Supplemental Materials

Tetrathionate-forming thiosulfate dehydrogenase from the acidophilic, chemolithoautotrophic bacterium *Acidithiobacillus ferrooxidans*

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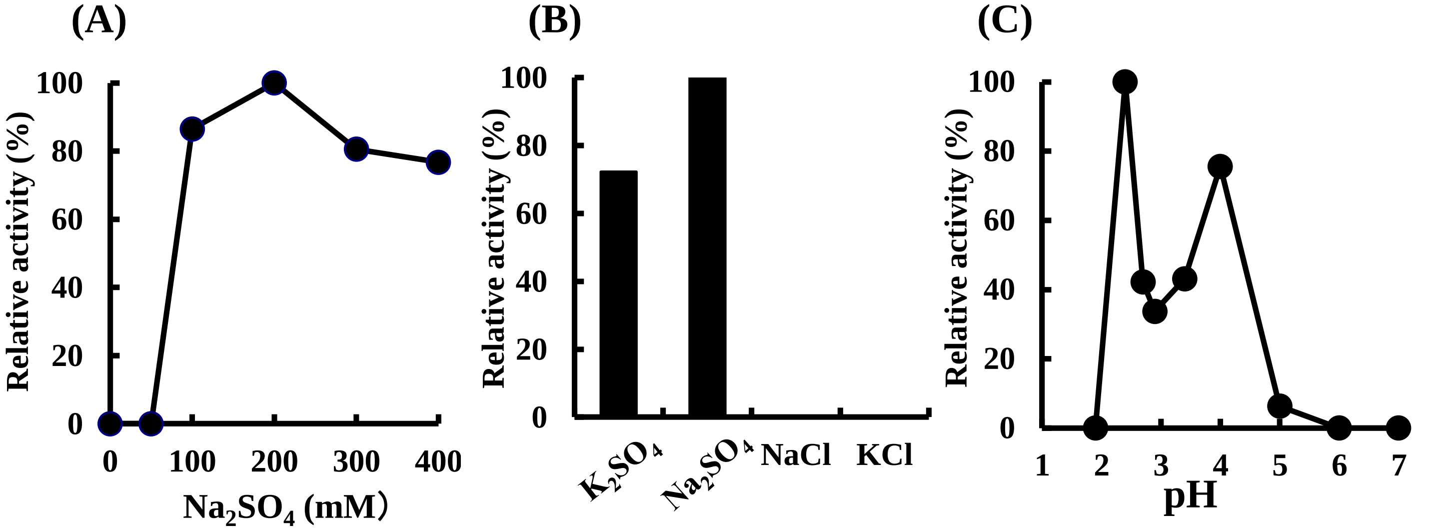


FIG S1 Effect of sulfate ion concentration (A), ions (B), or pH (C) on TSD activity in cell-free extracts from *A. ferrooxidans*. (A) Activity was measured at pH 2.5 and 40°C; (B) activity was measured in the presence of 200 mM of K_2SO_4 or Na_2SO_4 and 100 mM of NaCl or KCl; (C) activity was measured in the presence of 200 mM Na-sulfate at 40°C. Experiments were carried out in triplicate in a mixture containing 10 mM Na-thiosulfate and 1 mM K-ferricyanide. Each data point is given as the arithmetic mean value. Specific activity for the 100% value was 0.04 U·mg^{-1}.

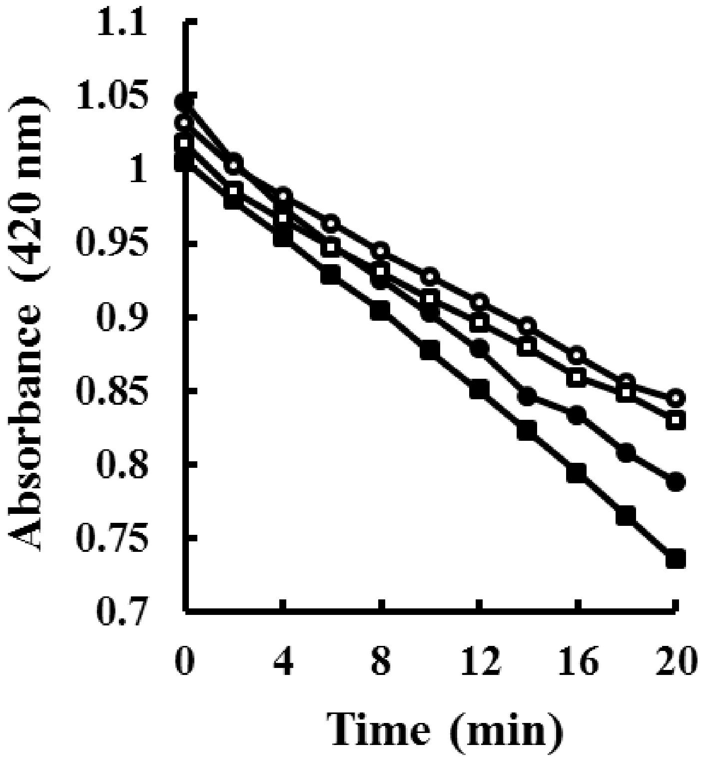
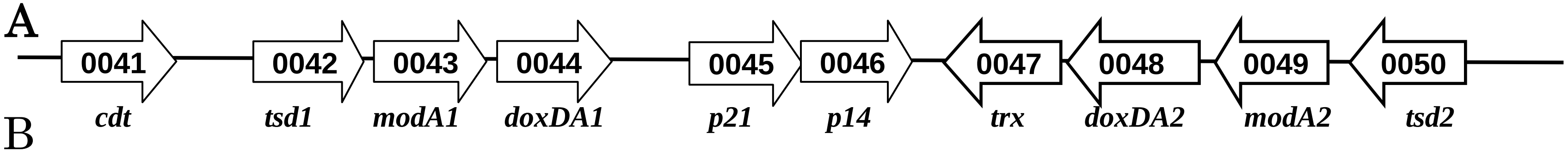


