

Proteomics reveal enhanced oxidative stress responses and metabolic adaptation in *Acidithiobacillus ferrooxidans* biofilm cells on pyrite

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Supplementary Table 1. Proteins of COG energy production and conservation (C) plus selected proteins associated with sulfur metabolism, iron(II)- and RISC oxidation in *A. ferrooxidans*^T

Locus tag	Protein ID	Description	log ₂ pyrite/Fe	q-value
AFE_0029	WP_012535754.1	tetrathionate hydrolase	1.12	0.01
AFE_0043	WP_009566763.1	glycine/betaine ABC transporter substrate-binding protein	1.66	0.02
AFE_0044	WP_009566762.1	DoxD family quinol oxidase	2.55	0.00
AFE_0045	WP_009566760.1	sulfur/pyrite/thiosulfate/sulfide-induced protein	1.00	0.00
AFE_0151	WP_012535979.1	sulfurtransferase	3.98	0.00
AFE_0177	WP_012536002.1	glutathione S-transferase	0.69	0.03
AFE_0267	WP_009565299.1	sulfide/quinone oxidoreductase, sqr	0.44	0.19
AFE_0366	WP_012536110.1	glutathione-disulfide reductase	-0.03	0.98
AFE_0423	WP_012536129.1	aconitate hydratase	0.77	0.01
AFE_0424	WP_012536130.1	isocitrate dehydrogenase (NADP(+))	-0.28	0.15
AFE_0425	WP_009564695.1	succinyl-CoA ligase subunit beta	-0.24	0.26
AFE_0427	WP_012536131.1	succinyl-CoA synthetase subunit alpha	0.48	0.13
AFE_0492	WP_009566885.1	inorganic pyrophosphatase	-0.16	0.84
AFE_0536	WP_012536189.1	phosphoribulokinase	-0.97	0.03
AFE_0539	WP_012536192.1	adenylyl-sulfate kinase	-1.79	0.01
AFE_0545	WP_012536195.1	thiol:disulfide interchange protein	2.21	0.00
AFE_0631	WP_012536247.1	cytochrome o ubiquinol oxidase	5.03	0.00
AFE_0632	WP_012536248.1	cytochrome o ubiquinol oxidase	2.56	0.01
AFE_0633	WP_012536249.1	cytochrome o ubiquinol oxidase	0.73	0.48
AFE_0660	WP_009560818.1	malate dehydrogenase	1.44	0.09
AFE_0674	WP_009566457.1	iron-sulfur cluster scaffold-like protein	-1.96	0.01
AFE_0678	WP_009566453.1	ferredoxin, 2Fe-2S type, ISC system	-0.05	0.93
AFE_0692	WP_012536289.1	oxidoreductase alpha (molybdopterin) subunit	-0.35	0.29

AFE_0697	WP_012536293.1	S-(hydroxymethyl)glutathione dehydrogenase	-1.59	0.01
AFE_0701	WP_012536297.1	hydrogenase	-0.88	0.05
AFE_0702	WP_012536298.1	cytochrome-c3 hydrogenase	-1.01	0.00
AFE_0808	WP_009561607.1	oxidoreductase	0.99	0.01
AFE_0809	WP_012536374.1	oxidoreductase	-0.60	0.31
AFE_0810	WP_012536375.1	oxidoreductase	0.00	1.00
AFE_0859	WP_009564795.1	pyridine nucleotide-disulfide oxidoreductase	5.34	0.00
AFE_0861	WP_009564313.1	globin	6.87	0.00
AFE_0954	WP_012536460.1	cytochrome d ubiquinol oxidase subunit II	4.80	0.01
AFE_0955	WP_009567364.1	cytochrome bd oxidase subunit I	2.18	0.00
AFE_0958	WP_009565875.1	pyridine nucleotide-disulfide oxidoreductase	-0.76	0.03
AFE_0984	WP_012536482.1	ferredoxin	-0.50	0.17
AFE_1388	WP_012536491.1	thiol reductant ABC exporter subunit CydD	1.86	0.00
AFE_1390	WP_009566343.1	thiol reductant ABC exporter subunit CydC	1.92	0.00
AFE_1546	WP_012536595.1	NAD-dependent succinate-semialdehyde dehydrogenase	1.98	0.01
AFE_1652	WP_012607156.1	oxidoreductase molybdopterin subunit	0.58	0.06
AFE_1662	WP_009566373.1	NADH dehydrogenase	-1.89	0.00
AFE_1663	WP_009566374.1	glycolate oxidase iron-sulfur subunit	-1.63	0.00
AFE_1664	WP_012607162.1	glycolate oxidase subunit GlcE	-0.07	0.96
AFE_1683	WP_012607172.1	carbon dioxide-concentrating protein CcmK	0.14	0.83
AFE_1685	WP_012536700.1	carbon dioxide-concentrating protein CcmK	-0.25	0.31
AFE_1686	WP_009567525.1	carboxysome peptide B	0.97	0.08
AFE_1687	WP_009567526.1	carboxysome peptide A	1.03	0.71
AFE_1690	WP_009567528.1	ribulose biphosphate carboxylase small subunit	-1.44	0.01
AFE_1723	WP_009563486.1	general stress protein	0.30	0.07
AFE_1769	WP_012536743.1	FAD-linked oxidase	-1.40	0.03
AFE_1792	WP_012536761.1	sulfide:quinone reductase	1.01	0.00
AFE_1803	WP_009568931.1	pyridine nucleotide-disulfide oxidoreductase	3.10	0.01
AFE_1808	WP_012536772.1	phosphate butyryltransferase	2.28	0.05
AFE_1810	WP_012607242.1	acetate kinase	-0.49	0.58
AFE_1811	WP_012536774.1	2-oxo acid dehydrogenase acyltransferase	1.26	0.01

