

**Anaerobic Sulfur Metabolism Coupled to Dissimilatory Iron Reduction in the
Extremophile *Acidithiobacillus ferrooxidans***

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Supplemental Material Below

TABLE S1

TABLE S2

TABLE S3

FIG. S1

FIG. S2

FIG. S3

Table S1. Genes interrogated by qPCR and their respective PCR primers.

Gene	Primer (5' to 3')
<i>sreA</i> (forward)	ATGAGGACTTTCTCGTCAGCCACA
<i>sreA</i> (reverse)	TGCTGCCTTGCAGATTGTAGTTGC
<i>sreB</i> (forward)	TTGCCAAGCCTGTGCC
<i>sreB</i> (reverse)	TCCAGGTGGCGCCAG
<i>sreC</i> (forward)	GCCTTTGTTACCGTTTCC
<i>sreC</i> (reverse)	CAGGACCAGGGCAGGAAG
<i>sreD</i> (reverse)	GGCAATCCCTGAATGGC
<i>rpoC</i> (forward)	AATGCGGTGTTGAGGTAACC
<i>rpoC</i> (reverse)	AGGTACTGGTCTTCGGTAAG
<i>cbbL2</i> (forward)	ACGTCTTCGGCTTCAAGGCG
<i>cbbL2</i> (reverse)	GCAGGCGCGACCATAGTTCTT
<i>cbbS1</i> (forward)	TAGAACATAACCGAACCGGAAAACG
<i>cbbS1</i> (reverse)	GCCCCGATAGACTACCAGGGAAG
<i>cbbp</i> (forward)	AGACACCATCCTGCGCCGTAT
<i>cbbp</i> (reverse)	GCAGGAGGGTGGGGAAATTCT
<i>fba</i> (forward)	ATCAGGCCCTCATTCTGCAGC
<i>fba</i> (reverse)	GATCCATCTGGCAGGTCACACC

Table S2. Protein expression data from cytoplasmic and membrane enriched fractions of *Acidithiobacillus ferrooxidans*^T grown in anaerobic and aerobic conditions.

Spot ID ^a	Protein name	Predicted function	Accession ^b	Anova p-value ^c	Fold change ^d	Mowse score ^e	Sequence coverage [%] ^f	MW [Da] ^g	IP ^h
Up-regulated in aerobic conditions									
Energy metabolism									
60	HdrB	Heterodisulfide reductase subunit B, homolog	AFE_2586	0.001	9.4	86	18	50976	5.88
61	HdrB	Heterodisulfide reductase subunit B, homolog	AFE_2586	0.004	13.5	74	18	50976	5.88
45	HdrA	Pyridine nucleotide-disulfide oxidoreductase	AFE_2553	0.018	3.3	133	35	37657	5.58
47	HdrA	Pyridine nucleotide-disulfide oxidoreductase	AFE_2553	0.004	8.1	128	36	37657	5.58
49	Opr	Conserved hypothetical protein (clusters with TetH)	AFE_0030	0.005	8.5	108	18	43573	7.85
34	Orf2	Conserved hypothetical protein (clusters with Hdr)	AFE_2552	0.012	5.4	67	26	32473	5.82
59	AtpD	ATP synthase F1, β subunit [3.6.3.14]	AFE_3203	0.029	2.3	101	37	50564	5.01
68 ⁱ	AtpA	ATP synthase F1, α subunit [3.6.3.14]	AFE_3205	0.019	2.1 ⁱ	91 ⁱ	33 ⁱ	55570	5.28
68 ⁱ	NtrX	Putative nitrogen assimilation regulatory protein ⁱ	AFE_0024	0.019	2.1 ⁱ	129 ⁱ	41 ⁱ	51934	5.22
58	Cyc2	Outer membrane cytochrome <i>c</i> , Cyc2	AFE_3153	0.013	5.6	69	20	52358	5.54
Carbon management									
5	RfbA	Glucose-1-phosphate thymidyltransferase [2.7.7.24]	AFE_3295	0.010	2.8	181	48	32314	5.24
14	Fba	Fructose-bisphosphate aldolase, class II [4.1.2.13]	AFE_3248	0.023	2.7	89	27	38366	5.76
38	DhnA	Fructose-bisphosphate aldolase, putative	AFE_1802	0.002	4.9	139	51	32912	5.77
50	Fba	Fructose-bisphosphate aldolase, class II [4.1.2.13]	AFE_3248	0.005	3.4	192	58	38366	5.76
51	Fba	Fructose-bisphosphate aldolase, class II [4.1.2.13]	AFE_3248	0.022	3.0	170	45	38366	5.76
93	PdhB2	Dehydrogenase complex, E1 component, β subunit	AFE_1812	0.015	unique ⁱ	125	47	36335	5
94	PdhB2	Dehydrogenase complex, E1 component, β subunit	AFE_1812	0.001	unique ⁱ	140	49	36335	5
42	PdhB	Pyruvate dehydrogenase, E1 component, β subunit	AFE_3069	0.020	2.4	139	38	35568	5.36
52	PdhB	Pyruvate dehydrogenase, E1 component, α subunit	AFE_1813	0.005	7.1	115	27	38960	5.51
53	PmbA	Regulates CrsA in carbon storage	AFE_0600	0.022	2.1	136	25	47568	5.55
55	Icd	NADP-dependent isocitrate dehydrogenase [1.1.1.42]	AFE_0424	0.007	2.2	94	17	45756	5.64
65	CbbL2	RuBisCo, large subunit 2 [4.1.1.39]	AFE_2155	0.017	2.9	176	43	50351	5.69
69	Pgi	Glucose-6-phosphate isomerase [5.3.1.9]	AFE_2924	0.018	10.9	142	30	59405	6.04
78	Rpe	Ribulose-phosphate 3-epimerase [5.1.3.1]	AFE_3247	0.004	2.5	97	37	23537	5.16
79	Tal	Transaldolase [2.2.1.2]	AFE_0419	0.007	2.2	184	55	39495	5.4