FIG S2 Time course of absorbance changes at 420 nm after mixing thiosulfate and ferricyanide with the soluble fraction (circle) or soluble fraction at pH 4 (square). The enzymatic and non-enzymatic reactions by heat-inactivated enzymes (10 min at 100°C) are shown in black and white, respectively. Reactions were carried out in triplicate in a mixture containing 10 mM Na-thiosulfate, 1 mM K-ferricyanide, 200 mM Na-sulfate, and 60 $\mu g \cdot mL^{-1}$ of protein at pH 2.5 and 40°C. Each data point is given as the arithmetic mean value.

A



B

AFE	Description	MW (kDa)	pI	Putative function	Predicted subcellular location	Identification in previous studies	References[a]
0041	*cdt*	44.6	6.6	C4-dicarboxylate transporter	Membrane	*tehA*	S1, S4, S7
0042	*tsd1*	25.7	6.2	Thiosulfate dehydrogenase	Periplasm	*hypA1* *hyp4*	S3 S1, S7 S4
0043	*modA1*	33.9	9.2	Putative solute-binding protein (Sulfate/thiosulfate binding protein)	Periplasm	*sbp1* *modA-1*	S2 S1, S6, S7 S4
0044	*doxDA1*	38.8	9.6	DoxDA-like protein	Membrane	*doxDA-1*	S1, S7 S5
0045	*p21*	20.9	9.3	Rhodanese-like protein	Periplasm	*p21*	S5, S6, S7 S4
0046	*p14*	14.3	4.6	Rhodanese domain containing protein	Cytoplasm	*p14*	S1, S4
0047	*trx*	18.1	8.8	Thioredoxin	Periplasm		
0048	*doxDA2*	39.0	9.7	DoxDA-like protein	Membrane		
0049	*modA2*	33.8	8.8	Putative solute-binding protein	Periplasm		
0050	*tsd2*	25.7	6.0	Tat pathway signal sequence domain protein	Periplasm		

FIG S3 (A) Schematic map of the contig region containing the putative gene cluster context around gene *tsd* *from A. ferrooxidans.* (B) Putative functions of AFEs present in this region (see reference S6). AFE, derived from the NCBI numbering. Molecular weight (MW), pI, and export signal prediction was done with sequences from a local database. MW was estimated using the putative amino acid sequence without the signal peptide. [a] see reference section.