AFE_1812	WP_012536775.1	pyruvate dehydrogenase subunit beta	0.97	0.00
AFE_1813	WP_009563431.1	dehydrogenase	0.78	0.07
AFE_1844	WP_009561766.1	ferredoxin	1.83	0.01
AFE_1883	WP_012536819.1	phosphoenolpyruvate carboxylase	-2.14	0.00
AFE_1893	WP_012536825.1	dihydrolipoyl dehydrogenase	2.81	0.00
AFE_2075	WP_012536951.1	3-isopropylmalate dehydrogenase	0.83	0.00
AFE_2120	WP_012536987.1	hopanoid biosynthesis associated radical SAM protein HpnJ	-1.51	0.00
AFE_2126	WP_012536992.1	glycerol-3-phosphate dehydrogenase (NAD(P)(+))	-0.60	0.22
AFE_2131	WP_012536996.1	aldehyde dehydrogenase	0.62	0.05
AFE_2211	WP_012537054.1	oxidoreductase	-0.33	0.64
AFE_2256	WP_012537084.1	MBL fold metallo-hydrolase	-0.13	0.70
AFE_2321	WP_012537120.1	monooxygenase	0.24	0.69
AFE_2328	WP_012537125.1	cytochrome b561	-1.86	0.00
AFE_2364	WP_009562958.1	sulfurtransferase	-2.31	0.10
AFE_2388	WP_012537164.1	2-octaprenyl-6-methoxyphenyl hydroxylase	0.42	0.22
AFE_2389	WP_012537165.1	2-octaprenyl-6-methoxyphenyl hydroxylase	-1.29	0.01
AFE_2481	WP_012607441.1	mercury(II) reductase	1.03	0.00
AFE_2533	WP_012537244.1	oxidoreductase	0.34	0.11
AFE_2547	WP_012537252.1	glycine cleavage system protein H	-0.01	0.98
AFE_2548	WP_012537253.1	sulfur reduction protein DsrE	3.45	0.01
AFE_2549	WP_009565261.1	glycine cleavage system protein H	0.58	0.13
AFE_2550	WP_012537254.1	disulfide reductase	1.27	0.00
AFE_2551	WP_012607468.1	heterodisulfide reductase subunit C	0.57	0.13
AFE_2552	WP_012537255.1	hypothetical protein	0.76	0.08
AFE_2553	WP_012537256.1	pyridine nucleotide-disulfide oxidoreductase	1.81	0.00
AFE_2554	WP_049756725.1	heterodisulfide reductase subunit B	0.83	0.04
AFE_2555	WP_041647471.1	heterodisulfide reductase subunit C	2.27	0.00
AFE_2556	WP_009562304.1	NADH dehydrogenase	1.61	0.00
AFE_2557	WP_009562307.1	transcriptional regulator	3.06	0.00
AFE_2558	WP_012537258.1	sulfurtransferase	-0.84	0.10
AFE_2586	WP_012537276.1	heterodisulfide reductase subunit B	1.53	0.00
AFE_2594	WP_012537282.1	glutathione S-transferase	0.86	0.01
AFE_2621	WP_009565427.1	NADH:ubiquinone oxidoreductase subunit J	1.07	0.17
AFE_2622	WP_012537299.1	NADH-quinone oxidoreductase	0.01	0.99

