Supporting Information

Debieux et al. 10.1073/pnas.1105959108

SI Materials and Methods

Growth of *Thauera selenatis* and *Escherichia coli*. *T. selenatis* was cultured anaerobically (at 30 °C) on mineral salts medium containing yeast extract (0.1%), with either selenate or nitrate (10 mM) as terminal electron acceptors and acetate (10 mM) as the electron donor in 1-L batch cultures. Cultures were also grown under aerobic conditions on Luria-Bertani (LB) medium supplemented with selenate, selenite, or nitrate (all at 10 mM unless otherwise indicated). All *E. coli* cultures were grown on LB medium at 37 °C.

Isolation of SefA. Cultures were harvested during late log phase (after 16–18 h growth) at $OD_{600\,nm}$ 0.6–0.7 by centrifugation (25,000 × *g*, 20 min), and the supernatant, containing the Se nanospheres and extracellular media, was retained. The supernatant was passed through a 0.2-μm filter, to remove bacterial cells, and concentrated using an Amicon ultra centrifugation filtrate unit with a 10-kDa cutoff (Millipore). Isolated extracellular protein was analyzed by SDS-PAGE. Protein samples were tested for selenite reductase activity as described by Ridley et al. (1).

Identification of the *sefA* Gene. Isolated SefA was resolved by SDS-PAGE, excised, and sent for N-terminal sequencing at Pinnacle Proteomic facility, Newcastle University. In addition, gel slices containing SefA were sent to mass spectrometry facilities at the Universities of Exeter and York. To identify the *sefA* gene, the protein sequencing data obtained was blasted against the draft genome sequence of *T. selenatis* (2) using CLC Genomics Workbench 3 gene prediction software.

Generation of Recombinant SefA. The coding region of SefA was amplified using forward (5′-GTTCATATGGCTATCACTGCG ACTCAACGC-3′, underlined sequence *Nde*I restriction site) and reverse (5′-GGACTCGAGTTAGAACAGGTAGATGTT-GCC-3′, underlined sequence *Xho*I restriction site) primers and cloned into pET33b(+) using *Nde*I and *Xho*I restriction sites. Insertion of *sefA* into pET33b(+) using these restriction sites inserted an N-terminal 6 X His-tag (His-SefA). The plasmid was designated pET33b-*sefA*. Cloning of *sefA* was confirmed by analytical restriction digestion and DNA sequencing. SefA was overexpressed in *E. coli* BL21 CodonPlus (DE3)—RIPL cells and purified by immobilized metal affinity chromatography in buffer A (20 mM Tris-HCl pH 8.0, 0.5 M NaCl) and gel filtration. Extracellular proteins were concentrated from 50-mL culture to 1-mL samples using U-Tube 20–30 concentrators (Novagen). Samples were resolved by SDS-PAGE, and Western blotting was used to detect His-tagged protein to determine localization. Immuno-cross-reactive proteins were detected using 1′ monoclonal antipolyHistidine antibody (Sigma) and 2′ antimouse IgG (H + L) AP conjugate (Promega). The samples were detected by the addition of Western blue stabilized substrate for alkaline phosphatase (Promega).

In Vitro Formation of Selenium Nanospheres. Selenium nanoparticle formation assays were performed in 50 mM Tris-HCl buffer (pH 7.0) supplemented with protein, 4 mM reduced gluthathione, and 0.5 mM selenite at room temperature in quartz cuvettes (3). The formation of selenium nanospheres was monitored spectrometrically at 400 nm. All solutions were sparged with nitrogen before use.