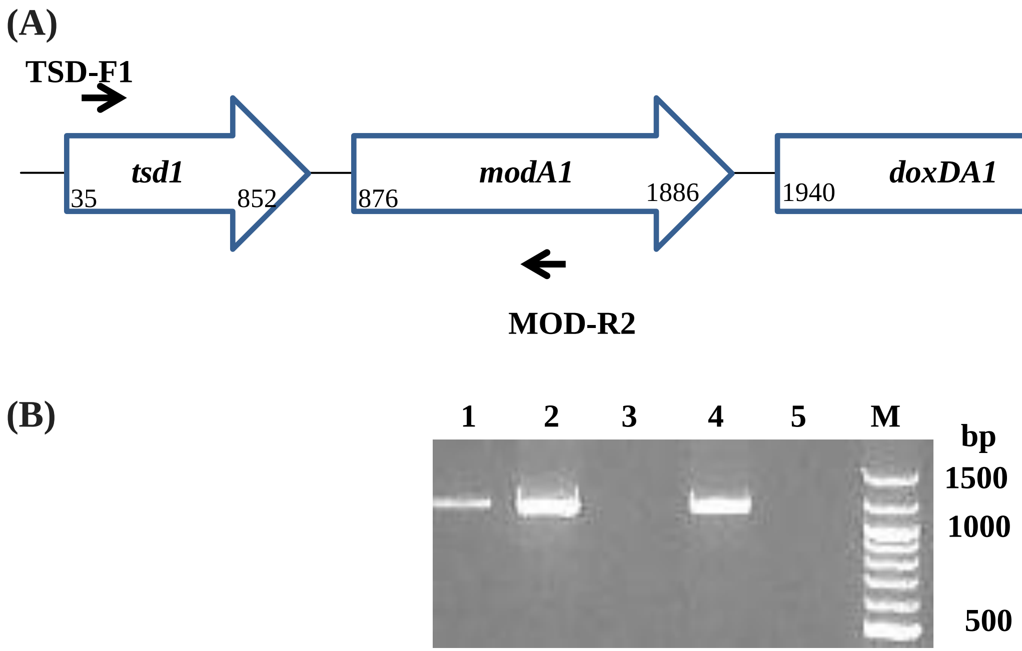


FIG S4 Transcriptional analysis of *tsd* gene in *A. ferrooxidans* ATCC 23270 by RT-PCR.

(A) Schematic map of the genetic context around the *tsd* gene in *A. ferrooxidans* ATCC 23270. Arrows indicate locations of primers used for the RT-PCR. The following primers were used; TSD-F1 (5’-GCTGCAGGAATTGAGTAACC-3’) and MOD-R2 (5’-TCCACGTCATGTTGCCGATG-3’).

(B) Agarose gel electrophoresis of DNA fragments amplified by RT-PCR. PCR products were analyzed by electrophoresis on 2% agarose gel. The following DNAs were used as the template DNA: Lane 1, cDNA prepared from sulfur-grown cells; Lane 2, cDNA prepared from tetrathionate-grown cells; Lane 3, cDNA prepared from iron-grown cells; Lane 4, gDNA prepared from iron-grown cells; Lane 5, control (without DNA); lane M, 100 bp DNA ladder marker.

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AFE_0042              1    MSN------ESNL-LSRREAMKRTVL-GAGAVAVGALGANASAMAAGMSGNPANLLPTGAGTLQELSNRLAKAPRRRDFK  72
Acife_2603            1    MIN------ESNL-LNRREAMKRAVL-GAGVVAVGAMGASASAMAAGMSGNPGNLLPPGASTLQELSNRLAKAPRRRDFK  72
AFE_0050              1    MSN------EQNL-LSRRDAMKHALW-GAGALAVGAVGTQTSAVAAGLSGNPGNLLPTGARSLKELGERLAKTPRRRDFK  72
Tint_0029             1    MST------HSPVQPSRRLALQSTLVAGLGLTALGA--KTAQAASAQERF-----MPQGASTLAELGKKLAAAPRRRDFK  67
ACMV_19130            1    MSDTGQTERDSSLQLSRRTALSLPAV-GVGTAVIGAM--IMPAAEAANSAPPPNLITPESKKLRALAAALAKAPRRRNYK  77
HYPMC_2183            1    MT------MTG-----RRQALQAFGL-ALAAASVGGFTREADAAAAHD-------IPAGASALADLTDRLAKTPRRRDFK  61
HY04AAS1_0084         1    MDQ------------SRRNVLKGAIL-GLGAGMVASALNPTAAKAEEVGA----LIPKGARTLKELAERLAKAPRRRNFK  63
BCAM2440              1    MSD-----RHA-----RRDALKTLGL-AAGTVLLAA-TRPAHAAAPDTG----TLLPGGAARLADLTRRLAATPRRRDFK  64
Gobo1_010100016936    1    MND-----MTSNLRADRRHALLGLMA-ATAGATIAT-TSTALAARSSSS----H--ADGTGNLAVLTSQLARSPRRRDFR  67
GeurL1_010100014279   1    MND-----MTSNLRADRRHALLGLMA-ATAGATIAT-TSTALAARSSSS----H--ADGTGNLAVLTSQLARSPRRRDFR  67
GOX2656               1    MND-----MTSNLRADRRHALLGLMA-ATAGATIAT-TSTALAARSSSS----H--ADGTGNLAVLTSQLARSPRRRDFR  67
UBAL3_43660050        1    M----------SRSMSRREMLGMLAMMGTAGVALGLGGGKVPLASASEHL---DLQPRGDHHLHALLSALARAKRRRDYR  67
LFE_1996              1    M----------SESVSRREMLGMVATIGAAGAALGLGVGKLPEAAASEKL---DLEPKGDHHLKALLRALERAKGRRDYK  67

