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GENOME SEQUENCES

Genome Sequence of the Extremely Acidophilic Fungus *Acidomyces richmondensis* FRIK2901

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ABSTRACT *Acidomyces richmondensis* is an extremophilic fungal species found in warm, acidic, and metal-rich environments. To improve upon the existing reference genome, we used PacBio and Illumina sequencing to assemble a highly contiguous 29.3-Mb genome of *A. richmondensis* FRIK2901.

Acidomyces richmondensis is an acidophilic dothideomycete capable of growing at a pH as low as 0.1 and optimally at a pH range of 2 to 5. *A. richmondensis* is part of an extraordinary microbial community present in the warm, acidic, and metal-rich acid mine drainage environment from the Richmond Mine at Iron Mountain in California (1–3). Here, we present a high-quality highly contiguous whole-genome assembly of *A. richmondensis* FRIK2901. This assembly is a significant upgrade from the existing highly fragmented *A. richmondensis* reference genome (3) and provides a basis for future genomic and molecular characterization of genes and pathways involved in acid and heavy metal tolerance.

We isolated *A. richmondensis* FRIK2901 by diluting samples of Richmond Mine water to extinction in growth medium, distributing diluted samples to microtitration plates, and incubating at 37°C for 14 days. *A. richmondensis* FRIK2901 was grown aerobically at pH 1.0 at 37°C in medium described previously, with some modifications (4). The medium contained (per liter): 20 g $FeSO_4 \cdot 7H_2O$, 800 mg $(NH_4)_2SO_4$, 800 mg $Ni(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 400 mg KH_2PO_4, 160 mg $MgSO_4 \cdot 7H_2O$, 85 mg $Na_2MoO_4 \cdot 2H_2O$, 70 mg $ZnCl_2$, 31 mg H_3BO_3, 10 mg $MnCl_2 \cdot 4H_2O$, 10 mg $CoCl_2 \cdot 6H_2O$, and 0.1% (wt/vol) yeast extract (Becton, Dickinson, Sparks, MD). Genomic DNA was extracted following the method described by Lee et al. (5). A PacBio circular consensus sequence (CCS) library was constructed and sequenced at the University of Wisconsin—Milwaukee on a PacBio RS II platform. A paired-end Illumina library with an average insert size of 450 bp was constructed with the Nextera DNA library preparation kit and sequenced to produce 152-bp reads on an Illumina NextSeq 500 system at ProteinCT (Madison, WI). Raw PacBio sequences were adapter trimmed and error corrected using Canu version 1.6 (6), resulting in a high-quality long-read data set of 112,430 PacBio reads, with an average read length of ~7 kb. Raw Illumina sequence reads were adapter and quality trimmed using Trim Galore! (7) and then error corrected using Quake (8), resulting in a high-quality short-read data set of 11,603,552 read pairs.

Genome assembly was conducted by first using Canu version 1.6 (6) with the preassembly-improved PacBio data. Next, genome polishing and error correction were performed on the initial PacBio assembly with the preassembly-improved Illumina data using Pilon version 1.21 (9). This hybrid assembly approach resulted in a highly

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contiguous ~150× coverage assembly composed of 34 scaffolds, with a GC content of 49.45%, an N_{50} value of 1,214,476 bp, a nuclear genome size of 29.25 Mb, and a mitochondrial genome size of 97.70 kb. Genome assembly quality was assessed using BUSCO version 2.0 (10) with the dikarya odb9 data set. Of the 1,312 BUSCO group genes, 97.5% were completely (93.6%) or partially (3.9%) recovered in the *A. richmondensis* FRIK2901 assembly, indicating a high-quality genome assembly. Gene prediction and annotation were performed using the Funannotate pipeline (11). A total of 8,336 protein-coding genes were predicted, including 306 carbohydrate-active enzymes and 281 peptidases. Interestingly, only 3 secondary metabolite-encoding gene clusters were identified via antiSMASH 3.0 (12). We identified 703 genes in the *A. richmondensis* FRIK2901 genome that did not have significant BLAST hits (E value cutoff = 1e−6) to the closely related species *Hortaea werneckii*, the dothideomycete *Mycosphaerella graminicola*, and the budding yeast *Saccharomyces cerevisiae* (13). Several of these lineage-specific genes possess domains with potential roles in acid tolerance and heavy metal tolerance.

Data availability. This whole-genome shotgun project has been deposited at GenBank under the accession number QVLQ00000000. Raw Illumina and PacBio data have been deposited in the NCBI Sequence Read Archive under the BioProject accession number PRJNA486357.

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