

## Supplementary Materials: Dissolution and Passivation of Chalcopyrite during Bioleaching by *Acidithiobacillus ferrivorans* at Low Temperature

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Table S1. Conditions for the bioleaching experiments.

Group	Initial pH	Initial Volume (mL)	Temperature (°C)	Pulp Density (%)	Initial Cell Density (cells/mL)
A-6	2.0	250	6	3	$2 \times 10^7$
A-28	2.0	250	28	3	$2 \times 10^7$
B-6	2.0	250	6	3	Non-inoculated
B-28	2.0	250	28	3	Non-inoculated

Table S2. Selected functional genes and primers. The glyceraldehyde-3-phosphate dehydrogenase gene (*gapdh*) gene was used as a reference.

Gene	Locus tag	Product	Iron/Sulfur Oxidation	Primer	Sequence (5'-3')	Product Length (bp)	Annealing Temperature
<i>rusA</i>	BBC27_RS14280	Rusticyanin	Iron oxidation	RusA-F RusA-R	GGCGGTCACTGTGGGTATGG TGGACTTCAAAGCTCGGGAATG	204	55
<i>cycA1</i>	BBC27_RS13565	cytochrome C	Iron oxidation	cycA1-F cycA1-R	CCTATCGTGCTCGTCAGTATGG TTGGGTATCCGCCGTTGTT	195	55
<i>hdrA</i>	BBC27_RS02855	Heterodisulfide reductase subunit A	Sulfur oxidation	HdrA-F HdrA-R	TCCCATTATCTCGGGCTATGC CGACCTTATCGCCGTTGCT	185	55
<i>doxDA</i>	BBC27_RS05135	Thiosulfate:quinone reductase	Sulfur oxidation	Dox-F Dox-R	TTCCACTACCACTTTCCGATGC TGATGTTTGCCAGGACTTACCG	151	55
<i>cyoC1</i>	BBC27_RS01730	Cytochrome o ubiquinone oxidase	Sulfur oxidation	CyoC1-F CyoC1-R	ATCATTGGGCATCACCGTAGC ATCCAGATCAACCCGAAGAAAAT	99	55
<i>gapdh</i>	BBC27_RS12850	glyceraldehyde-3-phosphate dehydrogenase	/	Gapdh-F Gapdh-R	CCCTCGTTGACCTCACCTGTATC GGTGGAGGAGTGCGGATTATGG	161	55

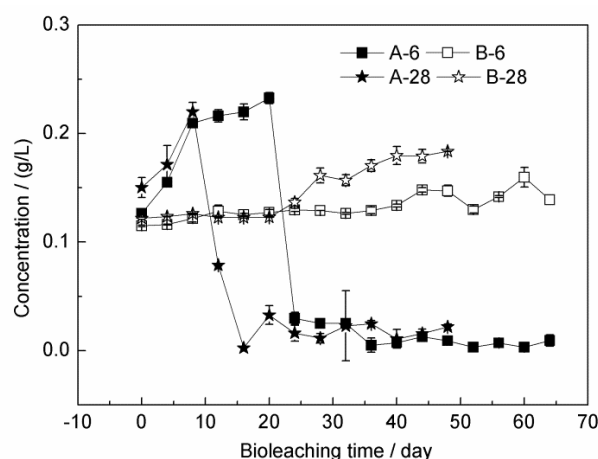
**Table S3.** Taxonomic traits of YL15 and the reported isolates of the species *Acidithiobacillus ferrivorans*. Data of strains SS3, NO-37 and ACH were referenced to [14,34–36].

Trait	YL15	SS3	NO-37 <sup>T</sup>	ACH
Cell shape	Rod	Rod	Rod	Rod
Cell size (µm)	2.09 × 1.02	ND	2.4 × 0.5	2.2
pH range for growth (optimum)	1.5–3.5 (2.0)	ND	1.9–3.4 (2.5)	1.7–2.5 (2.0–3.0)
Temperature range for growth (°C) (optimum)	4 <sup>a</sup> –35 (28)	5–30 (~25)	4–37 (28–33)	4–30 (28)
Growth using electron donor				
Sulfur	+	+	+	+
Thiosulfate	+	+	+	+
Sulfide minerals	+	+	+	+
Ferrous iron	+	+	+	+
Tetrathionate	+	+	+	+
Growth with organic carbon				
Glutamic acid	-	NR	-	NR
Glycerol	-	-	-	NR
Citric acid	-	NR	-	NR
Glucose	-	-	-	NR
Sucrose	-	-	NR	NR
Arabinose	-	-	NR	NR
Fructose	-	-	NR	NR
Galactose	-	-	NR	NR
Xylose	-	-	NR	NR
Yeast extract	-	-	-	NR
Cas amino acid	-	-	NR	NR

+ able to utilize it as a sole energy source; - unable to utilize it as a sole energy source or did not support or inhibit growth with inorganic energy sources (ferrous iron or tetrathionate); ND—not determined; NR—not reported; <sup>a</sup> the lowest temperature tested.

**Table S4.** A comparison of the relative gene expression at 6 °C and 28 °C in the mid-log phase during bioleaching of chalcopyrite. The relative expression level was the absolute cDNA copy number of functional gene relative to *gapdh*.

Temperature	<i>rusA</i>	<i>hdrA</i>	<i>cyoC1</i>	<i>doxDA</i>	<i>cycA1</i>
6 °C	1032.8	33.0	50.1	1.12	27.3
28 °C	107.7	13.4	19.1	0.17	14.4
Fold change (6 °C/28 °C)	9.59	2.45	2.62	6.59	1.90



**Figure S1.** The dynamics of ferrous iron concentration at 6 °C and 28 °C during bioleaching of chalcopyrite.