Supporting Information

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SI Materials and Methods

Resonance Raman, Surface-Enhanced Resonance Raman Spectoscropy, and Surface-Enhanced Raman Spectroscopy Materials and Experimental Methods. For resonance Raman and surface-enhanced resonance Raman spectroscopy (SERRS) spectra collection, samples were loaded into capillary tubes. Resonance Raman and SERRS spectra were recorded using 413.1-nm excitation from a Kr laser (Spectra-Physics; model 2025). Raman scattering was detected with a cooled back-illuminated CCD (LN/CCD-1100/PB; Roper Scientific) controlled by a ST-133 controller coupled to a subtractive dispersive double spectrograph. All SERRS and resonance Raman spectra were collected with a laser power of 9 mW with data acquisition times of 1 and 5 min for cytochrome *c* and bacteria, respectively. Raman spectra were corrected for wavelength-dependence of the spectrometer efficiency with a white lamp, and the instrument was calibrated using the Raman frequencies from cyclohexane, CCl_4, and toluene. The reported frequencies are accurate to $\pm$ 1 cm^{-1}, and the spectral bandpass was set to 8 cm^{-1}. For each Raman spectrum, the raw data were baseline-corrected because of a reflection background.

For surface-enhanced Raman spectroscopy (SERS) and Raman microscope data collection, experiments were performed with an epi-illumination Raman microscope (LabRAM) with a 633 nm HeNe laser as the excitation source. A 100×, numerical aperture of 0.8, microscope objective (Olympus) was used. The diameter of the laser spot was around 400 nm. The laser power was 9 mW and the data acquisition time was 90 s.

For Raman microscope experiments on a smooth Au surface, a Au (99.999% purity) disk electrode with a diameter of 10 mm was mechanically polished with 1, 0.3. and 0.05 mm-sized alumina powders. The polished electrode was then sonicated in 1 M KOH (to dissolve any embedded alumina) and rinsed in H_2O. Samples were spotted directly onto the electrode surface and allowed to dry.

For SERS experiments on a rough Au surface, the polished Au surface was roughened electrochemically in 0.1 M KCl, using 25 triangular potential scans from −0.28 to 1.22 V (vs. Ag/AgCl reference) at a rate of 500 mV/s. During each cycle, the potential was held at the positive and negative limits for 10 and 5 s, respectively. At the end of this procedure, the electrode was held at −0.3 V for 5 min before rinsing it with a copious amount of ultrapure H_2O. Samples were spotted directly onto the electrode surface and allowed to dry.

Spectral analyses of all resonance Raman, SERRS, and SERS traces were performed using Igor Pro (WaveMetrics).

Cell Suspensions to Determine Effect of Trypsin Shaving on Hydrous Ferric Oxides Reduction. *Thermincola potens* cells from 600 mL of anthraquinone-2,6-disulfonate (AQDS) media were pelleted at 4,000 × *g* anaerobically and washed with 100 mM NH_4HCO_3 buffer (Sigma). The cells were split into three parts and resuspended in a 500 μL of 100 mM NH_4HCO_3 buffer, and one third was incubated with 20 ng of trypsin gold porcine protease (Promega) at 37 °C for 1 h. The cells were again pelleted anaerobically at 4,000 × *g* and resuspended in basal phosphate buffer with ∼6 mM hydrous ferric oxides (HFO). To stimulate HFO reduction, 10 mM acetate was added from a 1 M stock and Fe(II) formation was monitored by the ferrozine assay, as previously described (1, 2).

Liquid Chromatography-MS/MS Materials. Acetonitrile (Optima grade, 99.9%) and formic acid (Pierce; 1 mL ampules, 99+%) purchased from Thermo Fisher Scientific and water-purified to a resistivity of 18.2 MΩ·cm (at 25 °C) using a Milli-Q Gradient ultrapure water purification system (Millipore) were used to prepare mobile phase solvents for liquid chromatography (LC)-MS.

LC-MS/MS Sample Preparation. Partially digested supernatants from trypsin shaving experiments were prepared for LC-MS/MS as follows: The supernatants containing partially digested proteins and peptides were treated in two sequential 30 min steps with 1 mM DTT (Sigma) and 1 mM iodoacetamide (Pierce) to block cysteines. The samples were then incubated overnight at 37 °C with 20 ng of trypsin (Promega). Before LC-MS/MS analysis, C_{18} Zip Tips (Millipore) were used to concentrate peptides and remove salts. 85% acetonitrile (Optima LC/MS grade; Fisher) in water (Optima LC/MS grade; Fisher) with 0.1% trifluoroacetic acid (Sigma) was used to elute the peptides and acetonitrile was removed by speed vac before LC-MS/MS analysis.