		subunit I		
AFE_2623	WP_009565424.1	NADH-quinone oxidoreductase subunit H	0.06	0.94
AFE_2624	WP_012537300.1	NADH-quinone oxidoreductase subunit G	0.20	0.23
AFE_2625	WP_012537301.1	NADH-quinone oxidoreductase subunit F	-0.43	0.11
AFE_2626	WP_009566653.1	NADH dehydrogenase subunit E	-0.27	0.80
AFE_2627	WP_012537302.1	NADH-quinone oxidoreductase subunit D	-0.22	0.14
AFE_2628	WP_009566652.1	NADH-quinone oxidoreductase subunit C	-0.06	0.91
AFE_2629	WP_009566651.1	NADH-quinone oxidoreductase subunit B 2	-0.96	0.06
AFE_2673	WP_009565770.1	class II fumarate hydratase	0.03	0.89
AFE_2959	WP_009564008.1	Fe(2+)-trafficking protein	-0.74	0.23
AFE_3052	WP_009566929.1	ribulose biphosphate carboxylase small subunit	2.55	0.00
AFE_3065	WP_012537576.1	citrate synthase	0.27	0.46
AFE_3068	WP_012537578.1	pyruvate dehydrogenase	0.92	0.00
AFE_3069	WP_012537579.1	pyruvate dehydrogenase subunit beta	-0.34	0.27
AFE_3070	WP_012537580.1	pyruvate dehydrogenase (acetyl-transferring) E1 component subunit alpha	-0.69	0.03
AFE_3079	WP_009567865.1	nitroreductase	1.70	0.02
AFE_3107	#NV	cytochrome c4, CycA-1	-3.49	0.00
AFE_3108	WP_009566406.1	short-chain dehydrogenase, Sdr_1	-2.51	0.00
AFE_3109	#NV	ubiquinol--cytochrome c reductase, iron-sulfur subunit, PetA-1	-9.49	0.00
AFE_3110	WP_009566409.1	cytochrome b, PetB-1	-4.85	0.00
AFE_3111	WP_012537599.1	ubiquinol cytochrome C oxidoreductase, PetC-1	-6.93	0.00
AFE_3112	WP_012537600.1	cytochrome c biogenesis protein, ResB	-1.48	0.00
AFE_3113	WP_009566490.1	c-type cytochrome biogenesis protein CcsB, ResC	-2.33	0.00
AFE_3121	WP_012537605.1	sulfite reductase subunit alpha	-0.98	0.15
AFE_3122	WP_009566801.1	sulfite reductase subunit beta	-9.44	0.00
AFE_3123	WP_012537606.1	phosphoadenosine phosphosulfate reductase	-5.48	0.00
AFE_3124	WP_041646660.1	sulfate adenylyltransferase small subunit	-6.87	0.00
AFE_3125	WP_012537608.1	sulfate adenylyltransferase	-7.98	0.00
AFE_3146	WP_012537622.1	rusticyanin	-1.79	0.00
AFE_3149	WP_009561084.1	cytochrome c oxidase subunit I,	-0.72	0.07

		coxA		
AFE_3150	WP_009561085.1	cytochrome c oxidase subunit II, coxB	-0.86	0.01
AFE_3151	WP_012537626.1	hypothetical protein	-0.63	0.01
AFE_3152	WP_012537627.1	cytochrome c552	-1.58	0.00
AFE_3153	WP_012537628.1	cytochrome c	-0.17	0.64
AFE_3161	WP_009562388.1	lactate dehydrogenase	1.52	0.00
AFE_3202	WP_009569104.1	FOF1 ATP synthase subunit epsilon	-0.87	0.28
AFE_3203	WP_012537655.1	ATP synthase subunit beta	-0.21	0.54
AFE_3204	WP_012537656.1	ATP synthase subunit gamma	-3.75	0.00
AFE_3205	WP_012537657.1	ATP synthase subunit alpha	0.66	0.03
AFE_3206	WP_009561112.1	ATP synthase subunit delta	-0.35	0.58
AFE_3207	WP_009561113.1	ATP synthase subunit B	0.16	0.63
AFE_3238	WP_009567147.1	ferredoxin	-1.32	0.45
AFE_3286	WP_012537694.1	hydrogenase 2 large subunit	2.54	0.02

Supplementary Table 2. Proteins with functions related to oxidative stress management and repair mechanisms