Imaging Se Nanospheres by Transmission Electron Microscopy. *T. selenatis* and *E. coli* cultures were centrifuged at 6,000 × *g* for 3 min in a 50-mL falcon tube, supernatant was removed, and a fixative of 2% (wt/vol) paraformaldeyhde, 2,5% (vol/vol) glutaraldehyde in 0.1 M cacodylate buffer pH 7.2 was added. Fixation time was a minimum of 2 h. Post fixation was carried out with 1% (vol/vol) osmium tetroxide in 0.1 M cacodylate buffer pH 7.2 followed by embedding in 3% (wt/vol) agarose LM in 0.1 M cacodylate buffer pH 7.2. After cooling on ice, the plug of agar was removed and cut into 3-mm^2 pieces, washed, stained with 1% (wt/vol) uranyl acetate for 1 h, washed, and dehydrated. After embedding in TAAB Low Viscosity Resin Hard (TAAB Laboratories Equipment Ltd.), sections of 80- to 90-μm thickness were cut with a diamond knife and collected on carbon-coated Formvar films on 300-mesh copper grids. Sections were stained with uranyl acetate and lead citrate before being examined at 80 kV with a Jeol 1400 transmission electron microscope. For in vitro generated selenium particles, droplets of sample were placed on carbon-coated Formvar films on 300-mesh copper grids for 1 min, after which excess liquid was withdrawn with filter paper. Grids were placed on filter paper and air-dried. Specimens were examined at 80 or 100 kV with a Jeol 1400 transmission electron microscope.

RNA Isolation. For transcriptional studies, bacteria were grown aerobically at 30 °C in LB broth supplemented with various concentrations of selenite. Total RNA was extracted from 10 mL of exponentially growing cultures ($OD_{600\,nm}$ of 1) and 5 mL of stationary phase cultures at 16 h after inoculation, respectively, using a hot-phenol extraction protocol (4). In brief, cells were harvested by centrifugation at 3,220 × *g* at 4 °C for 10 min. The cell pellet was resuspended in 1 mL of ice-cold resupension buffer (10 mM KCl, 5 mM $MgCl_2$, 10 mM Tris; pH 7.4) and 0.5 mL each were added to preheated tubes containing 0.4 mL of lysis buffer (0.4 M NaCl, 40 mM EDTA, 1% 3-mercaptoethanol, 1% sodium dodecyl sulfate, 20 mM Tris; pH 7.4) and 0.2 mL of acid phenol (pH 4.5; Ambion), using duplicate tubes per sample. The tubes were incubated at 90 °C for 5 min and then chilled on ice for 5 min. Phase separation was achieved by centrifugation at 18,000 × *g* for 2 min. RNA in the supernatant was extracted with two additional phenol-chloroform extraction and precipitated overnight at −20° C in isopropanol. The RNA was pelleted by centrifugation, washed with 70% ethanol, air-dried for 5 min at room temperature, and resuspended in nuclease-free water. Contaminating DNA was removed by DNase I (Ambion) digestion for 45 min at 37 °C, followed by phenol/chloroform extractions, isopropanol precipitation, and resuspension of the total RNA in nuclease-free water as described above.

RT-PCR. For cDNA synthesis, 4 μg of total RNA was mixed with 3 μL of random primers at 3 μg/μL (Invitrogen) and 1 μL of a dNTP mixture at 10 mM each (Promega). After primer annealing at 65 °C for 5 min, a mix of first-strand buffer, DTT, 40 U RNase OUT recombinant RNase inhibitor (Invitrogen), and 200 U Superscript III reverse transcriptase (Invitrogen) was added according to the manufacturer's recommendations. cDNA synthesis was performed at 50 °C for 60 min, followed by heat inactivation at 70 °C for 15 min. cDNA samples were 10× diluted in water and directly used for PCR amplification. *sefA* transcript levels were determined using primers sef-fw (CGACTCGAGGGCACCTT-CGGTACTGTAAC) and sef-rv (CGCTCTAGACGGAGGTCA-GCAGATCATTC). For the adjustment of cDNA amounts, 16S rRNA was used as an internal standard, using primers Ts-16S-RT-1 (GCAGTGAAATGCGTAGAG) and Ts-16S-RT-2 (TGTCAA-GGGTAGGTAAGG) for the PCR reaction. As a control for

DNA contaminations, PCRs were performed using total RNA without any reverse transcription reaction.

