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Draft Genome Sequence of Novel *Metschnikowia* sp. Strain JCM 33374, a Nectar Yeast Isolated from a Bumblebee

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ABSTRACT Here, we report the draft genome sequence of *Metschnikowia* sp. strain JCM 33374, a nectar yeast isolated from a bumblebee (*Bombus diversus*). The genome of 20.1 Mb is a naturally heterozygous diploid. Phylogenetic analysis with related taxa demonstrated that the strain is very likely a novel species.

The genus *Metschnikowia* (Saccharomycetaceae) consists of more than 80 validly described species of ascomycetous yeasts (1), characterized by the formation of needle-shaped ascospores as their sexual forms (2). *Metschnikowia* species show a high degree of ecological specialization (3), some of which are found at the plant-insect interface (4–7). We isolated *Metschnikowia* sp. strain JCM 33374 from nectar carried by a worker bumblebee (*Bombus diversus*) (Hymenoptera: Apidae) foraging flowers of red clover *Trifolium pratense* on a grassland in Sugadaira, Nagano Prefecture, Japan (36.523857°N, 138.348215°E). To retrieve nectar from a crop of the bumblebee, we gently squeezed her abdomen to regurgitate her crop content, which was then collected and stored using a sterile 10- μ l microcapillary tube (Drummond Scientific, USA). We cultivated the strain in yeast-malt (YM) liquid medium (Difco, USA) at 20°C for 3 days, and the genomic DNA was extracted using the Genomic-tip 100/G extraction kit (Qiagen, USA). Genome sequencing was performed on the MinION system (Oxford Nanopore Technologies, UK) using an R9.4 flow cell with the rapid sequencing kit (SQK-RAD002) protocol, or on the MiSeq platform (Illumina, USA) using approximately 5% of a flow cell with the 2 \times 300-bp paired-end protocol. The MinION system generated 105,151 reads (mean length, 5,514 bp), while the MiSeq platform generated 1,067,271 pairs of reads (mean length, 284 bp) after filtering by fastp v0.19.6 (8), both of which were processed for the following analyses. The combined assembly of the MinION and MiSeq reads was performed following the assembly-polish pipeline (<https://github.com/nanoporetech/ont-assembly-polish>), which integrated the assembler Canu v1.7 (9) for the long MinION reads with the consensus module Racon v1.3.1 (10) and the assembly improvement tool Pilon v1.22 (11) tailored for polishing based on the short Illumina reads.

The assembled draft genome consisted of 20,114,833 bp from 165 contigs, with a G+C content of 43.5%. The longest contig was 1,028,286 bp long, and the N_{50} value was 208,628 bp. Average coverages were 16.4 \times and 32.3 \times for the MinION and the MiSeq reads, respectively. This draft genome sequence contains 90.3% (1,473 complete and 72 fragmented) of the 1,711 benchmarking universal single-copy orthologs (BUSCOs) (BUSCO v3.0.2 [12]) using the OrthoDB v9 data set for Saccharomycetales. Protein-coding genes were annotated using AUGUSTUS v3.3.1 (13) with a *Saccharomyces cerevisiae* training set, which predicted 6,685 genes. tRNAscan-SE v2.0 (14) identified 240 tRNA genes. Genome Analysis Tool Kit (GATK v4.1.2.0) (15) best practices were

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performed to filter and accept a total of 148,417 single nucleotide polymorphisms (SNPs) and 30,795 insertion and deletions (indels). The observed distributions of allele depth ratios for all SNPs and indels exhibited a single peak at around 0.5, representing that the naturally heterozygous genome is diploid.

Phylogenetic analysis was performed to determine if the strain could be part of a novel species. A maximum likelihood tree was constructed using RAxML v8 (16) on the sequence of the D1/D2 domain of the large-subunit (LSU) rRNA gene from the draft genome with those from the *Metschnikowia* clade available in the DDBJ/ENA/GenBank database. The phylogenetic tree revealed that the strain located in the *Metschnikowia* clade but was different from any described *Metschnikowia* species. The sequence of the LSU D1/D2 from the strain was validated by Sanger sequencing and showed 6.3% divergence from that of the closely related species *Metschnikowia lachancei* (17). For ascomycetous yeasts, more than 1% divergence in LSU D1/D2 represents the threshold used to discriminate a species (18). Therefore, the strain is very likely a novel species.

