Supporting Information

Dynamic of active microorganisms inhabiting a bioleaching industrial heap of low-grade copper sulfide ore monitored by Real-time PCR and oligonucleotide Prokaryotic Acidophile Microarray (PAM)

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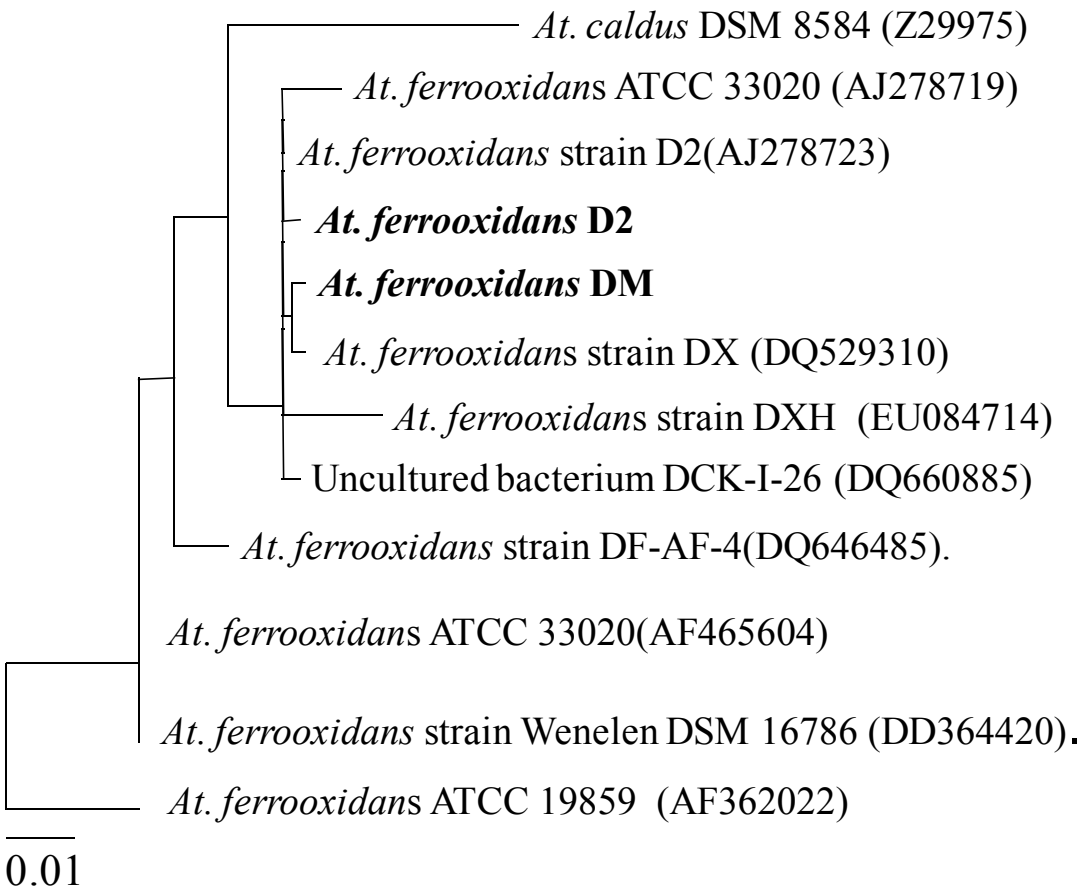
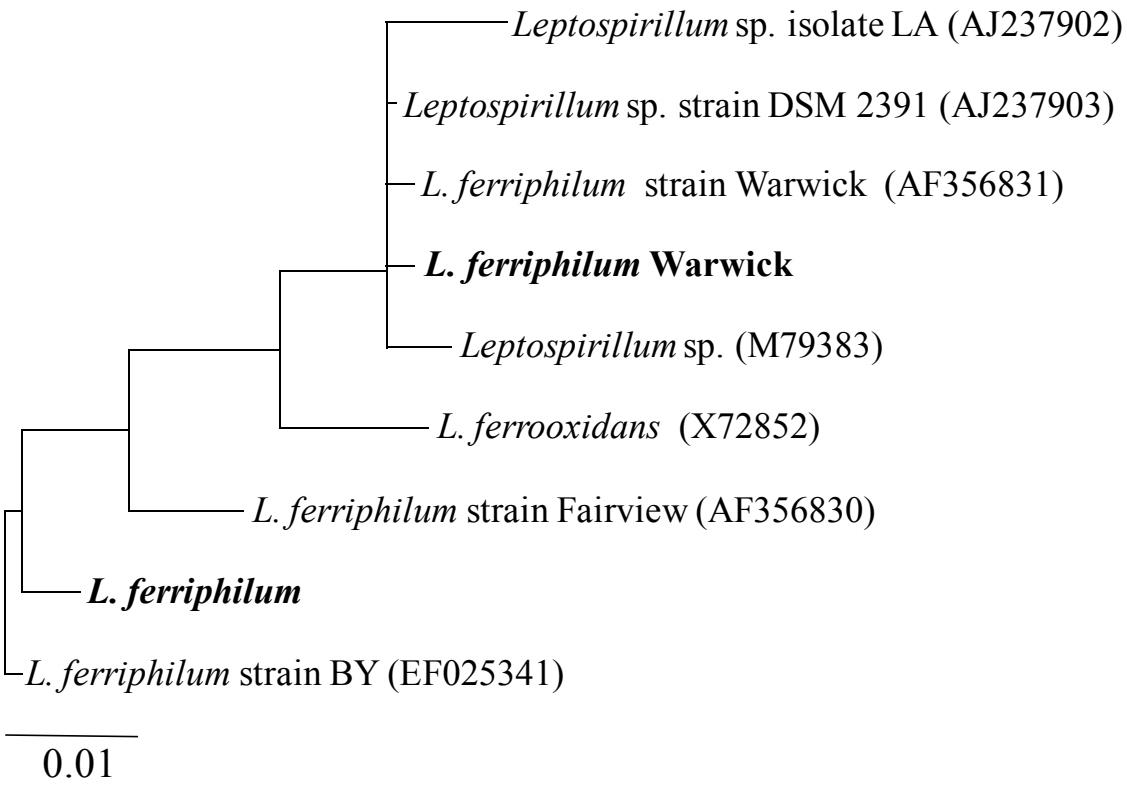
Supporting Figures

