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GENOME SEQUENCES

Draft Genome Assemblies of Ionic Liquid-Resistant *Yarrowia lipolytica* PO1f and Its Superior Evolved Strain, YlCW001

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ABSTRACT Adaptive laboratory evolution of *Yarrowia lipolytica* PO1f in the benchmark ionic liquid (IL; 1-ethyl-3-methylimidazolium acetate) produced a superior IL-tolerant microorganism, strain YlCW001. Here, we report the genome sequences of PO1f and YlCW001 to study the robustness of *Y. lipolytica* and its potential use as a microbial platform for producing fuels and chemicals.

Yarrowia lipolytica PO1f (ATCC MYA-2613), one of the most widely characterized strains of the *Yarrowia* clade (subphylum *Saccharomycotina*), is derivative of the French haploid strain W29 (ATCC 20460), engineered to remove extracellular protease production (1). This generally recognized as safe (GRAS) yeast (2) is regarded as an emerging bioenergy microbe due to its ability to consume complex sugars (3, 4) and produce specialty lipids (5–7), organic acids (8–10), and proteins (1, 11). Additionally, *Y. lipolytica* exhibits exceptional tolerance to high salinity (12), broad pH ranges (13), and ionic liquids (ILs) (14, 15). Microbial biocatalysis in IL is an attractive strategy for the production of high-value chemicals (16) and biofuels (17) because ILs effectively process recalcitrant biomass for fermentation (18–20). While ILs are toxic to most microbes at a concentration of 0.5 to 1.0% (vol/vol) (21, 22), PO1f exhibits robust growth and organic acid production in up to 10% (vol/vol) 1-ethyl-3-methyl imidazolium acetate (EMIM OAc) (14). Aiming to illuminate the underlying mechanisms of IL resistance, we report the genome sequences of the naturally IL-tolerant strain PO1f and its superior evolved mutant, strain YlCW001, generated from 200 generations of adaptive laboratory evolution (15). To our knowledge, YlCW001 is the most IL-tolerant microorganism, capable of growing in a variety of ILs at high levels, up to 18% IL.

PO1f and YlCW001 were cultured in rich medium overnight, and their genomic DNA samples were collected using a Zymo Research (Irvine, CA) fungal/bacterial DNA miniprep kit (catalog no. D6005). Sequencing was performed at the U.S. Department of Energy Joint Genome Institute (DOE JGI) using the Illumina HiSeq 2500 platform as previously described (23). Briefly, 100 ng of DNA was sheared to 500-bp inserts with Covaris LE220 (Woburn, MA) with size selection solid-phase reversible immobilization (SPRI) beads (Beckman Coulter, Brea, CA). The KAPA-Illumina library creation kit (Kapa Biosystems, Boston, MA) was used to treat the resulting fragments with end repair, A tailing, and ligation of Illumina-compatible adapters (IDT, Inc., Skokie, IL). A clustered flow cell was generated using a TruSeq paired-end cluster kit v4 from the multiplexed libraries, and sequencing was performed following a 2 × 100-bp indexed run recipe using the HiSeq TruSeq sequencing by synthesis (SBS) sequencing kits v4. The raw sequence data were filtered using the JGI quality control (QC) pipeline, which integrates BBDuk (v36.94) (http://bbtools.jgi.doe.gov) and BBMap. This pipeline removes contaminants, adapter sequences, right quality reads where quality drops to 0, reads containing 1 or more N bases, reads having an average quality score less than 13, and reads

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TABLE 1 Genome information and accession numbers

Strain	GenBank accession no.	SRA accession no.	BioProject accession no.	Genome size (Mbp)	Coverage (×)	No. of contigs	No. of gene models
PO1f	WHUU00000000	SRP115772	PRJNA347220	20.21	155.42	175	6,798
YlCW001	WEZX00000000	SRP115773	PRJNA347221	20.22	166.48	150	6,800

containing a minimum length less than or equal to 41 bp or 33% of the full read length. Further, reads that were mapped at 95% identity to masked human, cat, dog, mouse, or common microbial contaminants were removed. Genome assembly on JGI QC-filtered genomic reads was carried out using SPAdes v3.11.1 (24) with the following parameters: --phred-offset 33 --cov-cutoff auto -t 16 -m 115 --careful --12. The genomes of PO1f and YlCW001 were annotated with transcripts of FKP355 (25) using the JGI annotation pipeline (https://mycocosm.jgi.doe.gov/programs/fungi/FungalGenomeAnnotationSOP.pdf) (26, 27). The resultant genome assemblies are just over 20.2 Mbp, containing 49% median GC content and 6,798 to 6,800 gene models (Table 1).

Data availability. The whole-genome assemblies and annotation were deposited at DDBJ/EMBL/GenBank under the accession numbers listed in Table 1. The versions provided in this paper are the first versions.

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