

Supplementary Information

Possible Role of envelope components in the extreme copper resistance of the biomining *Acidithiobacillus ferrooxidans*

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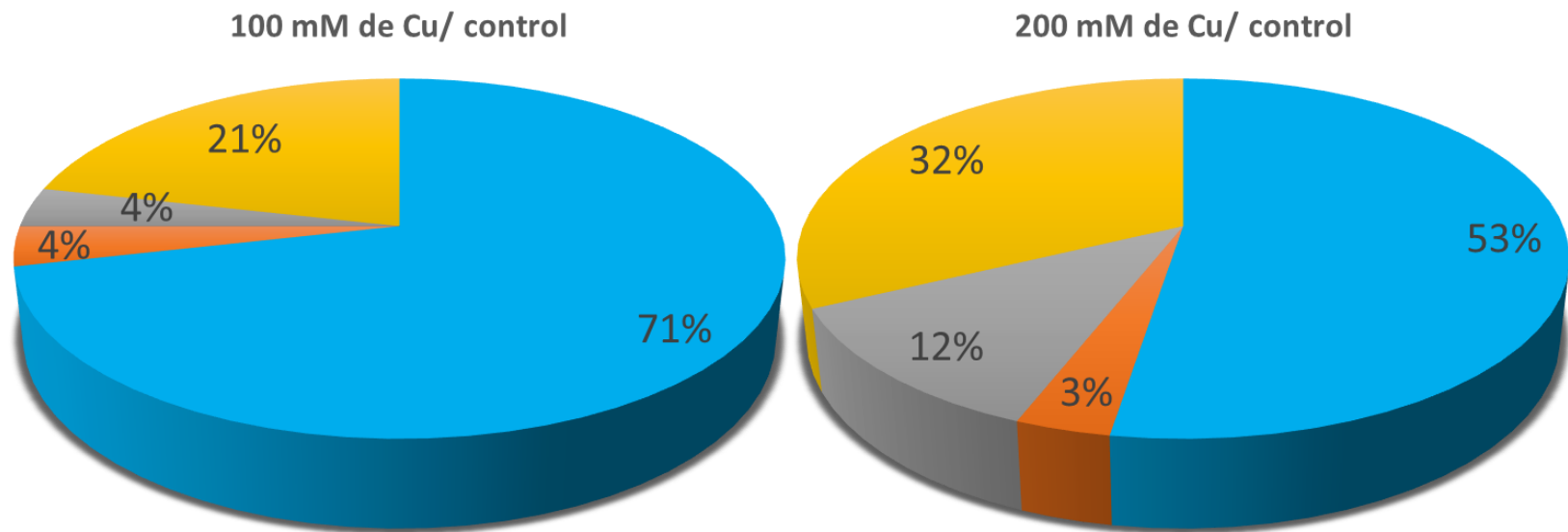


Figure S1. Functional categories of *A. ferrooxidans* proteins changing their synthesis levels in cells grown in the presence of 100 and 200 mM CuSO_4 . Blue: Metabolism; Orange: Cellular Processes and Signaling; Gray: Information Storage and Processing; Yellow: Poorly Characterized

Table S1. Functional categories and numbers of *A. ferrooxidans* ATCC 53993 proteins changing their synthesis levels in cells grown in the presence of 100 and 200 mM CuSO₄.

Functional category	Number of proteins changed			
	100 mM de Cu/ Control		200 mM de Cu /Control	
	Protein (-)	Protein (+)	Protein (-)	Protein (+)
METABOLISM				
Inorganic Ion Transport and Metabolism	3	9	5	10
Carbohydrate Metabolism and Transport	5	0	5	1
Energy Production and Conversion	1	0	4	4
Secondary Metabolites biosynthesis, Transport, and Catabolism	1	0	2	0
Lipid transport and metabolism	1	0	0	0
INFORMATION STORAGE AND PROCESSING				
Translation, Ribosomal structure and Biogenesis	0	1	7	0
CELLULAR PROCESSES AND SIGNALING				
Post-translational Modification, Protein turnover, and Chaperones	1	0	1	0
Cell wall/Membrane/Envelope biogenesis	0	0	1	0
POORLY CHARACTERIZED				
General function prediction only	0	0	1	0
Function Unknown	5	1	16	2
Total	17	11	42	17

(+) increased level. (–) decreased level.

