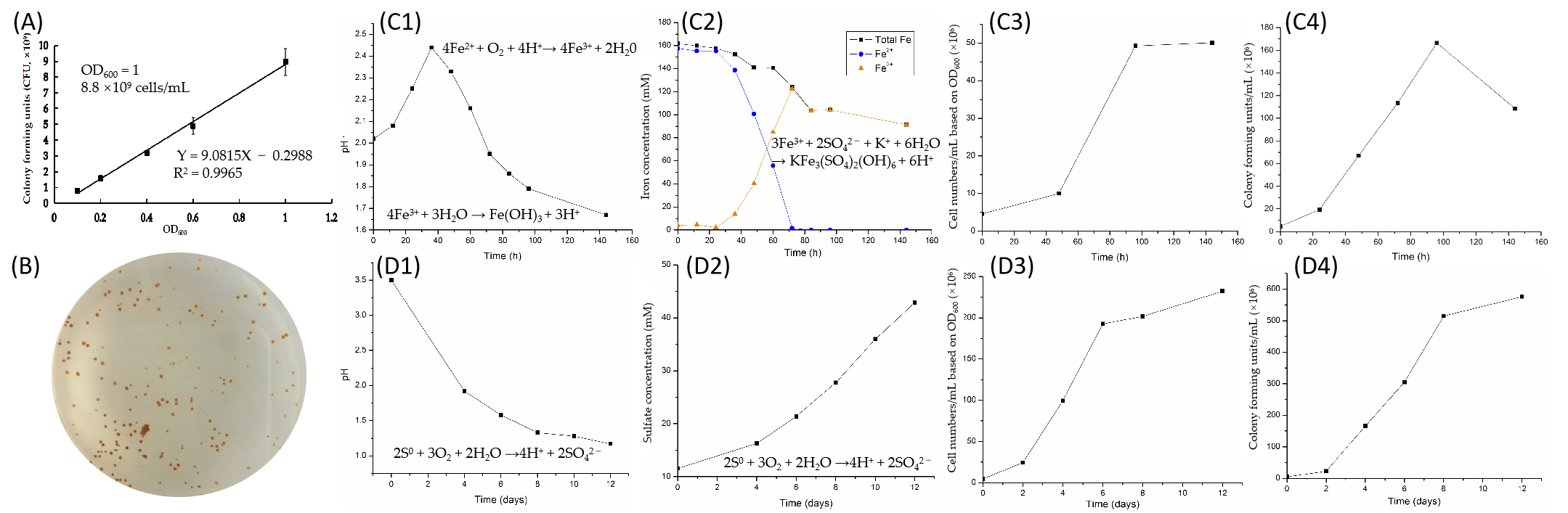
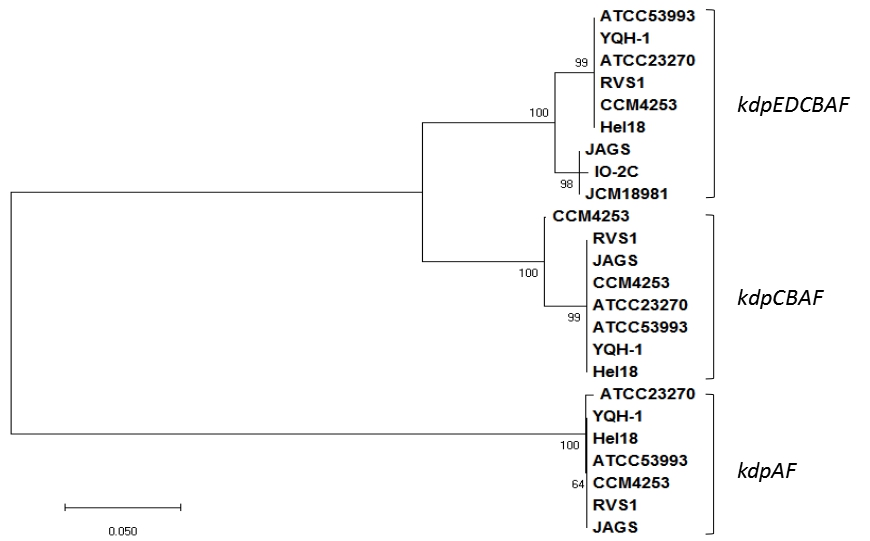
Supplementary Materials: Genomic Analysis of a Newly Isolated *Acidithiobacillus ferridurans* JAGS Strain Reveals Its Adaptation to Acid Mine Drainage

