

1    **An acid-tolerant heterotrophic microorganism role in improving tannery sludge**  
2    **bioleaching conducted in successive multi-batch reaction systems**

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10    **Supporting Information**

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**Capitons:**

Table S1. Morphological and biochemical characteristics of the strain D13

Figure S1. Neighbor-joining tree depicting the phylogenetic relationship of *Pichia spartinae* D13 with related species based on the 26S rRNA gene D1/D2 domain sequence. Bootstrap values > 50% from 1000 bootstrap replicates are shown at the branch points. Bar, 0.005 substituions per site. Reference sequences were retrieved from GeneBank under the accession numbers indicated.

Figure S2. Relationship of *P. spartinae* D13 with sludge DOM during  $\text{Fe}^{2+}$  was oxidized by *A. ferrooxidans* LX5 in the presence of tannery sludge DOM: DOC for pure culture of *A. ferrooxidans* LX5 ( $\Delta$ ); DOC for the mixed cultures of *A. ferrooxidans* LX5 and *P. spartinae* D13 ( $\blacktriangle$ ); counts of *P. spartinae* D13 for pure culture of *A. ferrooxidans* LX5 ( $\square$ ); counts of *P. spartinae* D13 for the mixed cultures of *A. ferrooxidans* LX5 and *P. spartinae* D13 ( $\blacksquare$ )

Figure S3. Relationship of *P. spartinae* D13 with sludge DOM during  $\text{S}^0$  was oxidized by *A. thiooxidans* TS6: DOC for pure culture of *A. thiooxidans* TS6 ( $\Delta$ ); DOC for the mixed cultures of *A. thiooxidans* TS6 and *P. spartinae* D13 ( $\blacktriangle$ ); counts of *P. spartinae* D13 for pure culture of *A. thiooxidans* TS6 ( $\square$ ); counts of *P. spartinae* D13 for the mixed cultures of *A. thiooxidans* TS6 and *P. spartinae* D13 ( $\blacksquare$ )

37 Figure S4. Schematic figure representing the successive multibatch reaction  
38 bioleaching systems  
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*Molecular phylogenetic analysis.* Total DNA was extracted from cells of strain D13 according to the method of Makimura et al. (1). The 18S rRNA gene was amplified with primer pair NS0 (5'-TATCTGGTTGATCCTGCC-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') (2). Purified PCR product was ligated into the linear vector pMD18-T (TakaRa biotechnology) and was sequenced with primer RV-M, primer 13-47, designed according to the sequence of vector pMD18-T, and another primer (5'-CGAGGAACAATTGGAGGGC-3') designed according to the 18S rRNA gene. A DNA fragment covering the ITS, 5.8S rRNA gene and 26S rRNA gene D1/D2 domain was amplified with the primer pairs ITS1 and NL4, the ITS region was sequenced directly with ITS1 and ITS4, the D1/D2 domain was sequenced directly with NL1 and NL4 (3). These sequences were compared with available sequences in the GeneBank database by BLAST system (<http://www.ncbi.nlm.nih.gov/BLAST/>). The D1/D2 domain sequence was aligned with closely related sequences using the CLUSTAL\_X program (4). Phylogenetic tree was constructed from evolutionary distance data that were calculated with Kimura's two-parameter model (5) by using the neighbor-joining method (6). Bootstrap analysis (7) was based on 1000 random resamplings. Reference sequences were retrieved from GeneBank with the accession numbers as indicated in the tree.