Table S2. Proteins with increased levels in *A. ferrooxidans* ATCC 53993 grown in the presence of 100 and 200 mM CuSO₄

Accession number	ORF	Function/similarity	qvalue (FDR)	Coverture (%)	Peptides number	Log2 Fold change (Cu 100/0 mM)	Log2 Fold change (Cu 200/0 mM)
METABOLISM							
Inorganic ion transport and metabolism							
B5EJG7	<i>Lferr_1619</i>	Outer membrane efflux protein (CusC1)	0.001	45	9	1.018	1.258
B5EJG6	<i>Lferr_1618</i>	Efflux transporter, RND family, MFP subunit (CusB1)	0.001	63.3	15	0.927	0.859
B5EM36	<i>Lferr_2057</i>	Periplasmatic Copper chaperone (CusF2)	0.001	60	3	1.084	1.92
B5EK73	<i>Lferr_0174</i>	Periplasmatic Copper chaperonea (CusF3)	0.001	60	3	0.68	1.63
B5EK71	<i>Lferr_0172</i>	Heavy metal efflux pump, CzcA family (CusA3)	0.001	39.09	10	1.06	1.019
B5EM41	<i>Lferr_2062</i>	Outer membrane efflux protein (CusC2)	0	44.5	12	0.823	1.084
B5EJG5	<i>Lferr_1617</i>	Heavy metal efflux pump, CzcA family (CusA1)	0.002	35	9	1.022	0.855
B5EM40	<i>Lferr_2061</i>	Efflux transporter, RND family, MFP subunit (CusB2)	0.003	68.3	6	0.801	0.968
B5EM39	<i>Lferr_2060</i>	Heavy metal efflux pump, CzcA family (CusA2)	0.003	38.3	9	0.927	0.846
B5EQ72	<i>Lferr_1075</i>	Periplasmic glucan biosynthesis protein (MdoG)	0.009	48.1	15	0	0.415
Carbohydrate metabolism and transport							
B5EL00	<i>Lferr_1865</i>	Carboxymethylenebutenolidase	0.011	88.43	16	0	0.347
Energy production and conversion							
B5EJH8	<i>Lferr_1630</i>	Cytochrome c class I	0.026	9.64	1	0	1.843
B5EQK0	<i>Lferr_2705</i>	Cytochrome c class I (CycA1)	0.013	27.31	5	0	0.515
B5EMP7	<i>Lferr_2183</i>	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	0.007	65.53	20	0	0.369
B5ES29	<i>Lferr_1469</i>	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	0.015	74.65	21	0	0.341

INFORMATION STORAGE AND PROCESSING							
Translation, ribosomal structure and biogenesis							
B5ENR6	<i>Lferr_2358</i>	Uncharacterized protein	0.014	17.02	1	1.593	0
POORLY CHARACTERIZED							
Function unknown							
B5ELE6	<i>Lferr_0408</i>	O-antigen polymerase (Wzy)	0.026	3.19	1	0	1.795
B5EM38	<i>Lferr_2059</i>	Uncharacterized protein	0.005	38.86	5	0	0.936
B5ELK0	<i>Lferr_1967</i>	Uncharacterized protein	0.025	4.17	1	1.081	0

Color represents change in log₂ fold change value: green, increase; yellow, no change; red, decrease.

Table S3. Proteins with lower levels of synthesis in *A. ferrooxidans* ATCC 53993 grown in the presence of 100 and 200 mM CuSO₄.

Accession number	ORF	Function/similarity	qvalue (FDR)	Coverture (%)	Peptides number	Log2 Fold change (Cu 100/0 mM)	Log2 Fold change (Cu 200/0 mM)
METABOLISM							
Inorganic ion transport and metabolism							
B5EJZ3	<i>Lferr_1702</i>	TonB-dependent receptor	0.039	43.46	18	0	-0.318
B5EQV4	<i>Lferr_1216</i>	Extracellular solute-binding protein family	0.049	69.38	15	-0.463	-0.343
B5ERT7	<i>Lferr_1375</i>	von Willebrand factor type A	0.003	64.11	35	0	-0.37
B5EPV1	<i>Lferr_2552</i>	TonB-dependent receptor	0.011	26.94	16	0	-0.407
B5EJF9	<i>Lferr_1611</i>	Phosphate-binding protein PstS	0.001	58.29	13	-0.934	-0.488
B5EK98	<i>Lferr_0199</i>	Periplasmatic Copper chaperone (CusF4)	0.007	51.61	6	-0.673	0
Carbohydrate metabolism and transport							
B5ERT6	<i>Lferr_1374</i>	Ketose-bisphosphate aldolase class-II	0.007	76.84	22	0	-0.374
B5ERV1	<i>Lferr_1389</i>	Ribulose-bisphosphate carboxylase	0	72.94	31	-0.501	-0.574
B5ERV0	<i>Lferr_1388</i>	Ribulose-bisphosphate carboxylase	0.001	90	9	-0.501	-0.53
B5ERS6	<i>Lferr_1364</i>	Transketolase central region	0.001	65.36	31	-0.699	-1.163
B5ERU9	<i>Lferr_1387</i>	Carboxysome structural protein CsoS2	0.002	68.67	47	-0.289	-1.185
B5EL78	<i>Lferr_0340</i>	Toluene tolerance family protein	0.024	67.45	9	-0.439	0
Energy production and conversion							
B5EQK2	<i>Lferr_2707</i>	Ubiquinol-cytochrome c reductase iron-sulfur subunit (petA1)	0.003	45.63	9	0	-0.478
B5ERS3	<i>Lferr_1361</i>	FAD linked oxidase domain protein	0.028	23.51	4	0	-0.588