Supporting Figure S1. Phylogenetic tree based on comparative analysis of 16S rRNA gene sequences representatives of *At. ferrooxidans* type strains. Sequences were aligned using the alignment tool of ARB program (Strunk and Ludwing, 1995). Phylogenetic tree was generated using the maximum-parsinomy algorithm in the ARB program. The bar indicates a 1 % estimated sequence divergence. The sequences obtained in this study are indicated in bold.

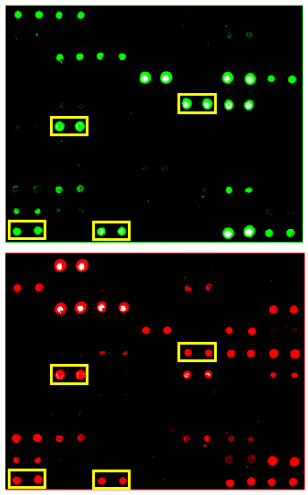


Supporting Figure S2. Phylogenetic tree based on comparative analysis of 16S rRNA gene sequences representatives of *Leptospirillum* strains. Sequences were aligned using the alignment tool of ARB program (Strunk and Ludwing, 1995). Phylogenetic tree was generated using the maximum-parsinomy algorithm in the ARB program. The bar indicates a 1 % estimated sequence divergence. The sequences obtained in this study are indicated in bold.