Locus tag	Protein ID	Description	log₂ pyrite/Fe	q-value
AFE_0861	WP_009564313.1	globin	6.87	0.00
AFE_0859	WP_009564795.1	pyridine nucleotide-disulfide oxidoreductase	5.34	0.00
AFE_3104	WP_012537597.1	DNA polymerase/3'-5' exonuclease PolX	4.58	0.00
AFE_0751	WP_009564668.1	universal stress protein	4.23	0.02
AFE_1862	WP_009568883.1	heavy metal-binding protein	3.53	0.03
AFE_2867	WP_012537441.1	thioredoxin	3.51	0.00
AFE_2183	WP_012537036.1	universal stress protein	3.36	0.00
AFE_2195	WP_009567357.1	iron donor protein CyaY	3.29	0.01
AFE_2021	WP_012536912.1	copper-translocating P-type ATPase	3.15	0.00
AFE_1803	WP_009568931.1	pyridine nucleotide-disulfide oxidoreductase	3.10	0.01
AFE_2259	WP_009562362.1	universal stress protein	2.76	0.01
AFE_0363	WP_012536107.1	peroxiredoxin	2.68	0.00
AFE_2086	WP_009567699.1	heat-shock protein Hsp20	2.29	0.00
AFE_0871	WP_012536407.1	heat-shock protein Hsp20	2.24	0.19
AFE_1970	WP_012536875.1	ATP-dependent chaperone ClpB	2.22	0.00
AFE_0545	WP_012536195.1	thiol:disulfide interchange protein	2.21	0.00
AFE_0590	WP_009564993.1	cold-shock protein	2.13	0.05
AFE_1979	WP_012536882.1	thiol reductase thioredoxin	2.04	0.00
AFE_1390	WP_009566343.1	thiol reductant ABC exporter subunit CydC	1.92	0.00
AFE_1648	WP_009569199.1	heat-shock protein Hsp20	1.86	0.00
AFE_1388	WP_012536491.1	thiol reductant ABC exporter subunit CydD	1.86	0.00
AFE_3241	WP_009567144.1	peroxiredoxin	1.86	0.02
AFE_0450	WP_012536142.1	DNA repair protein RecN	1.79	0.00
AFE_2375	WP_012537158.1	ATP-dependent helicase	1.74	0.01
AFE_2590	WP_012537279.1	thiol reductase thioredoxin	1.72	0.13
AFE_0178	WP_012536003.1	exodeoxyribonuclease III	1.64	0.35

AFE_2945	WP_012537491.1	peptide-methionine (R)-S-oxide reductase	1.44	0.02
AFE_1408	WP_012536503.1	molecular chaperone Hsp33	1.42	0.07
AFE_2773	WP_012607524.1	thiol:disulfide oxidoreductase	1.42	0.00
AFE_2665	WP_009563301.1	molecular chaperone DnaK	1.34	0.00
AFE_0553	WP_012536200.1	thioredoxin family protein	1.33	0.22
AFE_2372	WP_012537156.1	type I restriction-modification system subunit M	1.21	0.01
AFE_3116	WP_012537283.1	Peroxiredoxin family protein	1.20	0.07
AFE_0684	WP_012536282.1	restriction endonuclease EcoEI subunit M	1.19	0.03
AFE_3063	WP_012537575.1	glutathione synthase	1.07	0.02
AFE_2481	WP_012607441.1	mercury(II) reductase	1.03	0.00
AFE_2347	WP_009563737.1	ferritin	0.93	0.10
AFE_0677	WP_012536278.1	molecular chaperone HscA	0.93	0.00
AFE_3194	WP_009566737.1	ATP-dependent DNA helicase RecG	0.91	0.00
AFE_2526	WP_012537242.1	N-carbamoylputrescine amidase	0.88	0.01
AFE_1814	WP_009563432.1	alkylhydroperoxidase	0.78	0.06
AFE_3158	WP_012537630.1	molecular chaperone HtpG	0.75	0.02
AFE_1468	WP_009562391.1	peroxiredoxin	0.75	0.00
AFE_0657	WP_012536267.1	thiol reductase thioredoxin	0.75	0.08
AFE_0845	WP_012536389.1	excinuclease ABC subunit B	0.72	0.14
AFE_2738	WP_009561099.1	Co ²⁺ /Mg ²⁺ efflux protein ApaG	0.70	0.75
AFE_0744	WP_012536326.1	DNA mismatch repair protein MutS	0.67	0.07
AFE_1760	WP_012536740.1	DNA mismatch repair protein MutS	0.55	0.43
AFE_0156	WP_009567116.1	spermidine synthase	0.49	0.09
AFE_0267	WP_009565299.1	pyridine nucleotide-disulfide oxidoreductase	0.44	0.19
AFE_2946	WP_009560846.1	peptide-methionine (S)-S-oxide reductase	0.32	0.28
AFE_1723	WP_009563486.1	general stress protein	0.30	0.07
AFE_1898	WP_009566995.1	superoxide dismutase	0.17	0.54
AFE_0004	WP_012535741.1	pyridine nucleotide-disulfide oxidoreductase	0.17	0.41
AFE_2848	WP_012537429.1	thioredoxin	0.01	0.98

