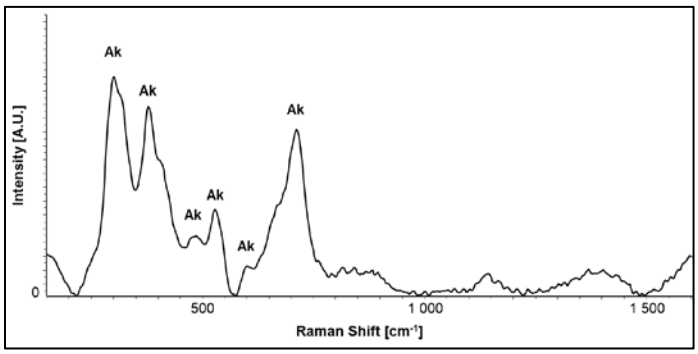
Supplementary Figure S1. Secondary elemental image of the crystals found on the abiotic control coupons, elemental composition, and corresponding Raman spectra identified as vivianite (Vi).



Supplementary Figure S2. Raman analysis of the abiotic control nail after the reduction test. On the left, the stereoscope images of the area sampled for the analysis; and, on the right, the corresponding Raman spectra identified as; 1: Goethite (Go), 2: Lepidocrocite (Le).



Supplementary Figure S3. Results of the average nucleotide identity (ANI) analysis performed between *Desulfitobacterium hafniense* strain LBE and the reference genome of *D. hafniense* strain DCB-2 (GenBank accession number NC_011830.1). The analysis was performed using the on-line ANI calculator tool available at http://enve-omics.ce.gatech.edu.



Supplementary Figure S4. Raman spectrum of the synthetic akaganeite. The analysis was carried out with a Horiba-Jobin Yvon Labram Aramis microscope equipped with a Nd:YAG laser of 532 nm at power lower than 1 mW (600 gr/mm). The spectral interval was 100 and 1600 cm^{-1} and the measurement conditions were 1000 µm hole, 100 µm slit and 5 accumulations of 100 s.

Supplementary material: Anaerobic standard medium for *Desulfitobacterium hafniense*

Standard minimal medium (MM) according to Prat L, Maillard J, Grimaud R, Holliger C. 2011. Physiological adaptation of Desulfitobacterium hafniense strain TCE1 to tetrachloroethene respiration. Applied and environmental microbiology **77:**3853-3859.

Mixture for 50 mL of culture, add aseptically with syringes the following solutions to 45 mL of **solution A**:

- 1.25 mL solution B
- 2.50 mL solution C
- 1.25 mL solution D
- 0.5 mL sodium lactate 40% (v/v)
- 1 mL disodium fumarate 16% (v/v)

Solutions

Solution A :

- $K_2HPO_4.3\ H_2O$ 0.958 g/L
- $NaH_2PO_4.2H_2O$ 0.218 g/L
- Peptone 0.1 g/L
- Resazurin 0.5g/L 1 mL

➔ Boil, cool down under N_2/CO_2, distribute to anaerobic flasks, gas exchange for N_2/CO_2, autoclave.

Solution B :

To 20 mL of anaerobic sterile H_2O, add the following solutions:

- 1 mL solution IV, filter sterilize
- 1 mL solution V
- 1 mL solution VI
- 1 mL solution VII
- 1 mL solution VIII

Solution C :

To 49 mL of solution IX, add :

- 1 mL solution X

Solution D :

- $CaCl_2 \cdot 2\ H_2O$ 4.40 g/L
- $MgCl_2 \cdot 6\ H_2O$ 4.06 g/L

➔ Gas exchange for N_2, autoclave.

Solution IV : Trace elements

- EDTA 500 mg/L, dissolve in 900 mL H_2O, adjust the pH to 7.0 with HCl, then add:
- $FeCl_2 \cdot 4\ H_2O$ 2000 mg/L
- $MnCl_2 \cdot 4\ H_2O$ 100 mg/L
- $CoCl_2 \cdot 6\ H_2O$ 190 mg/L
- $ZnCl_2$ 70 mg/L
- $CuCl_2 \cdot 2\ H_2O$ 2.55 mg/L
- $AlCl_3$ 5.52 mg/L
- H_3BO_3 6 mg/L
- $Na_2MoO_4 \cdot 2\ H_2O$ 41.4 mg/L
- $NiCl_2 \cdot 6\ H_2O$ 24 mg/L, add to 1 L with H_2O

Solution V : Vitamins-1

- Biotin 50 mg/L
- P-aminobenzoate (sodium salt) 250mg/L
- Pantothenate (sodium salt) 50mg/L
- Folic acid$\cdot 2\ H_2O$ 20mg/L
- Lipoic acid 50mg/L
- Pyridoxine 100mg/L
- Nicotinic acid 550mg/L

➔ Filter sterilize in sterile anaerobic flasks, gas exchange for N_2.

Solution VI : Vitamins-2

- Thiamine-HCl 100 mg/L

➔ Filter sterilize in sterile anaerobic flasks, gas exchange for N_2.

Solution VII : Vitamins-3

- Riboflavine 50 mg/L

➔ Filter sterilize in sterile anaerobic flasks, gas exchange for N_2.

Solution VIII : Vitamins-4

- Cyanocobalamin 250 mg/L

➔ Filter sterilize in sterile anaerobic flasks, gas exchange for N_2.

Solution IX :

- NH_4HCO_3 9.01g
- $NaHCO_3$ 76.11 g/L

➔ Boil, cool down under N_2/CO_2, distribute 49 mL to anaerobic flasks, gas exchange for N_2/CO_2, autoclave.

Solution X :

- $Na_2S \cdot 9H_2O$ 24.02 g in 100 mL

➔ Wash crystals with N_2-degased H_2O, weight, dissolve in degased H_2O, filter sterilize into anaerobic flasks, gas exchange for N_2.