Data availability. The draft genome sequence and annotation data of *Metschnikowia* sp. strain JCM 33374 have been deposited at DDBJ/ENA/GenBank under the accession number [BIMT00000000](https://doi.org/10.1093/bioinformatics/btv351). The version described in this paper is the first version, BIMT01000000. The raw reads were deposited in the SRA/DRA/ERA under the accession numbers [DRA008301](https://doi.org/10.1093/bioinformatics/btv351) and [DRA008302](https://doi.org/10.1093/bioinformatics/btv351). The strain is available from the Japan Collection of Microorganisms, RIKEN BioResource Research Center (Tsukuba, Ibaraki, Japan), under strain number JCM 33374.

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REFERENCES

- Lachance MA. 2016. *Metschnikowia*: half tetrads, a regicide and the fountain of youth. *Yeast* 33:563–574. <https://doi.org/10.1002/yea.3208>.
- Lachance MA. 2011. *Metschnikowia* Kamienski (1899), p 575–620. In Kurtzman CP, Fell J, Boekhout T (ed), *The yeasts, a taxonomic study*, 5th ed, vol 2. Elsevier Science, London, United Kingdom.
- Guzmán B, Lachance MA, Herrera CM. 2013. Phylogenetic analysis of the angiosperm-floricolous insect-yeast association: have yeast and angiosperm lineages co-diversified? *Mol Phylogenet Evol* 68:161–175. <https://doi.org/10.1016/j.ympev.2013.04.003>.
- Giménez-Jurado G. 1992. *Metschnikowia gruensis* sp. nov., the teleomorph of *Nectaromyces reukaufii* but not of *Candida reukaufii*. *Syst Appl Microbiol* 15:432–438. [https://doi.org/10.1016/S0723-2020\(11\)80218-3](https://doi.org/10.1016/S0723-2020(11)80218-3).
- Brysch-Herzberg M. 2004. Ecology of yeasts in plant-bumblebee mutualism in central Europe. *FEMS Microbiol Ecol* 50:87–100. <https://doi.org/10.1016/j.femsec.2004.06.003>.
- de Vega C, Guzman B, Lachance MA, Steenhuisen SL, Johnson SD, Herrera CM. 2012. *Metschnikowia proteae* sp. nov., a nectarivorous insect-associated yeast species from Africa. *Int J Syst Evol Microbiol* 62: 2538–2545. <https://doi.org/10.1099/ijs.0.040790-0>.
- Herrera CM, Pozo MI, Medrano M. 2013. Yeasts in nectar of an early-blooming herb: sought by bumble bees, detrimental to plant fecundity. *Ecology* 94:273–279. <https://doi.org/10.1890/12-0595.1>.
- Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34:i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>.
- Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res* 27:722–736. <https://doi.org/10.1101/gr.215087.116>.
- Vaser R, Sovic I, Nagarajan N, Sikic M. 2017. Fast and accurate de novo genome assembly from long uncorrected reads. *Genome Res* 27: 737–746. <https://doi.org/10.1101/gr.214270.116>.
- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>.
- Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210–3212. <https://doi.org/10.1093/bioinformatics/btv351>.
- Stanke M, Steinkamp R, Waack S, Morgenstern B. 2004. AUGUSTUS: a Web server for gene finding in eukaryotes. *Nucleic Acids Res* 32: W309–W312. <https://doi.org/10.1093/nar/gkh379>.
- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 25:955. <https://doi.org/10.1093/nar/25.5.955>.
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 20:1297–1303. <https://doi.org/10.1101/gr.107524.110>.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>.
- Giménez-Jurado G, Kurtzman CP, Starmer WT, Spencer-Martins I. 2003. *Metschnikowia vanudenii* sp. nov. and *Metschnikowia lachancei* sp. nov., from flowers and associated insects in North America. *Int J Syst Evol Microbiol* 53:1665–1670. <https://doi.org/10.1099/ijs.0.02470-0>.
- Kurtzman CP, Robnett CJ. 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie Van Leeuwenhoek* 73:331–371. <https://doi.org/10.1023/A:1001761008817>.