81	Tkt-1	Transketolase [2.2.1.1]	AFE_1843	0.007	2.1	129	20	74156	5.9
82	Xfp	Transketolase pyridine binding domain protein	AFE_1667	0.002	4.0	154	23	77857	5.75
83	Xfp	Transketolase pyridine binding domain protein	AFE_1667	0.007	7.0	106	20	77857	5.75
85	GlgB	1,4- α -glucan branching enzyme [2.4.1.18]	AFE_2836	0.018	4.6	85	17	83489	5.47
35	FghA	Esterase, putative/S-formylglutathione hydrolase	AFE_0698	0.016	3.9	84	26	31433	5.84
44	Hdh	2-hydroxyacid dehydrogenase family protein	AFE_3161	0.034	4.2	34	109	36359	5.59
39	PfkB	Carbohydrate kinase, PfkB family	AFE_0530	0.011	2.0	61	21	34507	5.41

Cell wall/membrane/envelope biogenesis

7 ⁱ	FadB	Malonyl CoA-acyl carrier protein transacylase	AFE_1907	0.029	2.0 ⁱ	54 ⁱ	38 ⁱ	33459	5.42
1	Lpp	Lipoprotein, putative	AFE_2811	0.030	2.2	65	48	10247	9.42
2	OmpA	Peptidoglycan-associated lipoprotein, putative	AFE_0071	0.011	2.2	90	39	19589	6.4
11	OmpA	Peptidoglycan-associated lipoprotein, putative	AFE_0071	0.012	4.1	122	39	19589	6.4
41	Gmd-1/Gmd-2	GDP-mannose 4,6-dehydratase	AFE_2977/3292	0.009	8.0	149/148	36	37986/38443	6.2/5.089
43	Gmd-1/Gmd-2	GDP-mannose 4,6-dehydratase	AFE_2977/3292	0.002	2.5	167/166	42	37986/38443	6.2/5.089

Post-translational modification/protein turnover/chaperone functions and Inorganic ion transport and metabolism

4	AhpC	Antioxidant, AhpC-Tsa family	AFE_0985	0.042	2.0	95	39	22363	5.69
7 ⁱ	GroEL	Chaperonin, 60 kDa	AFE_0542	0.029	2.0 ⁱ	114 ⁱ	41 ⁱ	58594	5.2
8	DeqQ	Serine protease, DO-DeqQ family	AFE_2849	0.045	2.0	67	17	53366	7.3
9	DeqQ	Serine protease, DO-DeqQ family	AFE_2849	0.040	2.4	66	17	53366	7.3
10	HscA	Fe-S protein assembly chaperone	AFE_0677	0.021	6.2	129	33	66023	5.09
13	GroEL	Chaperonin, 60 kDa	AFE_0542	0.029	2.1	89	21	58594	5.2
15	AhpC	Antioxidant, AhpC-Tsa family	AFE_0985	3.4e-5	unique ^j	60	28	22363	5.69
31	AhpC	Antioxidant, AhpC-Tsa family	AFE_0985	0.015	6.7	152	73	22363	5.69
32	SspA	Stringent starvation protein A	AFE_0221	0.024	2.2	89	38	23506	5.67
66	AldA	Aldehyde dehydrogenase (NAD) family protein	AFE_2131	0.003	3.1	168	33	55325	5.13
67	AldA	Aldehyde dehydrogenase (NAD) family protein	AFE_2131	0.006	4.1	106	30	55325	5.13
74	GroEL	Chaperonin, 60 kDa	AFE_0542	0.005	2.8	145	36	58594	5.2
75	DnaK	Chaperone protein Dnak	AFE_2665	0.002	3.3	167	33	68180	5.04
91	GstA	Glutathione S-transferase family protein	AFE_0177	0.001	unique ^j	71	29	22082	5.31
95	HscA	Fe-S protein assembly chaperone	AFE_0677	3.9e-4	unique ^j	57	14	66023	5.09
30	Sod	Superoxide dismutase [1.15.1.1]	AFE_1898	0.002	9.3	93	31	22934	6.05

Translation, transcription, and cell cycle

6	PspA	Phage shock protein A (IM30)	AFE_2017	0.002	2.4	101	42	26454	4.87
33	RplY	Ribosomal protein L25	AFE_2145	0.008	11.5	52	14	22124	5.86