AFE_0366	WP_012536110.1	glutathione-disulfide reductase	-0.03	0.98
AFE_0932	WP_012536446.1	DNA recombination/repair protein RecA	-0.10	0.62
AFE_0375	WP_009566072.1	thioredoxin-disulfide reductase	-0.12	0.87
AFE_0542	WP_012536194.1	molecular chaperone GroEL	-0.12	0.58
AFE_2362	WP_012537150.1	thiol reductase thioredoxin	-0.15	0.78
AFE_1872	WP_012536812.1	thiol:disulfide interchange protein	-0.23	0.51
AFE_2133	WP_009566245.1	universal stress protein	-0.27	0.82
AFE_2947	WP_012607576.1	thiol:disulfide interchange protein	-0.29	0.25
AFE_3038	WP_012537561.1	monothiol glutaredoxin, Grx4 family	-0.36	0.51
AFE_3118	WP_009566821.1	pyridine nucleotide-disulfide oxidoreductase	-0.40	0.03
AFE_0985	WP_012536483.1	peroxidase	-0.50	0.03
AFE_0006	WP_012535742.1	chaperone protein EcpD	-0.62	0.08
AFE_1682	WP_012607171.1	bacterioferritin	-0.73	0.01
AFE_2959	WP_009564008.1	Fe(2+)-trafficking protein	-0.74	0.23
AFE_0958	WP_009565875.1	pyridine nucleotide-disulfide oxidoreductase	-0.76	0.03
AFE_0543	WP_009567579.1	molecular chaperone GroES	-0.83	0.12
AFE_2847	WP_012537428.1	excinuclease ABC subunit A	-0.86	0.28
AFE_0199	WP_012536015.1	ferritin	-1.03	0.04
AFE_1548	WP_012536596.1	globin	-1.50	0.05
AFE_2246	WP_012537078.1	disulfide isomerase	-1.69	0.05
AFE_2664	WP_012537323.1	molecular chaperone DnaJ	-1.76	0.01
AFE_1943	WP_012607270.1	disulfide isomerase	-1.77	0.02
AFE_0367	WP_009566864.1	glutathione amide-dependent peroxidase	-2.41	0.00
AFE_1767	WP_012536741.1	exodeoxyribonuclease X	-2.74	0.01
AFE_3127	WP_009567512.1	cobalamin biosynthesis protein CbiX	-5.38	0.00

Supplementary Table 3. Transcription affecting proteins and transcriptional regulators with enhanced expression in pyrite biofilm cells ($\log_2 > 1$, $q\text{-value} \leq 0.10$) and iron(II)-grown cells ($\log_2 < -1$, $q\text{-value} \leq 0.10$).

Locus tag	Protein ID	Description	\log_2 pyrite/Fe	q- value
AFE_2557	WP_009562307.1	transcriptional regulator	3.06	0.00
AFE_0135	WP_012535969.1	LysR family transcriptional regulator	2.28	0.02
AFE_0590	WP_009564993.1	cold-shock protein	2.13	0.05
AFE_2915	WP_009567505.1	nitrogen regulatory protein P-II 1	2.02	0.00
AFE_0429	WP_009566205.1	nitrogen regulatory protein P-II	2.00	0.00
AFE_0024	WP_009566311.1	nitrogen assimilation regulatory protein NtrX	1.83	0.00
AFE_3060	WP_009566940.1	bifunctional pyr operon transcriptional regulator/uracil phosphoribosyltransferase	1.39	0.01
AFE_0255	WP_009566155.1	TetR family transcriptional regulator	1.12	0.01
AFE_0314	WP_012536079.1	transcription termination/antitermination protein NusG	-1.07	0.01
AFE_0638	WP_009564109.1	transcription elongation factor GreA	-1.14	0.01
AFE_0061	WP_009567692.1	Holliday junction branch migration protein RuvA	-1.16	0.09
AFE_0215	WP_012536023.1	division/cell wall cluster transcriptional repressor MraZ	-1.31	0.01
AFE_0911	WP_012536430.1	ATP-dependent Clp protease ATP- binding subunit ClpX	-1.83	0.00
AFE_2336	WP_012537129.1	RNA polymerase sigma factor RpoD	-1.85	0.01
AFE_2158	WP_009561657.1	LysR family transcriptional regulator	-2.30	0.01
AFE_0391	WP_012536117.1	translation initiation factor IF-2	-2.45	0.00
AFE_1668	WP_012607166.1	IclR family transcriptional regulator	-2.56	0.00
AFE_1463	WP_012536538.1	dihydroorotase	-3.10	0.00
AFE_0055	WP_009565541.1	FmdB family transcriptional regulator	-3.54	0.00
AFE_1689	WP_041645863.1	transcriptional initiation protein Tat	-3.83	0.00
AFE_0059	WP_009567690.1	transcriptional regulator	-4.15	0.00
AFE_0353	WP_012536098.1	30S ribosomal protein S4	-5.47	0.00

