Supplementary Material

**The Type IV Secretion System of ICE*Afe*1: formation**

**of a conjugative pilus in *Acidithiobacillus ferrooxidans***

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# Supplementary Figures and Tables

## Supplementary Tables

**Supplementary Table 1.** Primers used in this paper.

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**Supplementary Table 2**. Annotation of ICE*Afe*1 genes.

XLS file

**Supplementary Table 3**.Amino acidic sequence similarity values for proteins encoded in conjugative gene clusters in public *Acidithiobacilli* genomes.

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**Supplementary Table 4**. Log2 relative level of expression in *A. ferrooxidans*TY cells grown on different energy substrates.

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## Supplementary Figures

**Supplementary Figure 1**. Operon analysis of conjugative gene clusters by RT-PCR.

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**Supplementary Figure 2**. Comparison of TraA1 and TraA2 pilin primary and secondary structures. (A) Protein identity between pilin TraA1 and TraA2 of the *A. ferrooxidans* ATCC 23270 ICE*Afe*1 versus *A. ferrooxidans* Wenelen and other acidithiobacilli. Identity values for complete and mature pilin (in parentesis) are shown. (B) MAFFT-based amino acidic sequence alignment of mature TraA-like pilins from *A. ferrooxidans* ATCC 23270 ICE*Afe*1, other acidithiobacilli and *S. typhi* (5LEG). The secondary structures were predicted using Jpred. Hydrophobicity index and polarity were calculated using Geneious v11.0.2. Amino acids in the alignment are colored according to their polarity as follows: yellow, non-polar; green: polar, uncharged; red: polar, acidic; blue: polar, basic. Amino acids in the logo above are colored according to their hydrophobicity index as follows: red, hydrophobic; blue, hydrophilic.

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**Supplementary Figure 3**. Original images for immunogold to *A. ferrooxidans*TY treated with Mit C. (A) Cells incubated with pre-immune serum. (B) Cells incubated without anti-pilin serum.

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