AFE_0042             73    TVPMMLTSPDQWDSEALDEILHYAGGPKQVWDNTALTSPWLNLMRNSMNVQIWSFKHP--DFLALSATHGSAHFALYDNY  150
Acife_2603           73    TVPMILTSPDQWDSEALNEVLHYAGGPKQVWDNTVLKSPWLNLMRNAMNCQIWSFKHA--DFFCASATHGSAHLALYDNY  150
AFE_0050             73    TVPMILTKPDEWDDAALREIIHYAGGPKQVWDNTDVSSPWLNLMRNSMNVQVWSFRHP--DFLCVSATHGSAHWALYDDF  150
Tint_0029            68    TTQMVLENRDEWDAEALDLVLAYAGQPKQVWNNTNLEGPWLNLMRNAMNAQIWSFKHP--DFLAVSATHGSAHLALYDDA  145
ACMV_19130           78    SVPMILTNSDQWDSEALHILFTYAAGPKQIWDNTDLTGPWLNLMRNAMNAQIWSWKHP--NFIAVSATHGSAHLALYDNY  155
HYPMC_2183           62    TVPMILDHSDQWDDEALKEVLAYKPVTKQAWDVTDIAGPWLNVMRNSINAQIWSFKHP--DFLVVSATHGSAHYALYDQA  139
HY04AAS1_0084        64    SLPMVLTDKNQWDWEALDEVFNYKGGPKQVWDNYDIHSAWLNVMRNAMNAQIWSYKNP--DFLCVSATHGNAHFAIYDDY  141
BCAM2440             65    TVPMILERPDQWDHAALTEVIGYRGGPKQVWDNTELGGPWLNLMRNSLNAQIWSYGHP--DFLVVSATHGSAHLALFDRI  142
Gobo1_010100016936   68    TVPMVLDDPLMWDAQALDLVIAYRGERKQVWDNTAIDSPWLNLMRNSVNAQVFSFRHP--DFLAVSATHGSAHLALYDQT  145
GeurL1_010100014279  68    TVPMVLDDPHMWDAQALDLVIAYRGERKQVWDNTAIDSPWLNLMRNSVNAQVFSFRHP--DFLAVSATHGSAHLALYDQT  145
GOX2656              68    TVPMVLDDPLMWDAQALDLVIAYRGERKQVWDNTAIDSPWLNLMRNSVNAQVFSFRHP--DFLAVSATHGSAHLALYDQT  145
UBAL3_43660050       68    SLTMISLHQDEWDHEALALLLHYHAKPKQVWDNTIFGSPWLNLMRNSLNAQVYSFGKK--DALVLSATHGTAHLGLFDDT  145
LFE_1996             68    SVTMISLHKDEWDHEALSLLFHYRAKPKQVWDNTVIGSPWLNLMRNSLNAQVYSFGKK--DALVLSATHGTAHLALFDDH  145

AFE_0042            151    IWNKY-LATAT-------KGKWKANAWLKQPPASATNPADFEDPEGVFSPHDNSIIVLQRRGAVFLACHNEVWELTMGLH  222
Acife_2603          151    IWNKY-LATLT-------KGKWKANAWLKQPPASATNPADFEDAEGVFSPHDNSIIVLQRRGAVFLACHNEVWELTMGLH  222
AFE_0050            151    IWNKY-LAQMT-------GGKWKDNEWIKEPPAAAADPANFENPEGVFSPGDNSVTVLQRRGAVFLGCHNGIWEITEGVL  222
Tint_0029           146    LWAKYQLGSLT-------KGKAKTNTWIKTSLAADADPKSVEDPKGVYSPADNSITVLQERGAVFLACHNAIWELTGHLH  218
ACMV_19130          156    IWKKY-LSSFT-------GGKFSSNIWIEEPSAASSPASSFNDPKGVFSPHDNSIVVLQRRGAVFCACHNEVWELTMAVL  227
HYPMC_2183          140    MWDKYQFAKIV-------GKGFEKNTLIVDKPAASANPADYESPTGVFSPLNNSIPALQRRGAVFMSCHNAIWEQATKLH  212
HY04AAS1_0084       142    VWETY-ITPLT-------KGKLKKNVWLKMKP-GMLEKDSPTDESGAFSPADNSVEALIERGAVFLACHNQTWEMAGALL  212
BCAM2440            143    AWDKYGLAKFA-------GAAFPTNTLLDAKPAQAKGAQDHELPDGAFSSHDNGIAALQQRGIVFLSCHNAIWELAERLD  215
Gobo1_010100016936  146    IWDKYQLAKLA-------GPAFAFNTLIKT-PDGALDASAHEDPKGLFGAAGNTIPILQKRGVVFMACHNAIWEQTGKLI  217
GeurL1_010100014279 146    IWDKYQLAKLA-------GPAFASNTLIKT-PDGALDASAHEDTKGLFGAAGNTIPILQKRGVVFMACHNAIWEQTGKLI  217
GOX2656             146    IWDKYQLAKLA-------GPAFAFNTLIKT-PDGALDASAHEDPKGLFGAAGNTIPILQKRGVVFMACHNAIWEQTGKLI  217
UBAL3_43660050      146    MWEKYRLYKKA-------GVKSKTNPLIRLSPASTKPLSALNDPHGVLSPEDNNIPVLQARGVVFLACHNMIWELSGALK  218
LFE_1996            146    MWEKYKLHEKA-------GMKSAQNPLLKRSPASEKPLSDLNNPHGVLSPEDNNIPVLQARGVVFLGCHNMIWELSGALK  218