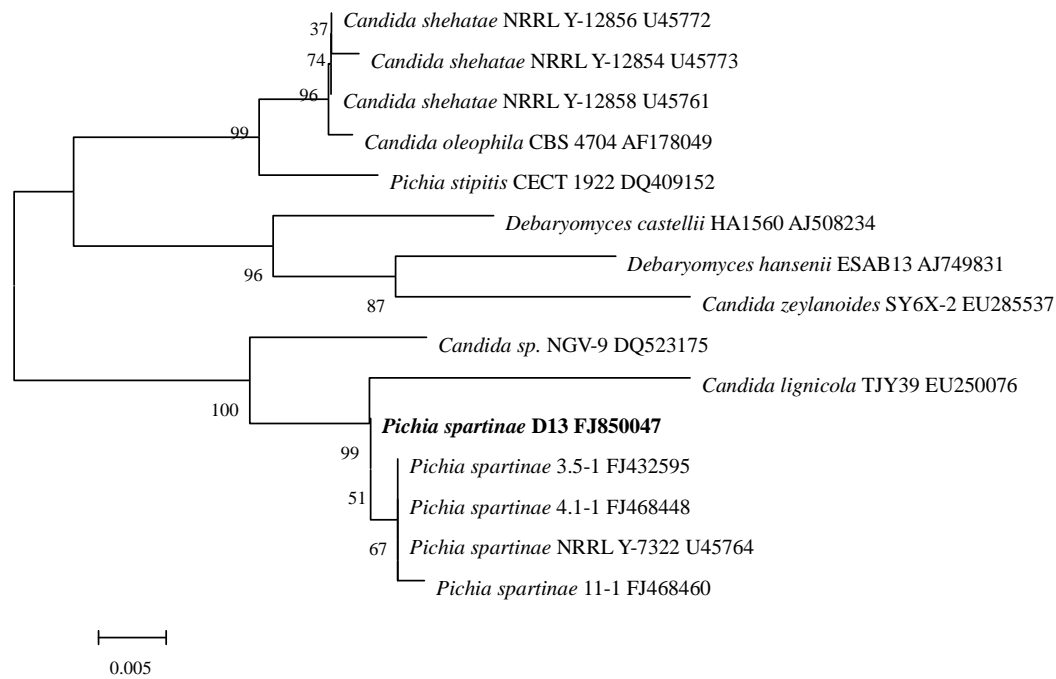
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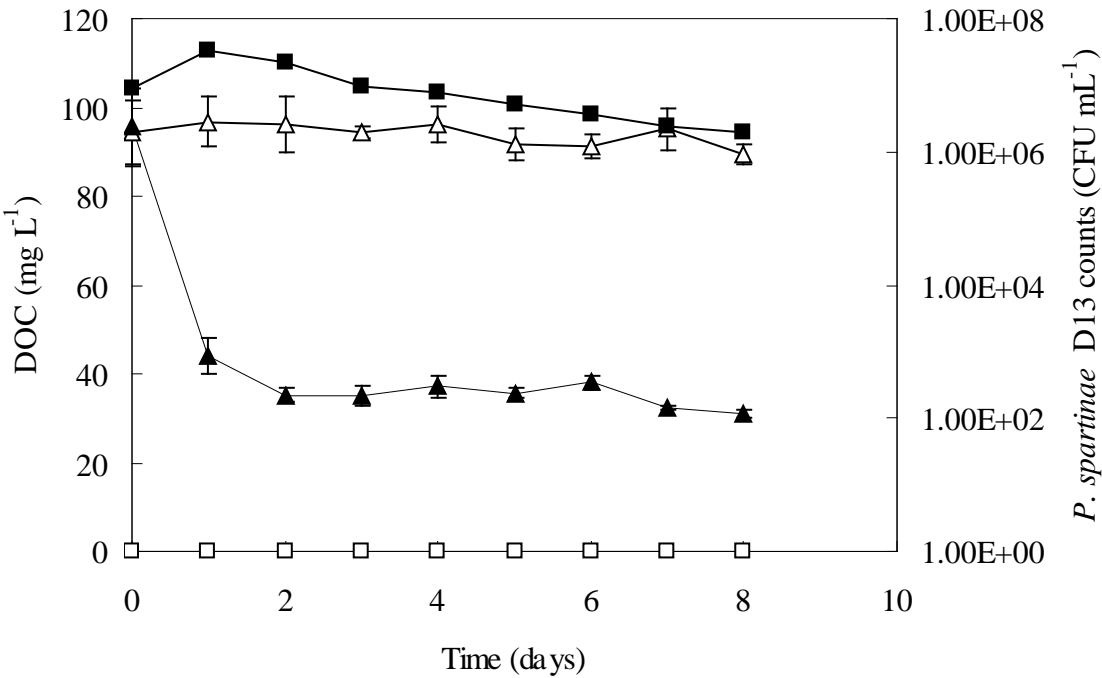
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84     FIGURE S2.

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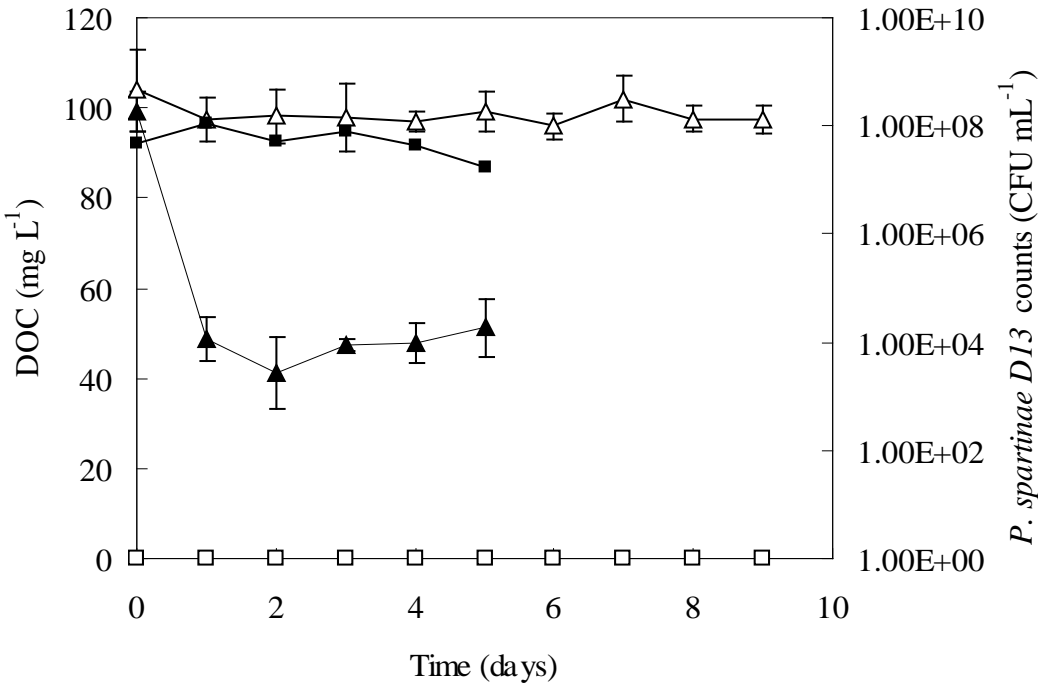
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88    FIGURE S3.

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92 FIGURE S4.

