





Draft Genome Sequence of the Yeast *Pichia manshurica* YM63, a Participant in Secondary Fermentation of Ishizuchi-Kurocha, a Japanese Fermented Tea

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ABSTRACT *Pichia manshurica* is common in fermentation; however, genome analysis has never been reported for the species. This yeast plays a role in the secondary fermentation of Ishizuchi-kurocha, a traditional Japanese fermented tea. This paper presents the draft genome sequence of *P. manshurica* YM63, isolated from the leaves of fermented tea.

Pichia manshurica (phylum Ascomycota, subphylum Saccharomycotina) is a common yeast found in fermented animal feed and foods such as fermented maguey juice (1), a traditional beverage of Burkina Faso (2), wines (3), and silages (4). Ishizuchi-kurocha is a traditional fermented tea in Shikoku Island, Japan (5). The yeast species *Pichia manshurica* naturally occurs during the production process of Ishizuchi-kurocha. The yeast species has important roles in fermentation and rotting plant materials. The genome sequences of *Pichia kudriavzevii* (6, 7) and *Pichia membranifaciens* (8), which are closely related to *P. manshurica*, and their comparative analysis (9) have been presented. Genome analysis of *P. manshurica*, however, has not been reported.

We used strain YM63, isolated from leaves in the secondary fermentation step of Ishizuchi-kurocha production. The strain was confirmed to be the correct species by 28S rRNA sequencing. YM63 was cultured in yeast extract-peptone-dextrose broth at 30°C for 14 h with shaking at 130 rpm. We prepared genomic DNA from the YM63 strain using a blood and cell culture DNA minikit (Qiagen, Inc.). Genome sequencing was performed using a MiSeq platform (Illumina, Inc.) and GridION with flow cell type R9.4.1 (Oxford Nanopore Technologies). For MiSeq sequencing, genomic DNA was sheared with a Covaris S2 sonicator (Covaris, Inc.) to obtain ~500-bp DNA fragments. A library was prepared from 200 ng of fragmented DNA using a preparation kit (Kapa HyperPrep kit; Kapa Biosystems) and an adapter kit (FastGene adapter kit; Nippon Genetics Co. Ltd.). After quantification and qualification, the prepared library was sequenced with MiSeq technology to produce 2 × 151-bp paired-end reads. A total of 2,327,748 reads with a Q30 of 83.4% were obtained by MiSeq sequencing. For GridION analysis, a library was prepared using a ligation sequence kit (Oxford Nanopore Technologies). By GridION analysis, 749,822 reads (average length, 16,203 bp) were obtained. MiSeq reads were trimmed using the parameters -q 20 -l 127 with Sickle v1.33 (10). A total of 2,170,013 filtered reads were used for a subsequent assembly. Adaptor sequences in the reads from GridION were trimmed with Porechop v0.2.3, and the trimmed reads were quality filtered using the parameters -min_mean_q 80.05 -min_length 1000 with Filtlong v0.2.0. Error-prone read data from GridION were processed using Canu v1.8 (11). A total of 668,602 reads was used for the subsequent assembly. Finally, assembly

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was done using MaSuRCA v3.2.8 (12), and polishing was done using Bowtie 2 v2.3.4.1 (13) and Pilon v1.22 (14).

The draft genome includes eight contigs with a total size of 12,405,322 bp. The GC content is 42.3%. The N_{50} contig and the longest contig sizes are 2,862,201 bp and 3,465,259 bp, respectively. The total contig size of *P. membranifaciens* KS47-1 is 11.4 Mb (8). The genome assembly size of *P. membranifaciens* NRRL Y-2026 is 11.5 Mb (9), which is slightly smaller than that of *P. manshurica* YM63. According to BLAST homology analysis, the scf718000000032 contig (GenBank accession no. [BJFO01000006](https://doi.org/10.1093/nar/nkz006)) is estimated to be a mitochondrial genome. Benchmarking Universal Single-Copy Orthologs (BUSCO) v3 (15, 16) analyses using Ascomycota and Saccharomycetales data sets showed 83% and 80.5% complete BUSCO, respectively.