Northern Blot. For Northern blotting, 5 μg of total RNA was separated on a 1.5% formaldehyde agarose gel prior to blotting onto a Hybond-N$^+$ membrane by capillary transfer. The *sefA* transcript was detected by hybridizing the membrane with a digoxigenin (DIG)-labeled DNA probe at 50 °C overnight. For amplification of the probe, the PCR DIG probe synthesis kit (Roche) was used with primers sef-fw and sef-rv (see above). The membrane was developed using an antidigoxigenin antibody and CDP-Star substrate (Roche) according to the manufacturer's recommendations. Chemiluminescent signals were visualized and quantified using a Chemidoc XRS system equipped with the QuantityOne software (BioRad).

1. Ridley H, Watts CA, Richardson DJ, Butler CS (2006) Development of a viologen based microtitre plate assay for the analysis of oxyanion reductase activity: Application to the membrane bound selenate reductase from *Enterobacter cloacae* SLD1a-1. *Anal Biochem* 358:289–294.
2. Lowe EC, et al. (2010) Quinol-cytochrome *c* oxidoreductase and cytochrome *c*4 mediate electron transfer during selenate respiration in *Thauera selenatis*. *J Biol Chem* 285:18433–18442.
3. Kessi J, Hanselmann KW (2004) Similarities between the abiotic reduction of selenite with glutathione and the dissimilatory reaction mediated by *Rhodospirillum rubrum* and *Escherichia coli*. *J Biol Chem* 279:50662–50669.
4. Chuang SE, Daniels DL, Blattner FR (1993) Global regulation of gene expression in *Escherichia coli*. *J Bacteriol* 175:2026–2036.