AFE_0042            223    SKGINPDHLSHEEMAAEFTNHLIPGVVLTPGIVGTLPELQLAG-YQYAK--------  270
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Acife_2603            223   NKGINPSKLSHVEMAAEFTNHLIPGAILTPGIVGTLPELELAG-FQYAK--------  270
AFE_0050              223   KKGINPDGLSHEQMAAEFTNHLIPGVILTPGVVGTLPELQLAG-FQYAK--------  270
Tint_0029             219   AIGHNPDKVSHAQMAAEFTNHLIPGAVLTPGIVGTLVELENAG-YRYAG--------  266
ACMV_19130            228   KRGINPDKLSHEQLAAEFTNHLIPGAILTPGIVGTIPQFELAG-FQYAK--------  275
HYPMC_2183            213   EKGVNPDKLEVDALAAELTNHLIPGVVLIPGAVATLPELQQAG-FHYAR--------  260
HY04AAS1_0084         213   KAGINPHKLTHDELSADLTNHIIPQAIVTPGVVATIPELQMRG-FHYINTADFPK--  266
BCAM2440              216   GANANPDKLPLDALAADLTNHVIPSAIVTPGAVGTLPELQQAG-FTYAK--------  263
Gobo1_010100016936    218   EKGINPDHLSHEQMAAELTNHLIDGVVLTPGIVATIPELQHAG-FGYVK--------  265
GeurL1_010100014279   218   EKGINPDRLSHEQMAAELTNHLIDGVVLTPGIVATIPELQHAG-FGYVK--------  265
GOX2656               218   EKGINPDHLSHEQMAAELTNHLIDGVVLTPG---------HRG-NRSLNCNMPVSAT  264
UBAL3_43660050        219   KEGTNPDHKTHGEIAAELTNHLIPGVVLTPGIVGTLPLLVEAG-YQYAHA-------  267
LFE_1996              219   KGGVNPDHKTHGEIAAELTNHLIPGVVLTPGIVGTIPLLSEAG-FHYARS-------  267
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FIG S5 Multiple alignment of thiosulfate dehydrogenase homologues.

Homologues of thiosulfate dehydrogenase (TSD) were obtained by BLASTP analysis and indicated in accession numbers. The alignment was made using the ClustalW program. Completely conserved residues across all the aligned sequences are indicated by red letters. Highly conserved residues are indicated by blue letters. The same residues as TSD of *A. ferrooxidans* ATCC 23270 were indicated by green letters.

AFE_0042: Tat pathway signal sequence domain-containing protein [*Acidithiobacillus ferrooxidans* ATCC 23270, YP_002424556]
Acife_2603: Tat pathway signal sequence domain-containing protein [*Acidithiobacillus ferrivorans* SS3, YP_004785011]
AFE_0050: Tat pathway signal sequence domain-containing protein [*Acidithiobacillus ferrooxidans* ATCC 23270, YP_002424564]
Tint_0029: Tat pathway signal sequence domain-containing protein [*Thiomonas intermedia* K12, YP_003641775]
ACMV_19130: hypothetical protein [*Acidiphilium multivorum* AIU301, YP_004284142]
HYPMC_2183: unnamed protein product [*Hyphomicrobium* sp. MC1, YP_004675973].
HY04AAS1_0084: hypothetical protein [*Hydrogenobaculum* sp. Y04AAS1, YP_002120754]
BCAM2440: hypothetical protein [*Burkholderia cenocepacia* J2315, YP_002235043]
Gobo1_010100016936: hypothetical protein [*Gluconacetobacter oboediens* 174Bp2, ZP_08900025]
GeurL1_010100014279: hypothetical protein [*Gluconacetobacter europaeus* LMG 18494, ZP_08903590]
GOX2656: hypothetical protein [*Gluconobacter oxydans* 621H, YP_190376]
UBAL3_43660050: protein of unknown function [*Leptospirillum ferrodiazotrophum*, EES53836]
LFE_1996: unnamed protein product [*Leptospirillum ferrooxidans* C2-3, YP_005469801]