LC-MS/MS Experimental Methods. Trypsin-digested proteins were analyzed using an orthogonal acceleration quadrupole time-of-flight (Q-tof) mass spectrometer that was connected in-line with an ultraperformance liquid chromatograph (UPLC). Peptides were separated using a nanoAcquity UPLC (Waters) equipped with C_{18} trapping (180 μm × 20 mm) and analytical (100 μm × 100 mm) columns and a 10-μL sample loop. Solvent A was 99.9% water/0.1% formic acid and solvent B was 99.9% acetonitrile/0.1% formic acid (vol/vol). Sample solutions contained in 0.3 mL polypropylene snap-top vials sealed with septa caps (Wheaton Science) were loaded into the nanoAcquity autosampler compartment before analysis. Following sample injection (10 μL), trapping was performed for 3 min with 100% A at a flow rate of 15 μL/min. The injection needle was washed with 500 μL each of solvents A and B after injection to avoid cross-contamination between samples. The elution program consisted of a linear gradient from 8 to 35% B over 60 min, a linear gradient to 95% B over 0.33 min, isocratic conditions at 95% B for 3.67 min, a linear gradient to 1% B over 0.33 min, and isocratic conditions at 1% B for 11.67 min, at a flow rate of 500 nL/min. The analytical column and sample compartment were maintained at 35 °C and 8 °C, respectively.

The UPLC column exit was connected to a Universal NanoFlow Sprayer nanoelectrospray ionization (nanoESI) emitter that was mounted in the nanoflow ion source of the mass spectrometer (Q-tof Premier; Waters). The nanoESI emitter tip was positioned ∼3 mm from the sampling cone aperture. The nanoESI source parameters were as follows: nanoESI voltage 2.4 kV, nebulizing gas (nitrogen) pressure 0.15 mbar, sample cone voltage 35 V, extraction cone and ion guide voltages 4 V, and source block temperature 80 °C. No cone gas was used. The collision cell contained argon gas at a pressure of 8×10^{-3} mbar. The Tof analyzer was operated in "V" mode. Under these conditions, a mass resolving power (3) of 1.0×10^4 (measured at m/z = 771) was routinely achieved, which was sufficient to resolve the isotopic distributions of the singly and multiply charged precursor and fragment ions measured in this study. Thus, an ion's mass and charge were determined independently (i.e., the ion charge was determined from the reciprocal of the spacing between adjacent isotope peaks in the m/z spectrum). External mass calibration was performed immediately before analysis using a solution of sodium formate. Survey scans were acquired in the positive ion mode over the range m/z = 400–1,500 using a 0.45-s scan integration and a 0.05-s interscan delay. In the data-dependent mode, up to five precursor ions exceeding an intensity threshold of 25 counts per second (cps) were selected from each survey scan for tandem mass spectrometry (MS/MS) analysis.

Real-time deisotoping and charge state recognition were used to select 2+, 3+, and 4+ charge state precursor ions for MS/MS. Collision energies for collisionally activated dissociation were automatically selected based on the mass and charge state of a given precursor ion. MS/MS spectra were acquired over the range m/z = 100–2,000 using a 0.20-s scan integration and a 0.05-s interscan delay. Ions were fragmented to achieve a minimum total ion current of 30,000 cps in the cumulative MS/MS spectrum for a maximum of 2 s. To avoid the occurrence of redundant MS/MS measurements, real-time dynamic exclusion was used to preclude reselection of previously analyzed precursor ions over an exclusion width of ±0.2 m/z unit for a period of 300 s.

Data resulting from LC-MS/MS analysis of trypsin-digested proteins were processed using ProteinLynx Global Server software (version 2.3; Waters), which performed background subtraction (threshold 35% and fifth-order polynomial), smoothing (Savitzky-Golay, 10 times, over three channels), and centroiding (top 80% of each peak and minimum peak width at half height four channels) of mass spectra and MS/MS spectra. Processed data were searched against the *Thermincola sp.* strain JR protein database (Microbial Genomics Program, Joint Genome Institute, US Department of Energy). The following criteria were used for the database search: precursor ion mass tolerance 100 ppm; fragment ion mass tolerance 0.1 Da; digest reagent trypsin, allowing for up to three missed cleavages, carbamidomethylcysteine as a fixed modification and methionine oxidation as a variable modification. The identification of at least three consecutive fragment ions from the same series [i.e., b or y-type fragment ions (4)] was required for assignment of a peptide to an MS/MS spectrum.