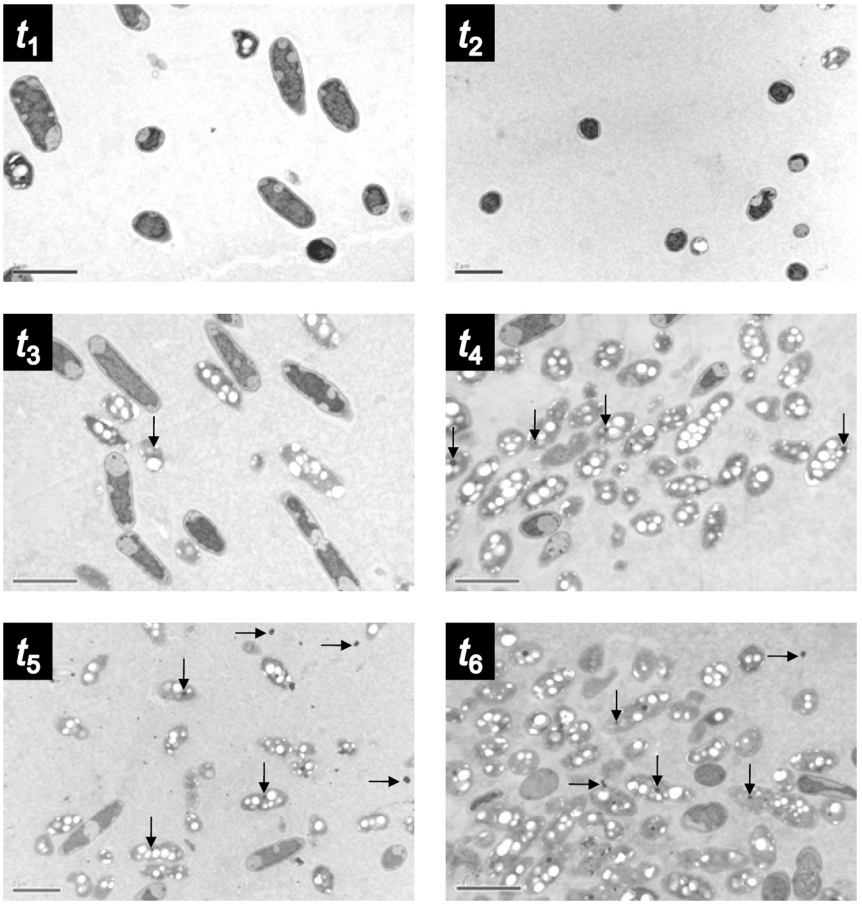

Fig. S1. Transmission electron micrographs of *T. selenatis* cells grown under selenate respiration conditions showing the formation of selenium deposits. Images show cell cohorts to indicate the distribution of typical cells shown in Fig. 1. Micrographs t_1 and t_2 show mid exponential phase, t_3 and t_4 show late exponential phase, and t_5 and t_6 show stationary phase. Scale bar, 2 μm in each case. Intracellular Se deposits are indicated by a vertical arrow. Extracellular Se deposits are indicated by a horizontal arrow.