Supplementary Table 4. Identified proteins associated with carbon fixation in *A. ferrooxidans*^T

gene	Locus tag	Protein ID	Description	log ₂ pyrite/Fe	q- value
Putative operon cbb1					
cbbL1	AFE_1691	WP_012536704.1	Ribulose bisphosphate carboxylase large chain 1	-2.27	0.00
cbbS1	AFE_1690	WP_009567528.1	ribulose bisphosphate carboxylase small subunit	-1.44	0.01
csoS2	AFE_1689	WP_041645863.1	transcriptional initiation protein Tat	-3.83	0.00
csoS3	AFE_1688	WP_012607174.1	carboxysome shell carbonic anhydrase	-1.61	0.01
csoS4A	AFE_1687	WP_009567526.1	carboxysome peptide A	1.03	0.71
csoS4B	AFE_1686	WP_009567525.1	carboxysome peptide B	0.97	0.08
csoS1C	AFE_1685	WP_012536700.1	carbon dioxide-concentrating protein CcmK	-0.25	0.31
csoS1B	AFE_1683	WP_012607172.1	carbon dioxide-concentrating protein CcmK	0.14	0.83
bfrA	AFE_1682	WP_012607171.1	bacterioferritin	-0.73	0.01
hyp1	AFE_1681	WP_012536697.1	hypothetical protein	-1.40	0.01
parA	AFE_1680	WP_012607170.1	cobyrinic acid a,c-diamide synthase	-1.09	0.01
hyp2	AFE_1679	ACK78664.1	hypothetical protein	-3.81	0.01
cbbQ1	AFE_1678	WP_012536694.1	ATPase AAA	0.17	0.59
cbbO1	AFE_1677	WP_012536693.1	VWA domain-containing protein	0.00	0.98
cbbA	AFE_1676	WP_009561318.1	fructose-bisphosphate aldolase	-1.84	0.00
Putative operon cbb2					
cbbL2	AFE_3051	WP_009566926.1	Ribulose bisphosphate carboxylase large chain 2	2.43	0.00
cbbS2	AFE_3052	WP_009566929.1	ribulose bisphosphate carboxylase small subunit	2.55	0.00
cbbQ2	AFE_3053	WP_009566930.1	ATPase AAA	1.51	0.00

cbbO2	AFE_3054	WP_009566932.1	VWA domain-containing protein	1.23	0.10
Putative operon cbb3					
cbbF	AFE_3253	WP_012537679.1	fructose 1,6-bisphosphatase	-0.88	0.02
cbbT	AFE_3252	WP_012537678.1	transketolase	0.27	0.09
cbbG	AFE_3251	WP_009567621.1	type I glyceraldehyde-3- phosphate dehydrogenase	-0.21	0.17
cbbK	AFE_3250	WP_012537677.1	phosphoglycerate kinase	-0.18	0.28
pykA	AFE_3249	WP_012537676.1	pyruvate kinase	-1.42	0.00
cbbA	AFE_3248	WP_009561009.1	fructose-1,6-bisphosphate aldolase	-0.05	0.81
cbbE	AFE_3247	WP_012537675.1	ribulose-phosphate 3- epimerase	0.98	0.05
cbbZ	AFE_3246	WP_009561007.1	phosphoglycolate phosphatase, bacterial	0.31	0.66
trpE	AFE_3245	WP_012537674.1	anthranilate synthase component I	1.06	0.13
trpG	AFE_3244	WP_009567142.1	glutamine amidotransferase	-0.12	0.87
Putative operon cbb4					
metK	AFE_0532	WP_012536187.1	S-adenosylmethionine synthase	0.71	0.02
sahA	AFE_0534	WP_009567191.1	adenosylhomocysteinase	-0.51	0.02
metF	AFE_0535	WP_012536188.1	methylenetetrahydrofolate reductase [NAD(P)H]	1.57	0.01
cbbP	AFE_0536	WP_012536189.1	phosphoribulokinase	-0.97	0.03
Putative operon cbb5					
cbbM	AFE_2155	WP_012537012.1	ribulose-bisphosphate carboxylase	-0.04	0.94
cbbQ3	AFE_2156	WP_012537013.1	ATPase AAA	-0.88	0.08
cbbR_M	AFE_2158	WP_009561657.1	LysR family transcriptional regulator	-2.30	0.01

Supplementary Table 5. Proteins in COGs cell wall/membrane biogenesis (M), carbohydrate transport and metabolism (G), intracellular trafficking and secretion (U), lipid transport and metabolism (I) and cell motility (N) with enhanced expression in pyrite biofilm cells ($\log_2 > 1$, q-value ≤ 0.10) and iron(II)-grown cells ($\log_2 < -1$, q-value ≤ 0.10).