37	Tsf	Translation elongation factor Ts	AFE_1445	4.7e-4	13.4	113	43	31227	5.13
46	RpoA	DNA-directed RNA polymerase, α -subunit	AFE_0354	0.041	7.4	212	34	36941	5.2
56	Tuf-1/Tuf-2	Translation elongation factor Tu	AFE_0325/0311	0.003	4.7	167	44	43152	5.35
57	Tuf-1/Tuf-2	Translation elongation factor Tu	AFE_0325/0311	0.005	3.0	120	36	43152	5.35
63 ⁱ	SerS	Seryl-tRNA synthetase [6.1.1.11]	AFE_0380	0.001	3.0 ⁱ	83 ⁱ	28 ⁱ	46529	5.76
76	ThrS	Threonyl-tRNA synthetase [6.1.1.3]	AFE_2614	0.011	3.0	115	17	72519	5.56
77	GlyS	Glycyl-tRNA synthetase, β -subunit [6.1.1.14]	AFE_2581	0.016	2.5	175	25	76381	5.87
80	FtsA	Cell division protein	AFE_0201	0.055	3.2	113	26	44997	5.45
84	Pnp	Polyribonucleotide nucleotidyltransferase [2.7.7.8]	AFE_0395	0.016	2.5	135	39	74344	5.72
87	FusA	Translation elongation factor G	AFE_0324	0.026	2.2	189	26	76507	5.13
88	FusA	Translation elongation factor G	AFE_0324	0.035	1.7	180	30	76507	5.13
97	RpoB	DNA-directed RNA polymerase, β -subunit [2.7.7.6]	AFE_0320	0.009	unique ^j	178	18	15167	5.42
99	AlaS	alanyl-tRNA synthetase [6.1.1.7]	AFE_0934	0.001	unique ^j	145	29	95466	5.32
Amino acid metabolism and transport									
16	MetZ	O-succinylhomoserine sulfhydrylase [4.2.99.-]	AFE_2063	5.5e-7	unique ^j	79	30	43586	6.19
40	IlvC	Ketol-acid reductoisomerase [1.1.1.86]	AFE_0747	0.045	2.1	175	40	36393	6.02
48	LeuB	3-isopropylmalate dehydrogenase [1.1.1.85]	AFE_2075	0.010	6.3	104	31	38384	5.04
64	PepA	Cytosol aminopeptidase [3.4.11.1]	AFE_2201	0.002	9.7	92	31	52811	5.3
70	SerA	D-3-phosphoglycerate dehydrogenase [1.1.1.95]	AFE_0896	0.008	2.0	142	35	56028	5.69
71	GlnA	Glutamine synthetase, type I [6.3.1.2]	AFE_0466	0.002	7.8	114	25	51743	5.1
72	GlnA	Glutamine synthetase, type I [6.3.1.2]	AFE_0466	0.017	8.1	155	31	51743	5.1
73	IlvD-2	Dihydroxy-acid dehydratase [4.2.1.9]	AFE_0662	0.010	2.2	140	22	65364	6.21
86	MetE	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase [2.1.1.14]	AFE_0288	0.005	10.3	102	22	87873	5.86
90	IscS-3	Cysteine desulfurase IscS [4.4.1.-]	AFE_0673	4.4e-4	3.4	71	15	45174	5.85
100	PyrA	Conserved hypothetical protein	AFE_0004	0.003	unique ^j	61	11	129489	5.92
Nucleotide metabolism and Transport									
62	PurA	Adenylosuccinate synthetase [6.3.4.4]	AFE_1889	0.010	4.9	126	31	46428	5.73
63 ⁱ	PurD	Phosphoribosylamine-glycine ligase [6.3.4.13]	AFE_2261	0.001	3.0 ⁱ	56 ⁱ	29 ⁱ	44316	6.08
Other proteins									
3	TtgA	Toluene tolerance protein, putative	AFE_1830	0.006	2.6	94	44	25160	9.07
12	TtgB	Toluene tolerance protein, putative	AFE_0161	0.024	3.2	83	47	23455	9.33
36	YbjQ	Conserved domain protein	AFE_2019	1.1e-4	2.2	134	38	36503	6.46
54	PyrB	Pyridine nucleotide-disulfide oxidoreductase	AFE_2601/ AFE_3118	0.040	6.5	127	35	42226	6.19
89	TypA	GTP-binding protein TypA	AFE_2740	0.008	3.0	194	27	66924	5.26

17	Trp2	TPR domain protein	AFE_2762	2.0e-4	unique ^j	56	10	66424	6.21
92	Ppa	Inorganic pyrophosphatase [3.6.1.1]	AFE_0492	0.007	unique ^j	79	31	19125	5.07
96	Fer1	Oxidoreductase, molybdopterin binding subunit	AFE_0809	0.023	unique ^j	108	14	83660	6.02
98	Fer1	Oxidoreductase, molybdopterin binding subunit	AFE_0809	0.003	unique ^j	73	12	83660	6.02

Up-regulated in anaerobic conditions

Cell wall/membrane/envelope biogenesis

103	AmpC	Metallo-β-lactamase family protein (clusters with Sqr)	AFE_0269	0.011	5.4	158	74	25102	5.43
105	SreB	Sulfur reductase, iron-sulfur binding subunit	AFE_2178	0.040	unique ^j	53	57	22991	4.85
104	AtpF	ATP synthase F ₀ , b subunit [3.6.3.14]	AFE_3207	0.001	unique ^j	57	31	17888	6.77
19	ThiJ	4-methyl-5(B-hydroxyethyl)-thiazole monophosphate biosynthesis enzyme	AFE_0523	0.008	3.0	54	35	21501	4.73

