





## Draft Genome Assemblies of Five Robust Yarrowia lipolytica Strains Exhibiting High Lipid Production, Pentose Sugar Utilization, and Sugar Alcohol Secretion from Undetoxified **Lignocellulosic Biomass Hydrolysates**

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ABSTRACT Screening the genetic diversity of 45 Yarrowia lipolytica strains identified five candidates with unique metabolic capability and robustness in undetoxified switchgrass hydrolysates, including superior lipid production and efficient pentose sugar utilization. Here, we report the genome sequences of these strains to study their robustness and potential to produce fuels and chemicals.

arrowia lipolytica is a dimorphic, generally regarded as safe (GRAS) oleaginous budding yeast (subphylum Saccharomycotina). It possesses unique phenotypes, including hydrocarbon assimilation (1-5), specialty lipid and organic acid production (6-11), and resistance to harsh environments, including high salinity (12), broad-range pH (13), and ionic liquid (14). By screening a comprehensive set of 45 Y. lipolytica strains with genetic diversity from the Agricultural Research Service Culture Collection (https:// nrrl.ncaur.usda.gov/), we identified five promising candidate strains, YB-392, YB-419, YB-420, YB-566, and YB-567, exhibiting beneficial phenotypes for industrial biocatalysis, including biomass hydrolysate consumption, inhibitor tolerance, and lipid and fatty acid production (15). In this study, we sequenced the genomes of these robust Y. lipolytica strains to aid further research into their physiology, metabolism, and genetics as well as metabolic engineering and synthetic biology for industrial biocatalysis.

The genomes of five Y. lipolytica isolates were extracted with the Zymo Research fungal/bacterial DNA miniprep kit (catalog number D6005; Zymo Research, Irvine, CA). Sequencing was carried out by The Department of Energy Joint Genome Institute (DOE JGI) using Illumina 500-bp insert size fragments, for which 100 ng of DNA was sheared to 500 bp using the LE220 focused ultrasonicator (Covaris, Woburn, MA) and size selected using solid phase reversible immobilization (SPRI) beads (Beckman Coulter, Brea, CA). The fragments were treated with end repair, A tailing, and ligation of Illumina-compatible adapters (IDT, Inc., Skokie, IL) using the KAPA-Illumina library creation kit (Kapa Biosystems, Boston, MA). All prepared libraries were quantified using the Kapa Biosystems next-generation sequencing library quantitative PCR (qPCR) kit and run on a Roche LightCycler 480 real-time PCR instrument. The quantified libraries were then multiplexed with other libraries, and the pool of libraries was then prepared for sequencing on the Illumina HiSeq sequencing platform utilizing a TruSeq pairedend cluster kit v4 and Illumina's cBot instrument to generate a clustered flow cell for sequencing. Sequencing of the flow cell was performed on the Illumina HiSeq 2500 sequencer using the HiSeq TruSeq sequencing by synthesis (SBS) kits v4 following a

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TABLE 1 Whole-genome assemblies and annotation for five Yarrowia lipolytica strains

|        |                       |           |                | Genome     |              | No. of  | No. of gene |
|--------|-----------------------|-----------|----------------|------------|--------------|---------|-------------|
| Strain | GenBank accession no. | SRA no.   | BioProject no. | size (Mbp) | Coverage (×) | contigs | models      |
| YB392  | QPFG00000000          | SRP129817 | PRJNA370154    | 20.18      | 735          | 362     | 6,750       |
| YB419  | QPFH00000000          | SRP129816 | PRJNA370155    | 20.14      | 740          | 370     | 6,751       |
| YB420  | QPFI00000000          | SRP129815 | PRJNA370156    | 20.2       | 259          | 369     | 6,772       |
| YB566  | QPFJ00000000          | SRP129822 | PRJNA370157    | 20.27      | 148          | 271     | 6,764       |
| YB567  | QPFK00000000          | SRP129827 | PRJNA370158    | 20.27      | 756          | 263     | 6,776       |

 $2\times100$ -bp indexed run recipe. All raw Illumina sequence data were filtered for artifact/process contamination using the JGI quality control (QC) pipeline. Briefly, BBDuk v36.94 (http://bbtools.jgi.doe.gov) was used to remove contaminants, reads that contained adapter sequences, and right quality trim reads where quality dropped to 0. BBDuk was also applied to eliminate reads containing 1 or more "N" bases, having an average quality score across the read of less than 13 or containing a minimum length of  $\leq$  41 bp or 33% of the full read length. Using BBMap, reads that were mapped to masked human, cat, dog, and mouse references at 95% identity and aligned to common microbial contaminants were separated. Filtered genomic reads were assembled with SPAdes v3.11.1 (16) using the parameters –phred-offset 33 –cov-cutoff auto -t 16 -m 115 –careful –12 to produce the target nuclear assembly. All genomes were annotated with the reference genome FKP355 (https://genome.jgi.doe.gov/Yarlip1) using the JGI annotation pipeline (17), which integrates an array of tools for gene prediction, annotation, and analysis (18).