Locus tag	Protein ID	Description	\log_2 pyrite/Fe	q- value
Cell wall/membrane biogenesis M				
AFE_2967	WP_012607583.1	group 1 glycosyl transferase	3.04	0.04
AFE_2316	WP_012537117.1	membrane protein	3.01	0.10
AFE_3264	WP_012607659.1	RND transporter	1.96	0.00
AFE_1552	WP_012536600.1	sucrose synthase	1.89	0.01
AFE_3291	WP_012537698.1	NAD-dependent dehydratase	1.73	0.00
AFE_3265	WP_012537684.1	transporter	1.46	0.02
AFE_1880	WP_009564623.1	RND transporter	1.44	0.00
AFE_2368	WP_012537154.1	transporter	1.27	0.01
AFE_2923	WP_012607567.1	peptidase M15	1.20	0.00
AFE_2367	WP_012537153.1	RND transporter	1.16	0.03
AFE_2677	WP_009565767.1	3-deoxy-D-manno-octulosonic acid transferase	1.14	0.01
AFE_0442	WP_012536140.1	NAD(P)-dependent oxidoreductase	1.12	0.03
AFE_2358	WP_009561191.1	4-hydroxy-tetrahydrodipicolinate synthase	1.10	0.01
AFE_0848	WP_012536391.1	2-dehydro-3-deoxyphosphooctonate aldolase	1.07	0.04
AFE_1879	WP_012536818.1	MexH family multidrug efflux RND transporter periplasmic adaptor subunit	0.97	0.01
AFE_2113	WP_012536981.1	VacJ family lipoprotein	-0.92	0.01
AFE_2186	WP_009561052.1	prolipoprotein diacylglyceryl transferase	-1.04	0.02
AFE_2961	WP_012537503.1	capsule polysaccharide export inner- membrane protein	-1.09	0.01
AFE_0524	WP_012536181.1	peptidase S41	-1.18	0.01
AFE_3034	WP_009562824.1	organic solvent tolerance protein	-1.70	0.10
AFE_0377	WP_009566070.1	outer membrane lipoprotein carrier protein LolA	-1.71	0.00
AFE_3293	WP_012537700.1	mannose-1-phosphate	-1.74	0.01

		guanylyltransferase/mannose-6-phosphate isomerase		
AFE_2929	WP_012537480.1	cyclopropane-fatty-acyl-phospholipid synthase	-1.89	0.00
AFE_2883	WP_012537451.1	choloylglycine hydrolase	-2.23	0.10
AFE_2223	WP_012537062.1	penicillin-binding protein 1A	-2.74	0.01
AFE_1397	WP_009567236.1	elongation factor 4	-2.96	0.01
AFE_2268	WP_012537091.1	cell envelope biogenesis protein TonB	-3.32	0.00
AFE_1456	WP_012536532.1	acyl-[acyl-carrier-protein]-UDP-N-acetylglucosamine O-acyltransferase	-3.59	0.01
AFE_2267	WP_012607367.1	phospholipase C	-4.31	0.00
AFE_1827	WP_041646068.1	murein transglycosylase	-5.74	0.00
Carbohydrate transport and metabolism, G				
AFE_1799	WP_012536767.1	alpha-glucan phosphorylase	3.95	0.01
AFE_1802	WP_012536769.1	aldolase	3.66	0.03
AFE_1843	WP_012536797.1	transketolase	3.41	0.00
AFE_1815	WP_012536777.1	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	2.74	0.00
AFE_0451	WP_012606559.1	NAD kinase	2.67	0.02
AFE_3051	WP_009566926.1	Ribulose biphosphate carboxylase large chain 2	2.43	0.00
AFE_2836	WP_009562056.1	glycogen-branching enzyme	2.32	0.01
AFE_3302	WP_009566480.1	HIT family protein	2.07	0.00
AFE_3018	WP_012537546.1	phosphoenolpyruvate--protein phosphotransferase	1.84	0.00
AFE_0419	WP_012536126.1	transaldolase	1.74	0.00
AFE_1816	WP_012536778.1	phosphoenolpyruvate synthase	1.69	0.00
AFE_0669	WP_009564866.1	inositol monophosphatase	1.67	0.10
AFE_3023	WP_012537551.1	PTS sugar transporter	1.56	0.01
AFE_2324	WP_012537123.1	alpha-D-glucose phosphate-specific phosphoglucomutase	1.53	0.00
AFE_2025	WP_012536916.1	glucose-6-phosphate dehydrogenase	1.34	0.01
AFE_2839	WP_012537423.1	glycoside hydrolase	1.24	0.00
AFE_0074	WP_012606487.1	chain-length determining protein	1.23	0.01