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AFE_0043                 1   MKKSKTLQVATVAAVASLLFCGVAAQA-----ADMGWNGKAEAPRYQEQVFPPWQHGENNPAIHQGLEFTVPEVDDLADF  75
Acife_1383               1   MKKTTIARMTLLAACTSMGLWGMTAHA-----ADMGWHGKAEVPRYQEQVFPPWQHGANNPTVKKGLNFTVPEVDDLADF  75
AFE_0049                 1   MTKSSTLRLAPLLAAASLMVFGTCAVA-----ADNGWGAPGEFHGTKSQVFPPWQNGANNPATSKGLDFTIPEVDNLPDF  75
Tint_0030                1   MRRIP-LATGTPLLAALLLALPVLAKAQQVQQADTGWQGKVHAPQYKETIFPPWQHGANNPALNKGLEFTVPEVNDLPDF  79
GOX2657                  1   MTPSRIRKTLLATAAATFVLSTAAALADPVQ-----WNGKAQTPHYAEDVFPPWQHGQNNDATRRGFEFTVPEVDVLADF  75
Gobo1_010100016941       1   ----------MATAAATFVLSTAAALADPVQ-----WNGKAQTPHYAEDVFPPWQHGQNNDATRRGFEFTVPEVDVLADF  65
ACMV_19140               1   MRNRH----IKAVATGLFVLLAGAGLAHATSPHDTGWRGKVQPPQYRGAIFPPWQGGANNDTVNKGFVFTIPEVDDVADF  76
HYPMC_2184               1   MRANSIVKA-VFGAAIAGTVTLGAISAQSAE-----QN--------APNIFPPWQHGFNNDATDRGFEFTVPQVDNLADF  66
UBAL3_48660049           1   MKKSG----LASAFLASLTLFLHGGPLSP-----------AESLAASSEIFPPWSHGRNNPSPDKGIDVTVPDVDNLPDL  65
LFE_1995                 1   MNQRP----LMTILAVSLLL---GGVSSG-----------KTVWADETSTYPPWSHGHNNPSVGKGVNVTVPDVDNLPDL  62
METDI0749                1   MARLS---------LPAVLSGLALLAASPAG---------ADPQRDTNRAFPPWQGGANAPARDKGLEFTVHEADNLADF  62
ACP_2404                 1   MSMYR-------RLLTTLLLMTAGAALAAVQ-------------QASAQQGPPWSRGANDPAA-QGYVFQVPDVDNVPDL  59

AFE_0043                76   HGSIDNPQLTIFVGGNYYFAMAPLVKAFEKEYPALRGKIYYETLPPGILIKQMKQGGTITIGNMTWTVKPDVYAAGLKKV  155
Acife_1383              76   HGSIDHPQLVIFVGGNYFFAMAPLVAAFEKEYPEIKGKIYYETLPPGILIRQMQQGNTITVGNMTWTIQPDVYAAGLKKV  155
AFE_0049                76   HGSIDNPKLSIFVGGNYFFAMAPLVAAFEKEHPEIKGRIYYETIPPGLLIRQMEHGNTITVGNMTWTVKPDVYAAGLQKV  155
Tint_0030               80   HGDLDHPKLVIFVAGNYYFAMAPLVQAFEKEHPDFKGRVFYETLPPGILLKQMKAGGTITVGNMTWTVKPDVYAAGLKKI  159
GOX2657                 76   HGDITNPSLVLYVGGNYFFAMAPLVSAFEHEHPEFSGKIYWETIPPGLLIRQIEAGGTITSGNMTWTAKPDAYFAGLKKI  155
Gobo1_010100016941      66   HGDITNPSLVLYVGGNYFFAMAPLVSAFEHEHPEFSGKIYWETIPPGLLIRQIEAGGTITSGNMTWTAKPDAYFAGLKKI  145
ACMV_19140              77   HGDVDHPRLVLYIGGNYYFAVAPLVKMFEKENPQYLGKVFVVTIPPGMLIKAMNADGRFMIGNMSFTAKPDAYLAGLKKV  156
HYPMC_2184              67   HGSVTDPKLVLYVGGNYFFAMAPLVDEFEKAHPEFKGRIYWETLPPGLLVHQIKAHGVVTSGNMTWTAKPDAYFAGLVAV  146
UBAL3_48660049          66   HGNLSHPRLVLYVGGNYFFAMAPLVKAFEKKYPQIRGGLFYETLPPGLLAKQIGKKGRLTIGNATMTVPGDVYAAGLKKV  145
LFE_1995                63   HGNMSHPELVMYVGGNYFFAMAPLVEAFEKKYPKIRGKLFYETLPPGLLAKQIAEKGRLTVGNATMTVPGDLYAAGLGQV  142
METDI0749               63   HGDLTNARLILYVGGNYFFAMAPLVAAFEARHPELAGRVYYETIPPGLLVRQMRAGGRVTVGNMTWTAKPDAYLAGLGAD  142
ACP_2404                60   HGNPCDARLVLFIGGNQFFVLPRLIAAFEQQHPELKGHIFYETLPPGILRRQIAHDDTLTLGNLTLRVHPDVYEAGLRAV  139