**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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## **REFERENCES**

- Coelho M, Amaral P, Belo I. 2010. Yarrowia lipolytica: an industrial workhorse, p 930–940. In Mendez-Vilas A (ed), Current research, technology and education topics in applied microbiology and microbial biotechnology, vol 2. Formatex Research Center, Badajoz, Spain.
- Fickers P, Benetti P-H, Waché Y, Marty A, Mauersberger S, Smit M, Nicaud J-M. 2005. Hydrophobic substrate utilisation by the yeast *Yarrowia lipolytica*, and its potential applications. FEMS Yeast Res 5:527–543. https://doi.org/10.1016/j.femsyr.2004.09.004.
- Ryu S, Hipp J, Trinh CT. 2016. Activating and elucidating metabolism of complex sugars in *Yarrowia lipolytica*. Appl Environ Microbiol 82: 1334–1345. https://doi.org/10.1128/AEM.03582-15.
- Thevenieau F, Beopoulos A, Desfougeres T, Sabirova J, Albertin K, Zinjarde S, Nicaud J-M. 2010. Uptake and assimilation of hydrophobic substrates by the oleaginous yeast *Yarrowia lipolytica*, p 1513–1527. *In* Timmis KN (ed), Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, Germany.
- Ryu S, Trinh CT. 2018. Understanding functional roles of native pentosespecific transporters for activating dormant pentose metabolism in Yar-

- rowia lipolytica. Appl Environ Microbiol 84:e02146-17. https://doi.org/10.1128/AEM.02146-17.
- Gao C, Yang X, Wang H, Rivero CP, Li C, Cui Z, Qi Q, Lin CSK. 2016. Robust succinic acid production from crude glycerol using engineered *Yarrowia lipolytica*. Biotechnol Biofuels 9:179. https://doi.org/10.1186/s13068-016 -0597-8
- Tan M-J, Chen X, Wang Y-K, Liu G-L, Chi Z-M. 2016. Enhanced citric acid production by a yeast *Yarrowia lipolytica* over-expressing a pyruvate carboxylase gene. Bioprocess Biosyst Eng 39:1289–1296. https://doi.org/ 10.1007/s00449-016-1607-8
- 8. Yovkova V, Otto C, Aurich A, Mauersberger S, Barth G. 2014. Engineering the  $\alpha$ -ketoglutarate overproduction from raw glycerol by overexpression of the genes encoding NADP+-dependent isocitrate dehydrogenase and pyruvate carboxylase in *Yarrowia lipolytica*. Appl Microbiol Biotechnol 98:2003–2013. https://doi.org/10.1007/s00253-013-5369-9.
- Beopoulos A, Cescut J, Haddouche R, Uribelarrea J-L, Molina-Jouve C, Nicaud J-M. 2009. Yarrowia lipolytica as a model for bio-oil production. Prog Lipid Res 48:375–387. https://doi.org/10.1016/j.plipres.2009.08.005.

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- Blazeck J, Hill A, Liu L, Knight R, Miller J, Pan A, Otoupal P, Alper HS. 2014. Harnessing *Yarrowia lipolytica* lipogenesis to create a platform for lipid and biofuel production. Nat Commun 5:3131. https://doi.org/10.1038/ncomms4131.
- Qiao K, Wasylenko TM, Zhou K, Xu P, Stephanopoulos G. 2017. Lipid production in *Yarrowia lipolytica* is maximized by engineering cytosolic redox metabolism. Nat Biotechnol 35:173–177. https://doi.org/10.1038/ nbt.3763.
- Andreishcheva E, Isakova E, Sidorov N, Abramova N, Ushakova N, Shaposhnikov G, Soares M, Zvyagilskaya R. 1999. Adaptation to salt stress in a salt-tolerant strain of the yeast *Yarrowia lipolytica*. Biochemistry 64:1061–1067.
- Epova E, Guseva M, Kovalyov L, Isakova E, Deryabina Y, Belyakova A, Zylkova M, Shevelev A. 2012. Identification of proteins involved in pH adaptation in extremophile yeast *Yarrowia lipolytica*, p 209–224. *In* Heazlewood JL, Petzold CJ (ed), Proteomic applications in biology. In-Tech, Rijeka, Licko-Senjska, Croatia.
- 14. Ryu S, Labbé N, Trinh CT. 2015. Simultaneous saccharification and fermentation of cellulose in ionic liquid for efficient production of

- $\alpha$ -ketoglutaric acid by *Yarrowia lipolytica*. Appl Microbiol Biotechnol 99:4237–4244. https://doi.org/10.1007/s00253-015-6521-5.
- Quarterman J, Slininger PJ, Kurtzman CP, Thompson SR, Dien BS. 2017.
   A survey of yeast from the *Yarrowia* clade for lipid production in dilute acid pretreated lignocellulosic biomass hydrolysate. Appl Microbiol Biotechnol 101:3319–3334. https://doi.org/10.1007/s00253-016-8062-y.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
- Grigoriev IV, Nikitin R, Haridas S, Kuo A, Ohm R, Otillar R, Riley R, Salamov A, Zhao X, Korzeniewski F, Smirnova T, Nordberg H, Dubchak I, Shabalov I. 2014. MycoCosm portal: gearing up for 1000 fungal genomes. Nucleic Acids Res 42:D699–D704. https://doi.org/10.1093/nar/gkt1183.
- Grigoriev IV, Martinez DA, Salamov AA. 2006. Chapter 5. Fungal genomic annotation, p 123–142. *In Arora DK*, Berka RM, Singh GB (ed), Applied mycology and biotechnology, vol 6. Elsevier, Amsterdam, the Netherlands.

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