Supporting Figure S3. Assaying total industrial heap RNA with a Prokaryotic acidophile microarray (PAM). (A) Examples of two PAM images corresponding to hybridization with total RNA from strips S10 and S5. The yellow rectangles point out the universal probes UNI1392, Y2R and EU338 for a better orientation. (B) The name and position of each probe was described by Garrido *et al*. (2008). Different coloured rectangles indicate some relevant probes: universal (*yellow*), those showing higher intensity in earlier (S10, in *green*) or older (S5, *red*) stages of the industrial process. (C) Histograms showing the average relative signal intensity of two different hybridizations for the different analyzed strips, from younger to older ones (S10-S8-S5). BACT, Bacteria; Alpha, Alphaproteobacteria; HGC, High GC containing bacteria; LGC, Low GC containing bacteria; Acidob., Acidobacterium; Beta, Betaproteobacteria.

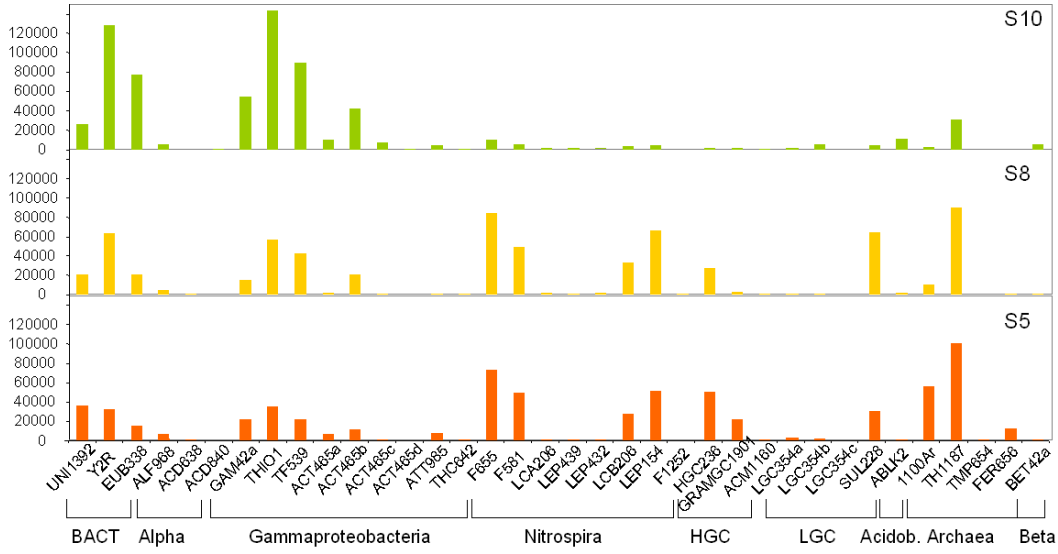
A



B

ABLK2	TH1187	Cren 537	MBGB 280	MBGB 335	MBGB 380	MBGB 525
SUL228	TM1G 138	TM2G 138	GALTS 0084	WJ2	NITROSO	ABLK 1
ACT465d	F655	F581	F1252	ACD 840	FRM0732	HGC 236
BET42a	E.coli DNA	Lfe DNA	TF539	THC642	ACT465b	ACT465c
MEB859	ICDH	EUK 502	CASEI F	Y2R	GAM42a	GRAMGC 1901 (23s)
Nonsense	UNI1392	NON 338	1Af	1100Ar	TMP 654	FER 656
Type 2b	NON338	NON 338	SREDBA C 647	NON338	NON338	NON338
DSP648	DFMI 229	Mb1007r	NON338	Mm1007r	Ms 1020r	Mm 835
LEP154	ALF968	ACD638	ACM1160	LGC354a	LGC354b	LGC 354c
ATT985	Nonsense	NON338	LCA 206	LEP439	LEP432	LCB 206
UNI1392	NON338	EUB 338	EUB 338II	EUB 338III	THI01	ACT465a

C



Supporting Table S1

Table S1. Acidophilic microorganisms identified in heap processes.