Carbon management

18	CbbS1	RuBisCo, small subunit [4.1.1.39]	AFE_1690	0.047	3.8	68	62	12869	5.9
23	CscE	Carboxysome shell peptide	AFE_1683	0.044	3.1	118	66	11357	5.25
25	p30	p30 protein containing a CBBQ domain	AFE_1678	0.036	2.4	98	33	29643	5.3
27	p30	p30 protein containing a CBBQ domain	AFE_1678	0.003	unique ^j	58	30	29643	5.3
22	AcnA	Aconitate hydratase, putative	AFE_0423	0.020	2.0	65	18	68181	5.84
101	Bfr	Bacterioferritin	AFE_1682	0.029	4.5	68	40	16741	5.22
21	ManB	Phosphomannomutase, putative	AFE_0943	0.016	3.0	56	18	51733	5.45

Cell wall/membrane/envelope biogenesis

20	Tat	Tat (twin-arginine translocation) pathway signal sequence domain protein	AFE_0042	0.001	2.2	k	k	29455	6.61
26	Tat	Tat (twin-arginine translocation) pathway signal sequence domain protein	AFE_0042	1.3e-6	unique ^j	k	k	29455	6.61
102	Slp	Outer membrane lipoprotein Slp, putative	AFE_2316	0.003	4.2	80	47	19169	8.44

Amino acid metabolism and transport

24	IlvD-1	Dihydroxy-acid dehydratase [4.2.1.9]	AFE_1673	0.011	7.4	102	20	58522	6.04
29	IlvD-1	Dihydroxy-acid dehydratase [4.2.1.9]	AFE_1673	4.5e-5	unique ^j	66	10	58522	6.04

Nucleotide metabolism and Transport

28	Dho	Dihydroorotase, homodimeric type [3.5.2.3]	AFE_1672	0.002	unique ^j	55	27	38548	6.73
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^aSpot IDs refer to the numbers in the supplemental figures.

^bAnnotation No. is that of Genbank genome annotation NC011761.

^cAnova test was performed for three replicates of each condition and spots with p-values < 0.05 were considered to be significant.

^dProteins with fold change ≥ 2.0 are regarded as differentially expressed.

^eMowse score is generated by Mascot and characterizes the hit of a peptide mass fingerprint (PMF) search giving the probability that the hit is a random match in the database (scores > 47 are significant with a significance threshold of 0.05).

^fSequence coverage (generated by Mascot) presents the percentage of the database hit that is covered by the submitted peptide masses of the experimentally acquired PMF.

^gMW is the predicted molecular weight of the database entry.

^hThe predicted isoelectric point (IP).

ⁱThe protein spot contains a mixture. No conclusions can be drawn regarding up-regulation of the individual protein. Mowse score and sequence coverage are given for the individual components.

^jUnique refers to spots present in either aerobic or anaerobic conditions that do not have a matching spot under the other condition (unique spots listed alongside up-regulated proteins under aerobic conditions do not have a match under anaerobic conditions and vice versa).

^kSpots identified by MS/MS and *de novo* protein sequencing.

Table S3. Microarray expression data of *Acidithiobacillus ferrooxidans*^T grown in aerobic and anaerobic conditions. Some of the reported gene transcripts were <2 fold differentially regulated; however, they have been retained as the results were reproducible and the T-test confirmed that the data were significant.