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**Figure S1.** Growth features of *A. ferridurans* JAGS. (**A**) Standard curve of optical density (OD600) versus cell numbers obtained by plate count method. (**B**) Colonies on 2:2 solid medium for cell count. (**C**) Growth on 9K-Fe2+ medium, (**C1**) pH value; (**C2**) Fe oxidation; (**C3**) cell numbers by OD600; (**C4**) cell numbers by plate count method. (**D**) Growth on 9K-S0 medium, (**D1**) pH value; (**D2**) sulfur oxidation; (**D3**) cell count by OD600; (**D4**) cell numbers by plate count method.



**Figure S2.** Neighbor-joining (NJ) phylogenetic tree of the kdpA protein sequences derived from nine *Acidithiobacillus* strains. Bootstrap values indicated at each node are based on a total of 500 bootstrap replicates.

**Table S1.** Average nucleotide identity (ANI) (%) based on whole-genome alignments among *Acidithiobacillus* strains by JSpeciesWS.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **\*** | ***A. ferriduxidans* JAGS** | ***A. ferriduxidans* JCM18981** | ***A. ferrooxidans* IO-2C** | ***A. ferrooxidans* ATCC53993** | ***A. ferrooxidans* RVS1** | ***A. ferrooxidans* CCM4253** | ***A. ferrooxidans* YQH1** | ***A. ferrooxidans* Hel18** | ***A. ferrooxidans* ATCC23270** |
| *A. ferridurans* JAGS | \* | 99.66 | 99.23 | 95.51 | 95.33 | 95.32 | 95.34 | 95.34 | 95.37 |
| *A. ferridurans* JCM18981 | 99.13 | \* | 99.22 | 95.13 | 95.08 | 95.07 | 95.05 | 95.06 | 95.12 |
| *A. ferrooxidans* IO-2C | 98.69 | 98.18 | \* | 95.32 | 95.27 | 95.26 | 95.26 | 95.26 | 95.52 |
| *A. ferrooxidans* ATCC53993 | 94.98 | 94.11 | 94.89 | \* | 99.86 | 99.78 | 99.8 | 99.8 | 99.87 |
| *A. ferrooxidans* RVS1 | 94.68 | 93.97 | 94.79 | 99.57 | \* | 99.94 | 99.95 | 99.95 | 99.84 |
| *A. ferrooxidans* CCM4253 | 94.70 | 93.83 | 94.79 | 99.49 | 99.92 | \* | 99.97 | 99.98 | 99.82 |
| *A. ferrooxidans* YQH1 | 94.71 | 93.83 | 94.8 | 99.5 | 99.93 | 99.97 | \* | 99.99 | 99.83 |
| *A. ferrooxidans* Hel18 | 94.69 | 93.83 | 94.77 | 99.49 | 99.91 | 99.97 | 99.97 | \* | 99.82 |
| *A. ferrooxidans* ATCC23270 | 94.76 | 94.00 | 94.81 | 99.64 | 99.56 | 98.93 | 99.1 | 99.04 | \* |

Note: lower matrix ANIb, upper matrix ANIm.