Heap, Location	Microorganisms identified	Reference
Cultures from Chalcopyrite overburden, Australia	*At. ferrooxidans*, *At. thiooxidans*, *Acidiphilium cryptum*	Goebel and Stackebrandt, 1994
Cultures and solutions from Lo Aguirre copper, Chile	*At. ferrooxidans, At. thiooxidans, L. ferrooxidans*	Espejo and Romero, 1997
Copper sulfide/oxide, SW USA	*Acidithiobacillus spp.*, *L. ferrooxidans*, *Acidiphilium spp.*, *'Ferrimicrobium acidiphilum'*	Bruhn *et al*., 1999
Run-of-mine test heap, Chile	*At. ferrooxidans*, *L. ferriphilum*, Firmicutes, *F. acidiphilum*, Crenarchaeotes	Demergasso *et al*., 2005
Chalcocite heap, Australia	*L. ferriphilum*, *Acidithiobacillus caldus*, *'Ferroplasma cyprexacervatum'*	Hawkes *et al*.,2006
Tong Shankou copper, China	Betaproteobacteria (uncultured Tui3-12 and *Acidivorax* sp.), *At. ferrooxidans*, *Acidiphilium* sp., *Leptospirillum* species, novel Firmicutes, *Thermoplasma* genus, *Ferroplasma* genus	Xie *et al*., 2007
Dongxiang copper mine, China	*L. ferriphilum, Leptospirillum* sp., *At. ferrooxidans, A. albertensis,* uncultured bacterium, *F. cyprexacervatum*, *F. acidiphilum*, uncultured archaeon, *Thermoplasma* group	He *et al.*, 2008; Xiao *et al.*, 2008
Run-of-mine industrial heap, Chile	*At. ferrooxidans*, *At. thiooxidans*, *L. ferriphillum*, *Acidiphilium*-like, Firmicutes, *Ab. disulfidooxidans*, *F. acidiphilum*, *Acidobacterium* spp., *Sulfobacillus* spp.	This study

Supporting Experimental Procedures

RNA amplification and cDNA labeling

For microarray analysis, the quality of RNA was checked using the Bioanalyzer 2100 (Agilent technologies). The total RNA of strips S5, S8 and S10 in August 2007 was amplified through a method based on T7 RNA polymerase linear amplification as described previously (Moreno-Paz and Parro, 2006), but using all the reagents and components of the Message Amp II aRNA kit (Ambion). After a second amplification round the sense RNA (2 μg) was labeled with the CyScribe first-strand cDNA labeling kit (Amersham Biosciences) with Cy5 or Cy3-dUTP following the supplier´s recommendations.

Microarray hybridization and analysis

The Prokaryotic Acidophile Microarray (PAM) was constructed with oligonucleotide probes obtained from the literature and printed with Microgrid II arrayer (BioRobotics, Genomic Solutions) as described previously (Garrido et al., 2008). Microarrays were pre-hybridized with 20 μL of 5× SSC, 0.1 % SDS, 0.1 mg mL^{-1} of herring sperm DNA, and 1 % BSA at 42 ^{o}C during 30-40 min. Slides were washed in distilled water, submerged in isopropanol before being dried by centrifugation. The hybridization was carried out at 50 ^{o}C during 12 hours and washed at room temperature as described (Garrido et al., 2008). Scanning was performed in a GenePix 4000B scanner (Genomic Solutions). Images were analyzed and spots signal was quantified using Genepix Pro 6.0 software (Genomic

Solution). Those spot having intensities 3-4 times over background were considered positive.

Supporting References

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Xie, X., Xiao, S., He, Z., Liu, J. and Qiu, G. (2007) Microbial populations in acid mineral bioleaching system of Tong Shankou Copper Mine, China. *J Appl Microbiol* **103:** 1227-1238.