Gene name	Predicted function	Accession ^a	Log ₂ ratio median ^b	t-Test (p-value) ^c
Up-regulated in aerobic conditions				
Energy metabolism				
<i>tetH</i>	Tetrathionate hydrolase	AFE_0029	4.3	1.4e-5
<i>hoxF</i>	[NiFe] hydrogenase β-subunit, putative	AFE_0940	1.0	1.0e-3
<i>hdrB</i>	Heterodisulfide reductase subunit B, homolog	AFE_2586	1.0	2.0e-3
<i>nuoI</i>	NADH-quinone oxidoreductase, subunit I	AFE_2622	0.7	0.030
<i>atpC</i>	ATP synthase F1, ε-subunit	AFE_3202	1.0	0.044
<i>tolQ</i>	MotA/TolQ/ExbB proton channel family protein	AFE_0768	0.8	0.001
<i>cyc1</i>	Cytochrome <i>c4</i> , <i>Cyc1</i>	AFE_3152	0.7	0.040
<i>cyc2</i>	Outer membrane cytochrome <i>c</i> , <i>Cyc2</i>	AFE_3153	0.9	3.0e-3
<i>coxA</i>	Cytochrome <i>c</i> , <i>aa</i> ₃ -type, subunit I	AFE_3149	0.9	0.046
<i>coxC</i>	Cytochrome <i>c</i> , <i>aa</i> ₃ -type, subunit III	AFE_3148	0.9	0.025
<i>rus</i>	Rusticyanin	AFE_3146	1.7	0.026
<i>hynD</i>	Hydrogenase maturation protease	AFE_3281	1.0	4.0e-3
<i>hynS</i>	[NiFe] hydrogenase small subunit	AFE_3283	1.3	4.0e-3
	Quinone interacting membrane-bound oxidoreductase subunit	AFE_3284	1.2	0.00
Carbon Management				
<i>pgd</i>	6-phosphogluconate dehydrogenase	AFE_2025	0.9	0.003
<i>zwf</i>	Glucose-6-phosphate 1-dehydrogenase	AFE_2024	1.2	0.004
<i>pdal</i>	Dehydrogenase complex, E1 component, α subunit	AFE_1813	0.8	3.0e-4
<i>gph-1</i>	Phosphoglycolate phosphatase	AFE_1823	0.7	0.003
<i>xfp</i>	Xylulose-5-phosphate/fructose-6-phosphate phosphoketolase	AFE_2053	0.9	9.2e-4
<i>amyA</i>	α-amylase family protein	AFE_2082	1.2	0.00
	Glycosyl transferase, group 1	AFE_2083	0.9	0.00
<i>adhP</i>	Zn-binding alcohol dehydrogenase family protein	AFE_2088	0.8	0.00
<i>pgm</i>	Phosphoglucomutase	AFE_2324	1.0	0.009
<i>glgB</i>	1,4-α-glucan branching enzyme	AFE_2836	0.7	0.02
Cell wall/membrane/envelope biogenesis				
<i>cfa</i>	Cyclopropane-fatty-acyl-phospholipid synthase domain protein	AFE_2437	0.7	0.026
<i>ftsQ</i>	cell division protein	AFE_0202	1.2	6.4e-4
<i>ftsI</i>	peptidoglycan synthetase	AFE_0212	1.1	0.007
<i>murC</i>	UDP-N-acetylmuramate--alanine ligase	AFE_0205	1.1	0.001
<i>mraY</i>	phospho-N-acetylmuramoyl-pentapeptide-transferase	AFE_0209	0.7	0.044
<i>murF</i>	UDP-N-acetylmuramoyl-tripeptide-D-alanyl-D-alanine ligase	AFE_0210	1.2	0.003
<i>murE</i>	UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase	AFE_0211	1.1	0.004
<i>mraW</i>	S-adenosyl-methyltransferase	AFE_0214	1.3	0.006
<i>mraZ^d</i>	MraZ protein (function unknown)	AFE_0215	1.1	0.002
<i>amiC</i>	N-acetylmuramoyl-L-alanine amidase, putative	AFE_2361	0.8	0.002
<i>mltB</i>	membrane-bound lytic murein transglycosylase B, putative	AFE_1827	0.8	0.020
Post-translational modification/protein turnover/chaperone functions and Inorganic ion transport and metabolism				
<i>copB</i>	copper-translocating P-type ATPase (copB)	AFE_2439	1.5	0.001
<i>copB</i>	copper-translocating P-type ATPase (copB)	AFE_2779	1.4	1.7e-4
<i>cic</i>	voltage-gated chloride channel	AFE_3129	1.2	0.002
<i>clpB</i>	clpB protein	AFE_1970	1.0	0.011
<i>cbpA</i>	curved DNA-binding protein	AFE_3102	0.9	0.007
<i>hsp</i>	heat shock protein, Hsp20 family	AFE_0871	1.2	0.014

<i>hsp</i>	heat shock protein, Hsp20 family	AFE_2086	0.9	0.001
<i>sod</i>	superoxide dismutase	AFE_1898	1.7	9.8e-5
<i>ahpC</i>	antioxidant, AhpC/Tsa family	AFE_0985	1.3	0.002
Translation, transcription, and cell cycle				
<i>ftsZ</i>	cell division protein FtsZ	AFE_0200	1.6	0.001
<i>ftsA</i>	cell division protein FtsA	AFE_0201	1.4	0.001
<i>ftsW</i>	cell division protein FtsW	AFE_0207	0.7	0.009
<i>ftsL</i>	cell division protein FtsL	AFE_0213	0.8	0.007
<i>ftsB</i>	septum formation initiator family protein	AFE_0850	1.3	0.003
<i>iscR</i>	Iron-sulfur cluster assembly transcription factor	AFE_0672	1.2	0.024
<i>iscA^d</i>	Iron-sulfur cluster assembly protein	AFE_0675	1.1	0.008
Nucleotide biosynthesis and transport				
<i>guaA</i>	glutamine amidotransferase, class I	AFE_2528	0.7	0.046
Gene regulation				
<i>arsR</i>	transcriptional regulator, ArsR family	AFE_2641	0.8	6.9e-4
<i>tetR</i>	transcriptional regulator, TetR family	AFE_0255	0.9	0.002
Up-regulated in anaerobic conditions				
Energy metabolism				
<i>ubiE</i>	Ubiquinone/menaquinone biosynthesis methyltransferase	AFE_0289	0.7	2.0e-3
	NADH:flavin oxidoreductase/NADH oxidase	AFE_2211	0.7	3.0e-3
<i>tusA</i>	Hypothetical protein (Similar SirA)	AFE_2557	0.9	0.001
<i>drsE</i>	Pyridine nucleotide-disulphide oxidoreductase (Dsr-like)	AFE_2556	1.3	0.004
<i>atpA</i>	ATP synthase F1, α -subunit	AFE_3205	0.9	0.01
<i>atpF</i>	ATP synthase F0, β -subunit	AFE_3207	1.5	5.0e-3
<i>atpE</i>	ATP synthase C chain	AFE_3208	1.4	0.009
<i>pdhA</i>	Pyruvate dehydrogenase complex, E1 component, α -subunit	AFE_3070	0.7	1.0e-3
<i>atpB</i>	ATP synthase F ₀ , A subunit	AFE_3209	1.0	0.002
<i>cycA2</i>	Cytochrome c4, CycA2	AFE_2727	0.9	0.027
<i>petA2</i>	Ubiquinol-cytochrome c reductase, iron-sulfur subunit	AFE_2729	0.9	0.005
<i>petB2</i>	Ubiquinol-cytochrome c reductase, cytochrome b subunit	AFE_2730	1.1	0.007
<i>cysI</i>	Sulfite reductase (NADPH) hemoprotein β -component	AFE_3122	0.8	0.01
<i>cysH</i>	Adenylylsulfate reductase	AFE_3123	1.5	0.0
Carbon Management				
<i>cbbp</i>	Phosphoribulokinase	AFE_0536	0.7	0.009
<i>cscC</i>	Carboxysome shell peptide	AFE_1685	1.6	0.002
<i>tkt-2</i>	Transketolase	AFE_3252	0.9	0.022
Post-translational modification/protein turnover/chaperone functions and inorganic ion transport and metabolism				
<i>surA</i>	Survival protein SurA	AFE_3035	1.4	0.003
Translation, transcription, and cell cycle				
<i>nusG</i>	Transcription termination/anti-termination factor	AFE_0314	1.0	0.002
<i>fusA</i>	Translation elongation factor G	AFE_0324	1.3	0.047
<i>tuf-1</i>	Elongation factor Tu	AFE_0325	1.2	4.4e-4
<i>tuf-2</i>	Elongation factor Tu	AFE_0311	1.8	3.0e-4
<i>infC</i>	Translation initiation factor IF-3	AFE_2613	1.0	0.021
<i>yfiA</i>	Ribosomal subunit interface protein	AFE_3024	1.1	4.3e-5
<i>rplU</i>	Ribosomal protein L21	AFE_0285	1.1	0.016
<i>rplK</i>	Ribosomal protein L11	AFE_0315	1.4	0.002
<i>rplA</i>	Ribosomal protein L1	AFE_0316	1.1	0.001
<i>rpsL</i>	30S ribosomal protein S12	AFE_0322	1.3	0.007
<i>rpsG</i>	30S ribosomal protein S7	AFE_0323	2.0	0.043
<i>rpsJ</i>	Ribosomal protein S10	AFE_0326	1.6	0.005
<i>rplC</i>	Ribosomal protein L3	AFE_0327	0.7	0.017
<i>rplD</i>	Ribosomal protein L4	AFE_0328	0.8	7.9e-4
<i>rpsS</i>	Ribosomal protein S19	AFE_0331	1.6	0.006