B5ERS1	<i>Lferr_1359</i>	NADH/Ubiquinone/plastoquinone (Complex I)	0.003	17.58	7	0	-0.956
B5ERS4	<i>Lferr_1362</i>	D-lactate dehydrogenase (Cytochrome)	0.01	8.2	2	0	-1.157
B5EQY4	<i>Lferr_2743</i>	Rusticyanin	0.002	74.87	14	-1.139	0
Secondary metabolites biosynthesis, transport, and catabolism							
B5ERU3	<i>Lferr_1381</i>	Microcompartments protein	0.004	95.54	4	0	-0.69
B5ERU5	<i>Lferr_1383</i>	Microcompartments protein	0.022	94.9	1	0	-0.851
B5EKF9	<i>Lferr_1773</i>	Hopanoid biosynthesis associated membrane protein HpnM	0.007	55.71	11	-0.485	0
Lipid transport and metabolism							
B5EKF4	<i>Lferr_1768</i>	Uncharacterized protein	0.011	37.16	5	-0.633	0
INFORMATION STORAGE AND PROCESSING							
Translation, ribosomal structure and biogenesis							
B5EMD3	<i>Lferr_0558</i>	30S ribosomal protein S15	0.004	48.31	2	0	-0.784
B5ELY3	<i>Lferr_0501</i>	30S ribosomal protein S19	0.001	70.33	7	0	-0.867
B5EKY1	<i>Lferr_1846</i>	30S ribosomal protein S20	0.003	25.27	3	0	-0.932
B5ELZ0	<i>Lferr_0508</i>	50S ribosomal protein L24	0.005	19.81	3	0	-0.982
B5ELZ7	<i>Lferr_0515</i>	50S ribosomal protein L30	0.002	45	3	0	-1.056
B5ENA6	<i>Lferr_2294</i>	50S ribosomal protein L33	0.002	47.06	2	0	-1.513
B5ELU3	<i>Lferr_0461</i>	50S ribosomal protein L27	0.044	58.14	5	0	-0.416
CELLULAR PROCESSES AND SIGNALING							
Post-translational modification, protein turnover, and chaperones							
B5EN20	<i>Lferr_0699</i>	10 kDa chaperonin groS	0.007	90.63	11	-0.445	-0.404
Cell wall/membrane/envelope biogenesis							
B5EL33	<i>Lferr_1898</i>	Carbohydrate-selective porin (OprB)	0.005	36.46	10	0	-0.635
POORLY CHARACTERIZED							

General function prediction only							
B5ERT8	<i>Lferr_1376</i>	CbbQ/NirQ/NorQ domain protein	0.018	83.15	13	0	-0.348
Function unknown							
B5ERD1	<i>Lferr_1295</i>	Uncharacterized protein	0.016	51.28	7	0	-0.458
B5ERS9	<i>Lferr_1367</i>	Uncharacterized protein	0.012	28.08	8	0	-0.519
B5EMQ4	<i>Lferr_2190</i>	Uncharacterized protein	0.007	56.44	6	0	-0.531
B5EP93	<i>Lferr_2439</i>	Uncharacterized protein	0.003	51.37	6	0	-0.569
B5EJI6	<i>Lferr_0030</i>	Pyrrolo-quinoline quinone	0.003	43.29	11	-0.468	-0.62
B5ERS0	<i>Lferr_1358</i>	UPF0753 protein	0	70.18	51	-0.401	-0.64
B5EQX9	<i>Lferr_2738</i>	Uncharacterized protein	0.003	63.43	9	0	-0.649
B5ERS5	<i>Lferr_1363</i>	Uncharacterized protein	0.003	64.94	17	0	-0.733
B5ERU8	<i>Lferr_1386</i>	Carboxysome shell carbonic anhydrase	0.001	52.72	21	0	-0.772
B5EL34	<i>Lferr_1899</i>	Pyrrolo-quinoline quinone	0.001	47.59	15	-0.373	-0.778
B5EM86	<i>Lferr_2116</i>	Uncharacterized protein	0.003	28	4	0	-0.909
B5EJU1	<i>Lferr_1650</i>	Uncharacterized protein	0.003	27.2	4	0	-0.975
B5EJ87	<i>Lferr_1539</i>	Uncharacterized protein	0.011	12.7	1	-2.336	-1.109
B5ELR1	<i>Lferr_0429</i>	Uncharacterized protein	0.03	36.89	9	0	-1.124
B5ERT9	<i>Lferr_1377</i>	Uncharacterized protein	0.016	10.71	1	0	-1.149
B5EJC5	<i>Lferr_1577</i>	Ribonuclease BN	0.046	2.71	1	0	-1.11
B5EN88	<i>Lferr_2275</i>	Uncharacterized protein	0.008	54.17	4	-0.818	0

Color represents the change in log₂ fold value: green, increased level; yellow, no change; red, decreased level.