1. Nevin KP, Lovley DR (2002) Mechanisms for accessing insoluble Fe(III) oxide during dissimilatory Fe(III) reduction by *Geothrix fermentans*. *Appl Environ Microbiol* 68:2294–2299.
2. Lovley DR, Phillips EJP (1986) Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal potomac river. *Appl Environ Microbiol* 52:751–757.
3. Marshall AG, Hendrickson CL (2008) High-resolution mass spectrometers. *Annu Rev Anal Chem (Palo Alto Calif)* 1:579–599.
4. Roepstorff P, Fohlman J (1984) Proposal for a common nomenclature for sequence ions in mass spectra of peptides. *Biomed Mass Spectrom* 11:601.

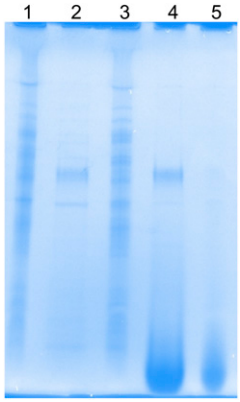

Fig. S1. SDS/PAGE gel of low pH extraction from *T. potens* cells before and after treatment with trypsin: (lane 1) Pellet after pH 2 extraction; (lane 2) pH 2 extract; (lane 3) pellet from pH 2 extraction from trypsin-shaved cells; (lane 4) pH 2 extract from trypsin shaved cells; (lane 5) trypsin (20 ng/μL) treatment of neutralized pH 2 extract from trypsin-shaved cells.