<i>rpsM</i>	Ribosomal protein S13	AFE_0351	1.0	0.029
<i>rpsD</i>	Ribosomal protein S4	AFE_0353	0.8	0.002
<i>rpmF</i>	Putative ribosomal protein	AFE_1903	1.1	0.002
<i>rplY</i>	Ribosomal protein L25	AFE_2145	1.6	0.001
<i>rpsF</i>	Ribosomal protein S6	AFE_2824	2.1	0.002
<i>rpS18</i>	Ribosomal protein S18	AFE_2823	0.8	0.004
<i>rpsP</i>	Ribosomal protein S16	AFE_2855	0.9	0.017
<i>rpsI</i>	Ribosomal protein S9	AFE_3074	1.5	0.018
<i>rplM</i>	Ribosomal protein L13	AFE_3075	2.0	0.001

Amino acid metabolism and transport

<i>metE</i>	5-methyltetrahydropteroyltri-glutamate-homocysteine S-methyltransferase	AFE_0288	1.1	0.006
<i>cysI</i>	sulfite reductase (NADPH) hemoprotein beta-component	AFE_3122	0.9	0.019
<i>cysH</i>	Adenylylsulfate reductase	AFE_3123	1.5	0.008
<i>dapA</i>	dihydrodipicolinate synthase	AFE_2358	0.8	0.009

Nucleotide biosynthesis and transport

<i>purB</i>	adenylosuccinate lyase	AFE_2356	1.5	0.001
<i>guaB</i>	inosine-5'-monophosphate dehydrogenase	AFE_1777	1.2	0.003

Other genes

<i>acpP</i>	Acyl carrier protein	AFE_1909	1.0	0.006
<i>Fad</i>	Fatty acid desaturase domain	AFE_1964	1.3	0.001
<i>yajC</i>	Pre-protein translocase, YajC subunit	AFE_2058	1.1	0.010

^a Annotation No. is that of Genbank genome annotation NC011761.

^b A 0.7 fold deviations from the 1:1 hybridization (\log_2) ratio are taken as indicative of differential gene expression.

^c Info on T-test and significance.

^d The gene is in COG designation S (function unknown) but is included due to its gene context.

Fig. S1. Representative aerobic (A) and anaerobic (B) *Acidithiobacillus ferrooxidans* 2D-PAGE soluble protein gel and insets giving comparisons between aerobic (C) and anaerobic gels (D). 200 μ g protein was applied to each gel. Differentially expressed protein spots circled (numbers refer to the spot IDs in Supplemental Table 1).