AFE_1923	WP_012536840.1	NAD(P)H-hydrate dehydratase	1.14	0.09
AFE_2009	ACK78253.1	sugar ABC transporter [WP_072452544.1, E-value: $2 \cdot 10^{-50}$]	1.12	0.04
AFE_2678	WP_009565766.1	starch synthase	1.07	0.00
AFE_3020	WP_012537549.1	PTS sugar transporter subunit IIA	1.03	0.01
AFE_2224	WP_012537063.1	glycosyl hydrolase	1.00	0.01
AFE_0629	WP_009560936.1	ribose-5-phosphate isomerase	0.99	0.02
AFE_3247	WP_012537675.1	ribulose-phosphate 3-epimerase	0.98	0.05
AFE_0434	WP_012536136.1	2,3-diketo-5-methylthiopentyl-1- phosphate enolase	0.95	0.06
AFE_0138	WP_012535972.1	lipopolysaccharide heptosyltransferase II	-0.96	0.01
AFE_2634	WP_009566647.1	phosphoglucosamine mutase	-1.11	0.02
AFE_3249	WP_012537676.1	pyruvate kinase	-1.42	0.00
AFE_1766	WP_009566988.1	aldolase	-1.44	0.03
AFE_0082	WP_012535780.1	rhamnosyltransferase	-1.62	0.01
AFE_1676	WP_009561318.1	fructose-bisphosphate aldolase	-1.84	0.00
AFE_1667	WP_009565308.1	transketolase	-1.98	0.00
AFE_1691	WP_012536704.1	Ribulose bisphosphate carboxylase large chain 1	-2.27	0.00
AFE_0081	WP_012535779.1	mannosyltransferase	-3.09	0.01
Intracellular trafficking and secretion, U				
AFE_1915	WP_009561366.1	pilus assembly protein PilZ	2.23	0.07
AFE_0286	WP_012536065.1	sulfate permease membrane protein	1.93	0.02
AFE_3265	WP_012537684.1	outer membrane efflux protein	1.46	0.02
AFE_1880	WP_009564623.1	RND efflux system, outer membrane lipoprotein, NodT family	1.44	0.00
AFE_2368	WP_012537154.1	outer membrane efflux protein	1.27	0.01
AFE_2753	WP_009568264.1	signal recognition particle-docking protein FtsY	1.12	0.03
AFE_0910	WP_012536429.1	ATP-dependent Clp protease proteolytic subunit	0.98	0.02
AFE_2057	WP_012536937.1	protein-export membrane protein SecD	0.94	0.01
AFE_1398	WP_009567235.1	S26 family signal peptidase	-1.01	0.07
AFE_0736	WP_012536320.1	pilus assembly protein PilP	-1.08	0.03

AFE_3049	WP_009566923.1	twin arginine-targeting protein translocase TatB	-1.18	0.04
AFE_0737	WP_012536321.1	pilus assembly protein PilO	-1.35	0.00
Lipid transport and metabolism, I				
AFE_1857	WP_012536805.1	glucose dehydrogenase	4.75	0.00
AFE_1651	WP_012536669.1	3-hydroxyisobutyrate dehydrogenase family protein	3.19	0.00
AFE_2542	WP_012537248.1	phosphatidylserine decarboxylase	3.10	0.00
AFE_0748	WP_009562365.1	glucose 1-dehydrogenase, putative	2.02	0.01
AFE_2225	WP_012537064.1	acetyl-CoA synthetase	1.36	0.01
AFE_1908	WP_009561353.1	acetyl-CoA carboxylase, carboxyl transferase, beta subunit	-0.61	0.03
AFE_3258	WP_009566635.1	holo-(acyl-carrier-protein) synthase	-0.68	0.06
AFE_1404	WP_009565421.1	acetyl-CoA carboxylase, carboxyl transferase, alpha subunit	-0.92	0.04
AFE_1462	WP_012536536.1	enoyl-(acyl-carrier-protein) reductase	-1.05	0.10
AFE_0420	WP_009562995.1	SCP-2 sterol transfer family protein	-1.14	0.00
AFE_1905	WP_009566798.1	fatty acid/phospholipid synthesis protein PlsX	-1.84	0.01
AFE_1448	WP_012536524.1	undecaprenyl diphosphate synthase	-2.03	0.01
AFE_1933	WP_009564018.1	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (flavodoxin)	-2.31	0.00
AFE_3108	WP_009566406.1	short-chain dehydrogenase	-2.51	0.00
Cell motility, N				
AFE_1915	WP_009561366.1	pilus assembly protein PilZ	2.23	0.07
AFE_0736	WP_012536320.1	pilus assembly protein PilP	-1.08	0.03
AFE_0737	WP_012536321.1	pilus assembly protein PilO	-1.35	0.00
AFE_0265	WP_012536053.1	twitching motility protein PilT	-1.19	0.01