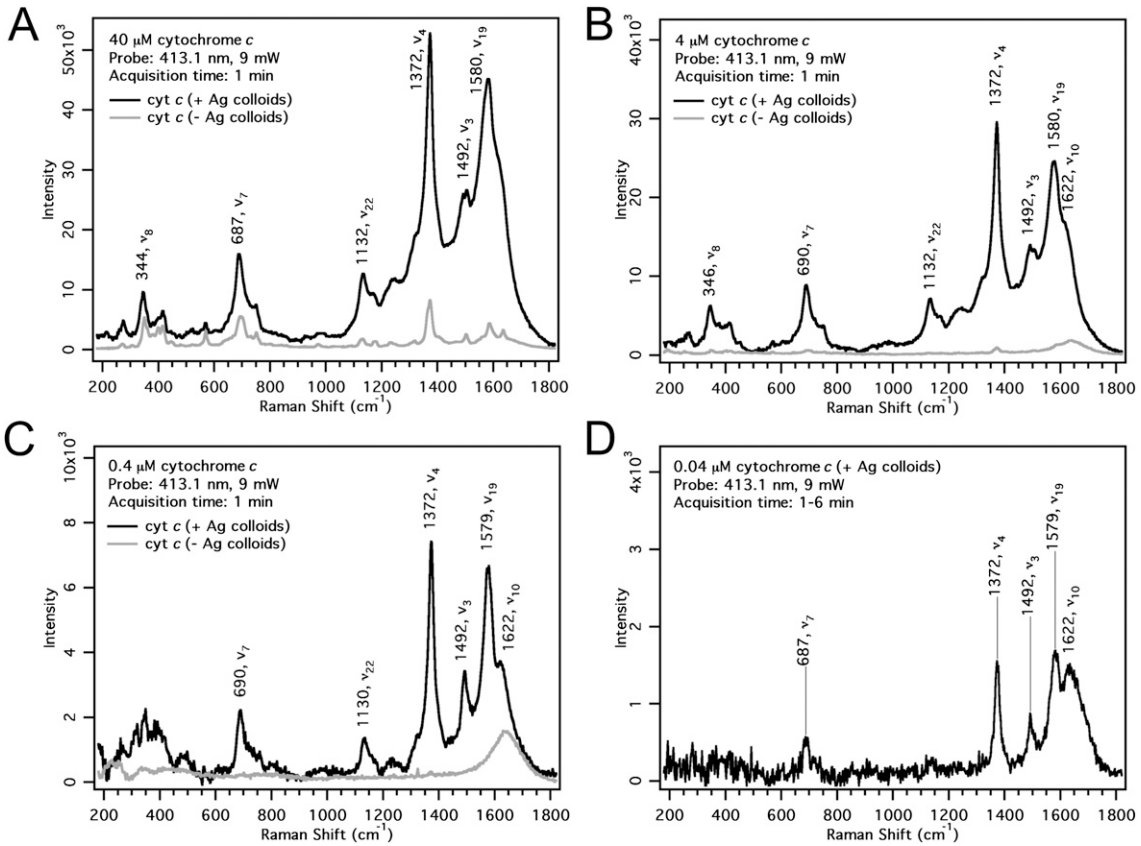

Fig. S2. Resonance Raman and SERRS spectra of cytochrome *c* with 413.1-nm laser excitation at decreasing concentrations of cytochrome *c*. (*A*) 40 μM cytochrome *c*, (*B*) 4 μM cytochrome *c*, (*C*) 0.4 μM cytochrome *c*, and (*D*) 0.04 μM cytochrome *c* in the presence (black traces) and absence (gray traces) of Ag colloids.

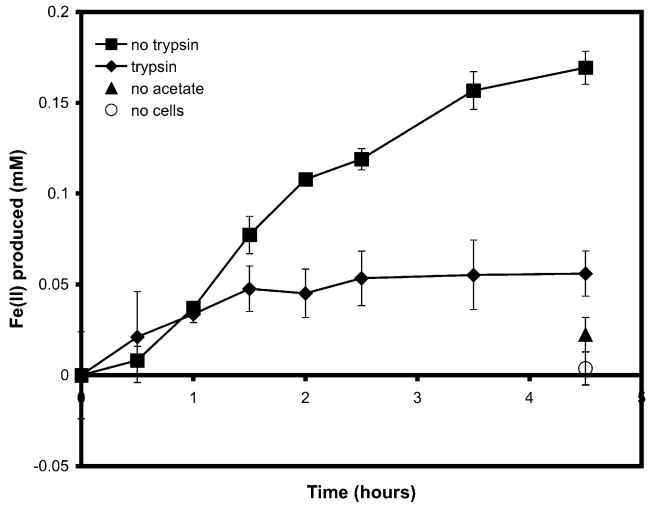

Fig. S3. Reduction of HFO coupled to acetate oxidation by washed cell suspensions of *T. potens*: trypsin-treated cells (◆), nontrypsin-treated cells (■), no cells control (▲), nontrypsin-treated cells without acetate (●). Error bars represent the SD of triplicate cell suspension experiments.

Table S1. All multiheme cytochromes in *Thermincola potens* genome

Accession no.	No. of heme motifs	MW (KDa)
TherJR_0117	15	50.6
TherJR_0120	7	41.2
TherJR_0122	7	45.3
TherJR_0233	5	30.0
TherJR_0333	10	40.6
TherJR_0493	2	31.3
TherJR_0510	5	18.8
TherJR_0930	2	10.8
TherJR_1022	6	43.2
TherJR_1024	6	90.0
TherJR_1025	42	33.9
TherJR_1043	10	39.1
TherJR_1085	9	59.2
TherJR_1117	10	38.6
TherJR_1118	12	45.1
TherJR_1119	17	57.4
TherJR_1122	6	39.3
TherJR_1123	14	59.5
TherJR_1124	58	22.3
TherJR_1130	11	68.2
TherJR_1197	4	16.7
TherJR_1198	4	46.7
TherJR_1421	6	47.8
TherJR_1423	6	118.2
TherJR_1425	15	170.8
TherJR_1844	6	51.2
TherJR_1847	12	43.5
TherJR_2096	6	32.0
TherJR_2221	16	158.7
TherJR_2595	9	56.5
TherJR_2863	42	340.1
TherJR_2864	6	88.0
TherJR_2866	6	42.5

Molecular weight and number of CXXCH heme anchoring motifs is indicated. MW, molecular weight (average mass).

Table S2. Conditions used to extract MHCs from *T. potens* and extent of extraction of TherJR_1122 and TherJR_2793

Treatment	Extraction of TherJR_1122 and TherJR_2793
0.2 M glycine, pH 2	+++
8 M urea	++
6 M guanidine HCl	++
5 mM EDTA	—
5 mM LiCl	—
1% Triton X-100	—
1% SDS	—

—+++, best extraction; ++, extraction; -, no extraction.