AFE_0043               156   NAFIKEGLLQGPAVPYVTNDLTIMIPKGNPAHITGLQDLGKPGVRLSMPNPAWEGVARQIKMSLTKAGGPALEKMVYDTK  235
Acife_1383             156   NYLIHQGLLQGPAVPYVTNDLTIMIAKGNPAHIETLRDLGKPGIRLSMPDPRWEGVARQIQISLHKAGGPALERMVYDTK  235
AFE_0049               156   DYYVQHGLLKGPAVPYVTNDLTIMVPKGNPAHISGLQDLGKPGMRLVMPNPAWEGIARQIKMSLTKAGGPALATAVYDTK  235
Tint_0030              160   EAGIHDGVLTGKAISYVSNDLTIMVPKGNPAHITGLADLGKPGVTLSMPNPAWEGVARQIEASLKKAGGDALEQAVYDTK  239
GOX2657                156   DQYIKSGLLAAPAVPYVTNTLTIMIPKDNPGHVESLKDLGRPGIRLAMPNPEFEGIARQIEASLDKAGGKALANAVYKTK  235
Gobo1_010100016941     146   DQYIKSGLLAAPAVPYVTNTLTIMIPKDNPGHVESLKDLGRPGIRLAMPNPEFEGIARQIEASLDKAGGKALANAVYKTK  225
ACMV_19140             157   KSLIAAGRLTAPAVAYATNDLTIMVPKGNPDHIRDLADLARKGVKVAMPNPAFEGIARQIEASLRKAGGKKLEHEIYNTG  236
HYPMC_2184             147   KRLVDDGTLAGPPVPYVKNTLTIMVPKDNPGHVSGLADLGRAGLRIAMPNPEFEGVARQIKASLKKAGGEALEKEVYETK  226
UBAL3_48660049         146   RILIAKKLLVGKPVVYATNDLTIMVPKGNPAGIKGLADLGKPGVRLVMPNPAWEGVARQIKGALEKAGGKKLVQAVYVDK  225
LFE_1995               143   RKLIAKKLLVGPPVAYATNDLTIMVPKGNPAGIKGLSDLGKPGVRLVMPNPAWEGVSRQIKGSLVKAGGEKLSKTVYEDK  222
METDI0749              143   ERLVREGDLIGPAAPYVTNQLAIMVPKGNPAKVTGLADLARPGLRLAMPNPEFEGVARQIKSALRKAGGEDLVRTVYETK  222
ACP_2404               140   RQMQTAGQLHG-VVAYATNDLAIMVAKGNPRGIHSLADLARPGIRLSMPNPQWEGVANQIAASLRKAGGESLYHAVYQAK  218
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AFE_0043             236   VKNGETILTHIHHRQTPLFLMQGLADAGVTWKSEAIFQEQAGHPIANVPIPAKDNTTAIYASAVVKGAAHPKWAKDWVNF  315
Acife_1383           236   VKNGETILTHVHHRQTPLFLMQGLADAGVTWKSEAIFQEQSGHPISNIPIPAKDNTTAIYASAVAKGALHPKWAKDWVNF  315
AFE_0049             236   VKNGETILTEIHHRQSPMFLMQGRAVAGVTWKSEAIFQEKIGNPIGNVAIPAQFNTKAIYAAAAVKDAPHPKWAEAWVNF  315
Tint_0030            240   VKNGETTLTHIHHRQTPLYLMQGVAQAGVTWKSEAIFQEQAGHPISNVPIPADQNTTAIYAAAVVKGAAHPKAGLMWAEF  319
GOX2657              236   IANGSTVLTHIHHRQTPLFLMQGLADAGVTWQSEAIFQEQTGHAISHVDIPADQNSTAIYAGAMVKGAAHPQAAKLWLDF  315
Gobo1_010100016941   226   IANGSTVLTHIHHRQTPLFLMQGLADAGVTWQSEAIFQEQTGHAISHVDIPADQNSTAIYAGAMVKGAAHPQAAKLWLDF  305
ACMV_19140           237   VKNGTTLLTHIHHRQTPLFLMQGLADAGVTWKSEAIFQEEIGHPISHVDLPARYNTTAIYGGAMVKNAPHPHAAKAWLSF  316
HYPMC_2184           227   VADGGTILTHIHHRQTPLFLMQGHVDAGVTWLSEAMFQEQVGNPITNVVIPADQNTTATYAGAMVKGAAHPEAAKMWLDF  306
UBAL3_48660049       226   VKNGETVLTRIHHRQSPMALMRKKADAGVTWRSEAVFQEEEGHPIENVSIPDSQNVRAIYAAGIIRGSRHVLWAKRWLTF  305
LFE_1995             223   VKNGETTLTRIHHRQSPMFLMAKKADAGVTWRSEAIFQEQVGHAIENVAIPDSQNVKAIYAASIIRGARHKLWAKRWLIF  302
METDI0749            223   VADGTTTLTRMHHRQTPVWLMQGRADAGVTWRSEAIFQAEANLPTERVDIPADENETGAYAGGAVKDAAHPAEALSWLNF  302
ACP_2404             219   VQSGQTVLTEIHHRQTPMRIMSGQADAGVTWASEVRFQESIGNPIQGLAIPAAQNATAIYAGGALNHAPHPAVAAAWLAF  298