**Table S2.** Genes predicted to be involved in acid and heavy metal tolerance in *A. ferridurans* JAGS.

|  |  |
| --- | --- |
| **Protein** | **Gene ID** |
| Acid Stress Resistance | - |
| OMP40, outer membrane protein. | F6A13\_00370 |
| HpnB, glycosyltransferase. | F6A13\_01630 |
| HpnMHNKJIAG cluster, hopanoid-associated proteins. | F6A13\_09105-09150 |
| Shc, squalene-hopene cyclase (EC 5.4.99.17). | F6A13\_09155 |
| Sqs, squalene synthase (EC 2.5.1.21). | F6A13\_09160 |
| kdpD, osmosensitive K+ channel histidine kinase | F6A13\_02550 |
| KdpEDFABC cluster, a kdp-type potassium uptake ATPase system. | F6A13\_09580-09605 |
| KdpCBAFAF cluster, a kdp-type potassium uptake ATPase system. | F6A13\_11005-11030 |
| TrkA, Voltage-gated potassium channel protein. | F6A13\_13725 |
| NhaA, Na+/H+ antiporter. | F6A13\_09475 |
| putative Na+/H+ antiporter. | F6A13\_04755 |
| SpeA, biosynthetic arginine decarboxylase (EC 4.1.1.19). | F6A13\_06090 |
| PanD, aspartate 1-decarboxylase (EC 4.1.1.11). | F6A13\_14595 |
| Psd, phosphatidylserine decarboxylase (EC 4.1.1.65). | F6A13\_04545 |
| ClpB, chaperone protein ClpB (ATP-dependent unfoldase). | F6A13\_08410 |
| DnaJK, chaperone proteins. | F6A13\_12450-12455 |
| GrpE, heat shock protein. | F6A13\_12460 |
| RadA, DNA repair protein. | F6A13\_13190 |
| DnaB, replicative DNA helicase (DnaB) (EC 3.6.4.12). | F6A13\_13200 |
| RecA, DNA repair protein. | F6A13\_05400 |
| LexA-ImuAB-DnaE cluster: LexA, SOS-response repressor and protease LexA  (EC 3.4.21.88); ImuA, RecA/RadA recombinase; ImuB, DNA polymerase IV-like protein;  DnaE, error-prone repair homolog of DNA polymerase III alpha subunit (EC 2.7.7.7). | F6A13\_07785-07800 |
| **Heavy-Metal Resistance** | - |
| Lead, cadmium, zinc and mercury transporting ATPase (EC 3.6.3.3) (EC 3.6.3.5);  Copper-translocating P-type ATPase (EC 3.6.3.4). | F6A13\_04860, 04905, 04925, 04945,  08625, 10865, 10890, 11740 |
| MgtA, Magnesium (Mg2+) transport ATPase, P-type (EC 3.6.3.2) | F6A13\_09840 |
| CopZ, Copper(I) chaperone | F6A13\_04870, 07760, 10900 |
| RND efflux system (Ni/Co/Cd/Zn). | F6A13\_08730-08740, 15065-15075 |
| CzcABC family RND transporter system (Ni/Mn/Fe/Mo). | F6A13\_00815-00825, 07835-07845,  08240-08250, 08695-08705,  10835-10845, 11760-11770 |
| CzcA/D, Co/Zn/Cd resistance proteins. | F6A13\_05895, 06205, 08215, 10785 |
| ZnuACBB, Zinc ABC transporter system. | F6A13\_03815-03830 |
| CorA, Mg/Co transport proteins. | F6A13\_14780-14795 |
| CorC, Mg/Co transport proteins | F6A13\_03800, 08900 |
| Cation-transporting ATPase | F6A13\_13815 |
| MerRTPA, Hg resistance. | F6A13\_00710-00725 |
| MerCAD, Hg resistance. | F6A13\_11040-11055 |
| ArsM, Arsenite methyltransferase (EC 2.1.1.137) | F6A13\_09070 |
| ArsADC, As resistance. | F6A13\_10625-10635; |
| ArsHBRCDA, As resistance. | F6A13\_13380-13405 |
| ArsRC, As resistance. | F6A13\_13430, 13435 |
| MntH, Manganese transport protein. | F6A13\_00800, 05345 |