Table S3. Proteins identified in trypsin-shaving experiments with >20 peptide hits

	Average no. of peptide hits						
Accession no.	Shed	Trypsin	Trypsin beads	Lysed	Trypsin/lysed	Export signal?	Annotation
TherJR_2595	1.25	2.5	1.25	0.25	10.00	Yes	Hypothetical protein
TherJR_2582	1.5	6.75	3	2.5	2.70	Yes	S-layer domain protein
TherJR_1061	14.25	18.25	12.5	11.25	1.62	Yes	S-layer domain protein
TherJR_0333	1	4.25	0.5	3.75	1.13	Yes	Hypothetical protein
TherJR_2793	7	7.25	6	6.5	1.12	Exported	Flagellin domain protein
TherJR_0765	9.25	10.5	4	10.25	1.02	Tat	Nickel-dependent hydrogenase large subunit
TherJR_0861	0.25	3.75	0.75	4	0.94	Yes	Extracellular solute-binding protein family 3
TherJR_0764	0.75	2.25	0.5	2.5	0.90	Tat	Hydrogenase (NiFe) small subunit HydA
TherJR_2944	1.25	2	1.5	4	0.50	No	Cobalamin B12-binding domain protein
TherJR_1161	5	4.75	3.25	10.5	0.45	No	Dihydropteroate synthase DHPS
TherJR_0276	2	5.25	3.75	12.25	0.43	No	Translation elongation factor Tu
TherJR_2871	0.25	1.25	1.25	3.25	0.38	Yes	S-layer domain protein
TherJR_0371	4.75	5.75	3.25	15	0.38	No	AMP-dependent synthetase and ligase
TherJR_1159	5.75	6.25	2.75	17	0.37	No	CO dehydrogenase/acetyl-CoA synthase, delta subunit
TherJR_1709	3	2.75	1.25	7.75	0.35	No	Methylenetetrahydrofolate dehydrogenase (NADP(+))
TherJR_1157	3.75	5.25	1.5	15.5	0.34	No	Carbon-monoxide dehydrogenase, catalytic subunit
TherJR_1985	2.25	3	3.25	9.75	0.31	No	Adenylate kinase
TherJR_0859	0.5	2	0.75	6.5	0.31	No	2-Hydroxyglutaryl-CoA dehydratase D-component
TherJR_2400	0	4	0.75	13.75	0.29	No	Pyruvate, phosphate dikinase
TherJR_0013	0.75	3.25	0	12	0.27	No	D-3-phosphoglycerate dehydrogenase
TherJR_1158	2	5.25	1.75	20	0.26	No	CO dehydrogenase/acetyl-CoA synthase, beta subunit
TherJR_0741	0	1	0.5	4	0.25	No	NADH dehydrogenase (quinone)
TherJR_0941	0	1	0	4.75	0.21	No	Electron transfer subunit, iron-sulfur cluster binding domain
TherJR_0208	1.5	2.25	0.5	10.75	0.21	No	Formate–tetrahydrofolate ligase
TherJR_2887	0	1	0.25	5.25	0.19	No	ATP synthase F1, beta subunit
TherJR_0775	0.75	1.75	0.25	11	0.16	No	Chaperonin GroEL
TherJR_2706	0	0.75	0	4.75	0.16	No	V-type H(+)-translocating pyrophosphatase
TherJR_1160	1.25	1.25	0.75	9.75	0.13	No	CO dehydrogenase/acetyl-CoA synthase, delta subunit
TherJR_0370	0	0.75	0.75	7.5	0.10	No	Acetate/CoA ligase

Average number of peptide hits observed for abundant *T. potens* proteins in trypsin-shaving experiments. Protein hits in this table had >20 peptides observed across four biological replicates. The proteins were further sorted in order of decreasing trypsin/lysed ratio. More peptide hits were observed in trypsin-shaved intact cells than lysed cells for proteins at the top of the table. A column is also included to indicate which proteins contain an export signal sequence. Tat, likely Twin-arginine translocated; Exported, secreted through flagellar apparatus.

Table S4. All multiheme *c*-type cytochromes and S-layer proteins identified in trypsin-shaving experiments

Accession no.	Annotation
TherJR_0333	Hypothetical protein
TherJR_1061	S-layer domain protein
TherJR_1117	Putative cytochrome *c*-type protein
TherJR_1122	Cytochrome C family protein
TherJR_2582	S-layer domain protein
TherJR_2595	Hypothetical protein
TherJR_2871	S-layer domain protein

Multiheme cytochromes (shaded rows) and S-layer homology domain-containing proteins identified in *T. potens* trypsin-shaving experiments. The same proteins were also identified in proteomes from *T. potens* cells grown on HFO (Dataset S1).

Dataset S1. Data from trypsin-shaving experiments with cells grown on AQDS and the proteome from lysed cells grown on HFO

Dataset S1