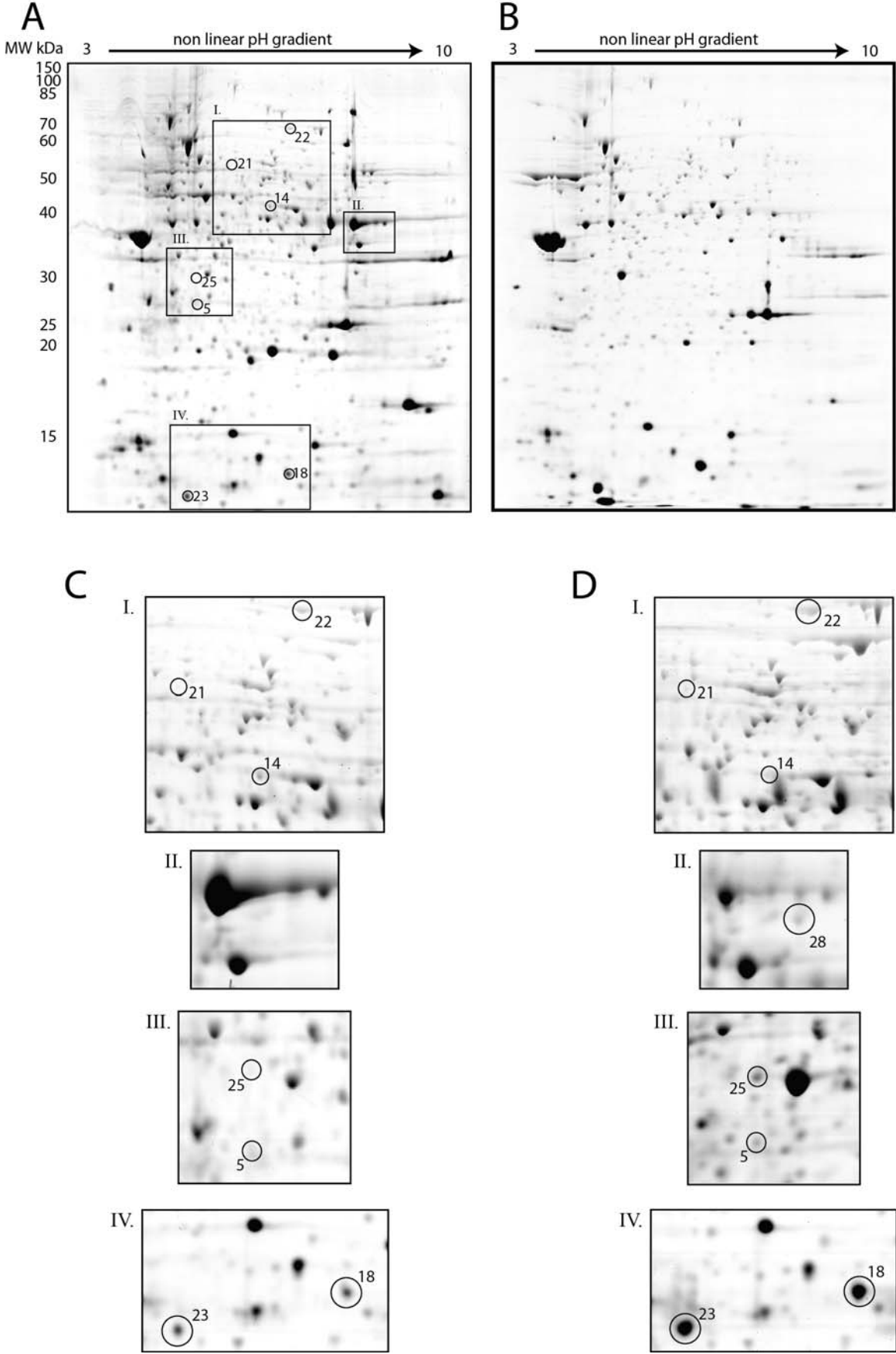


Fig. S2. Representative outer membrane protein enriched fraction (200 μ g protein applied to each gel) of *Acidithiobacillus ferrooxidans* cultured aerobically (A) and anaerobically (B). Differentially expressed protein spots in (A) and (B) are circled with numbers referring to the spot IDs in Supplemental Table 1. In part A, only the spots which are up-regulated in aerobic conditions are shown whereas part B displays spots up-regulated in anaerobic conditions. A train of protein spots (OMPP1/FadL/TodX family protein, indicated by solid arrows in B) as well as one large spot (major outer membrane protein 40, indicated by open arrow in A, B) stand out against the general protein pattern. To avoid a skewed analysis of membrane enriched gels, five spots (arrowed with an asterisk) were excluded from the analysis.