AFE_0043             316   LKSPTALQIFEHYGFKPY-T-GK--------  336
Acife_1383           316   LRSPVALHIFERYGFKAYHD-AK--------  337
AFE_0049             316   LKSPTALHIFEEYGFQPY-TQGK--------  337
Tint_0030            320   LRSPTALKIFERYDFKPVSA-HKPG------  343
GOX2657              316   IKSPTALAIFERYGFKAY--HGGSMPDHDSE  344
Gobo1_010100016941   306   IKSPTALAIFERYGFKAY--HGGSMPDHDSE  334
ACMV_19140           317   IQSPAALKIFEHYGFKQY----KRV------  337
HYPMC_2184           307   IHSPSALKIFEKYGF---------TPA----  324
UBAL3_48660049       306   LKSPEALHVFEHYGFKPV--TRKDL----K-  329
LFE_1995             303   LKSPEAQKVFRHFGFKSV--TKADL----KK  327
METDI0749            303   LRGAEAREIFARYGFEPY--RANAIPE--RR  329
ACP_2404             299   LKTPQAQAIYHQYGFRSLPAAASQK------  323
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FIG S6 Multiple alignment of putative sulfate-binding proteins.

Homologues of putative sulfate-binding protein (SBP) were obtained by BLAST analysis and indicated in accession numbers. The alignment was made using the ClustalW program. Completely conserved residues across all the aligned sequences are indicated by red letters. Highly conserved residues are indicated by blue letters. The same residues as SBP of *A. ferrooxidans* ATCC 23270 were indicated by green letters.

AFE_0043: putative sulfate-binding protein [*Acidithiobacillus ferrooxidans* ATCC 23270, YP_002424557]
Acife_1383: periplasmic solute-binding protein [*Acidithiobacillus ferrivorans* SS3, YP_004783859]
AFE_0049: putative sulfate-binding protein [*Acidithiobacillus ferrooxidans* ATCC 23270, YP_002424563]
Tint_0030: periplasmic solute-binding protein [*Thiomonas intermedia* K12, YP_003641776]
GOX2657: putative sulfate-binding protein [*Gluconobacter oxydans* 621H, YP_190377]
Gobo1_010100016941: putative sulfate-binding protein [*Gluconacetobacter oboediens* 174Bp2, ZP_08900026]
ACMV_19140: hypothetical protein ACMV_19140 [*Acidiphilium multivorum* AIU301, YP_004284143]
HYPMC_2184: unnamed protein product [*Hyphomicrobium* sp. MC1, YP_004675974].
UBAL3_48660049: ABC transporter, periplasmic component [*Leptospirillum ferrodiazotrophum*, EES53835]
LFE_1995: protein product [*Leptospirillum ferrooxidans* C2-3, YP_005469800].
METDI0749: sulfate-binding protein [*Methylobacterium extorquens* DM4, YP_003066433].
ACP_2404: hypothetical protein ACP_2404 [*Acidobacterium capsulatum* ATCC 51196, YP_002755444]

TABLE S1 Purification of recombinant TSD from soluble fraction of *E. coli* harboring pET-*tsd*.

Purification step	Total protein (mg)	Specific activity (μmol·min^{-1}·mg^{-1})	Total activity (μmol·min^{-1})	Recovery (%)	Purification (fold)
Cell-free extract	260.5	0.068	17.7	100	1
Soluble fraction at pH 4	32.6	0.26	8.5	48	3.8
Butyl-650M fraction	1.1	4.16	4.6	26	61.2

Enzyme activity was measured at pH 2.5 and 40°C in mixture containing 1 mM ferricyanide and 10 mM thiosulfate.

Supplemental References

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