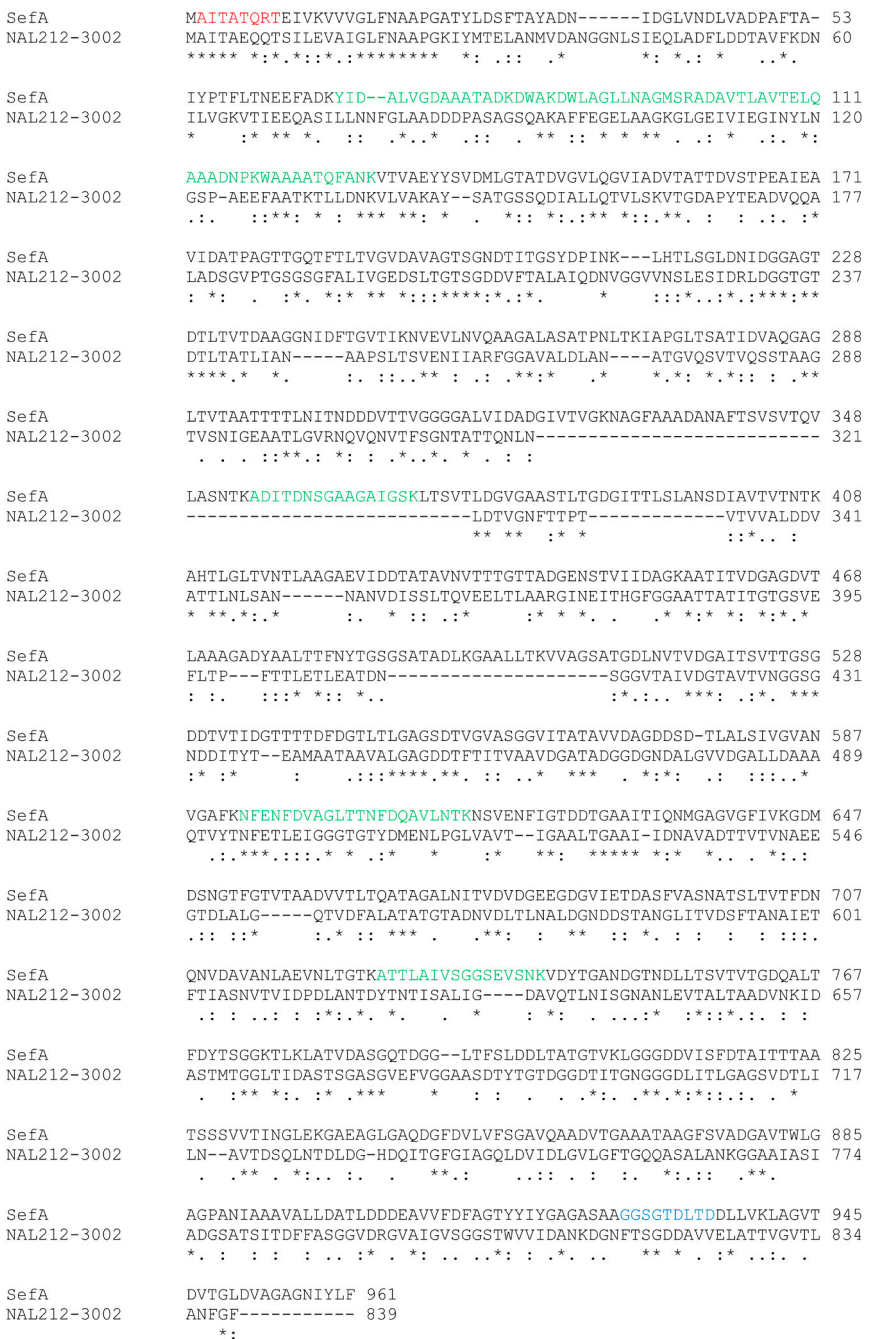

Fig. S2. Alignment of SefA with a hypothetical protein (NAL212_3002) from *Nitrosomonas* sp. AL212. Residues in red indicate the peptide determined by N-terminal sequencing of the mature peptide secreted from *T. selenatis*. Residues in green indicate the peptides generated during tryptic digest for MS analysis. Sequence in blue indicates the glycine-rich repeat (GGXGXDXXX) associated with T1SS substrates. Alignment performed using ClustalW2.

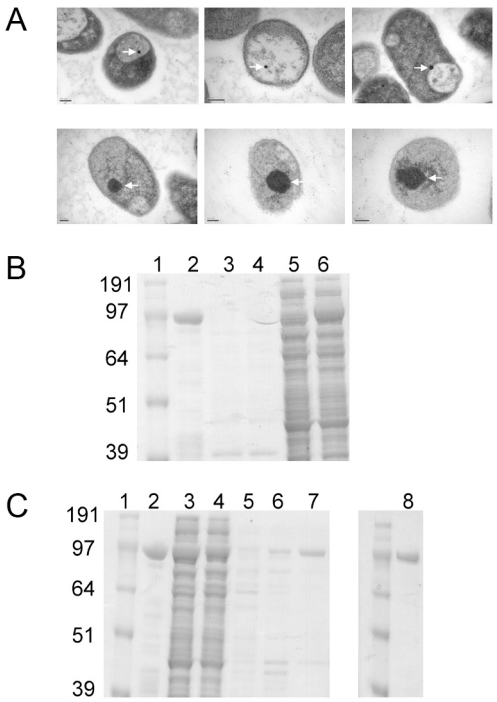

Fig. S3. Expression and purification of SefA from *E. coli*. Transmission electron micrographs of *E. coli* single cells harboring plasmid pET33b-*sefA* grown in the presence of selenite (10 mM), but not induced with IPTG. (*A*, *Upper*). Transmission electron micrographs of *E. coli* single cells harboring plasmid pET33b-*sefA* grown in the presence of both selenite (10 mM) and IPTG (*A*, *Lower*). Three replicate cells are shown. (*B*) SDS-PAGE analysis of SefA expression in *E. coli*. Lane 1, molecular weight markers; lane 2, purified SefA secreted from *T. selenatis*; lane 3, extracellular protein from noninduced cells; lane 4, extracellular protein from induced cells; lane 5, soluble cell extracts from noninduced cells; lane 6, soluble cell extracts from induced cells. (*C*) Purification of SefA from *E. coli* following induction with IPTG. Lane 1, molecular weight marker; lane 2, purified SefA secreted from *T. selenatis*; lane 3, total soluble protein; lane 4, flow through from nickel column; lane 5, wash through; lane 6, wash with 20 mM imidazole buffer; lane 7, elution with 250 mM imidazole buffer; lane 8, purified SefA post gel filtration.