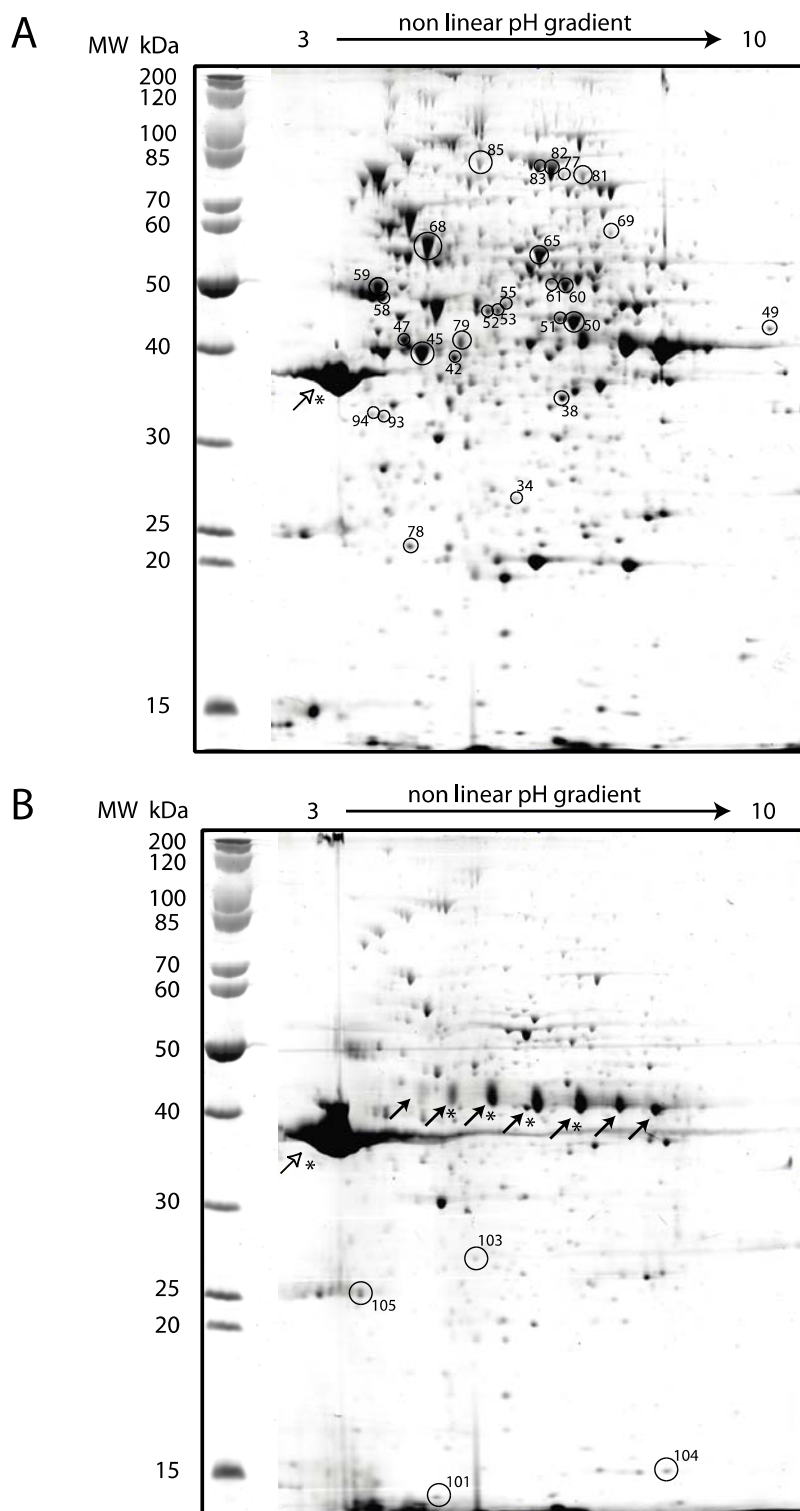
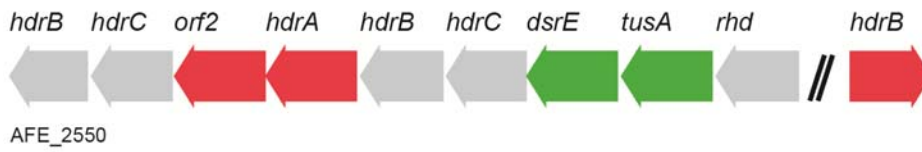
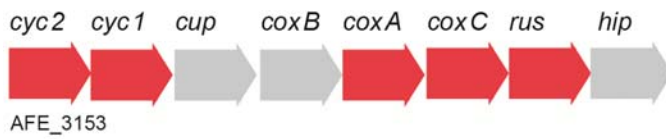


Fig. S3. Predicted genes and operons of energy metabolism and carbon management up-regulated in aerobic (red) and anaerobic (green) conditions. Genes that were not differentially expressed are in grey.

Heterodisulfide reductase complex



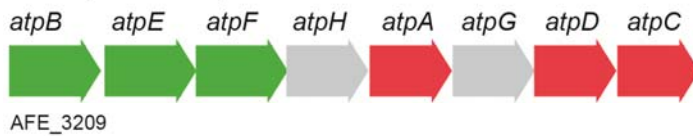
Rus operon



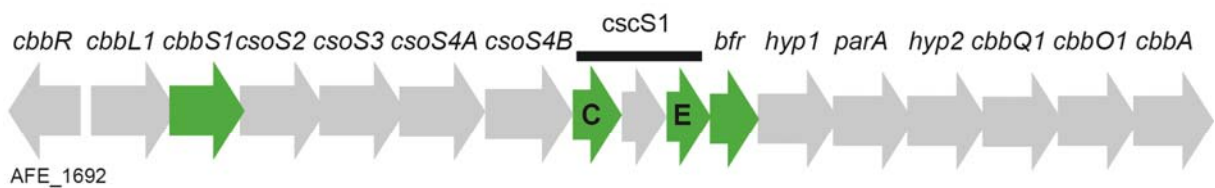
PetII operon



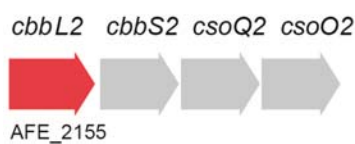
ATP synthase operon



cbb1 operon



cbb2 operon



cbb4