Data availability. The draft genome sequence of *Pichia manshurica* YM63 was deposited in DDBJ/ENA/GenBank under accession no. [BJFO01000001](https://doi.org/10.1093/nar/nkz006) to [BJFO01000008](https://doi.org/10.1093/nar/nkz006). The raw sequencing reads were submitted to the DRA under accession no. [DRA008229](https://doi.org/10.1093/nar/nkz006).

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REFERENCES

- Nolasco-Cancino H, Santiago-Urbina JA, Wachter C, Ruíz-Terán F. 2018. Predominant yeasts during artisanal mezcals fermentation and their capacity to ferment maguey juice. *Front Microbiol* 9:2900. <https://doi.org/10.3389/fmicb.2018.02900>.
- Sanata B, Adama Z, Ibrahim S, Apoline S, Mamoudou C, Constant S, Robert GT, Christophe H. 2017. Characterization of the fungal flora of dolo, a traditional fermented beverage of Burkina Faso, using MALDI-TOF mass spectrometry. *World J Microbiol Biotechnol* 33:172. <https://doi.org/10.1007/s11274-017-2335-1>.
- Ramírez-Castrillón M, Mendes SDC, Valente P. 2017. South Brazilian wines: culturable yeasts associated to bottled wines produced in Rio Grande do Sul and Santa Catarina. *World J Microbiol Biotechnol* 33:77. <https://doi.org/10.1007/s11274-017-2244-3>.
- Wang H, Hao W, Ning T, Zheng M, Xu C. 2018. Characterization of culturable yeast species associating with whole crop corn and total mixed ration silage. *Asian-Australas J Anim Sci* 31:198–207. <https://doi.org/10.5713/ajas.17.0183>.
- Horie M, Nara K, Sugino S, Umeno A, Yoshida Y. 2017. Comparison of antioxidant activities among four kinds of Japanese traditional fermented tea. *Food Sci Nutr* 5:639–645. <https://doi.org/10.1002/fsn3.442>.
- Chan GF, Gan HM, Ling HL, Rashid N. 2012. Genome sequence of *Pichia kudriavzevii* M12, a potential producer of bioethanol and phytase. *Eukaryot Cell* 11:1300–1301. <https://doi.org/10.1128/EC.00229-12>.
- Douglass AP, Offei B, Braun-Galleani S, Coughlan AY, Martos AAR, Ortiz-Merino RA, Byrne KP, Wolfe KH. 2018. Population genomics shows no distinction between pathogenic *Candida krusei* and environmental *Pichia kudriavzevii*: one species, four names. *PLoS Pathog* 14:e1007138. <https://doi.org/10.1371/journal.ppat.1007138>.
- Konishi M, Arakawa T, Kato Y, Ishida M, Horiuchi J. 2017. Draft genome sequencing of ascomycetes yeast *Pichia membranifaciens* KS47-1, which shows high acetate resistance in lignocellulosic feedstock hydrolysate. *Genome Announc* 5:e01672-16. <https://doi.org/10.1128/genomeA.01672-16>.
- Riley R, Haridas S, Wolfe KH, Lopes MR, Hittinger CT, Göker M, Salamov AA, Wisecaver JH, Long TM, Calvey CH, Aerts AL, Barry KW, Choi C, Clum A, Coughlan AY, Deshpande S, Douglass AP, Hanson SJ, Klenk H-P, LaButti KM, Lapidus A, Lindquist EA, Lipzen AM, Meier-Kolthoff JP, Ohm RA, Otiillar RP, Pangilinan JL, Peng Y, Rokas A, Rosa CA, Scheuner C, Sibirny AA, Slot JC, Stielow JB, Sun H, Kurtzman CP, Blackwell M, Grigoriev IV, Jeffries TW. 2016. Comparative genomics of biotechnologically important yeasts. *Proc Natl Acad Sci U S A* 113:9882–9887. <https://doi.org/10.1073/pnas.1603941113>.
- Joshi N, Fass J. 2011. Sickle: a sliding-window, adaptive, quality-based trimming tool for FastQ files (version 1.33).
- 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>.
- Zimin AV, Marçais G, Puiu D, Roberts M, Salzberg SL, Yorke JA. 2013. The MaSuRCA genome assembler. *Bioinformatics* 29:2669–2677. <https://doi.org/10.1093/bioinformatics/btt476>.
- Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9:357–359. <https://doi.org/10.1038/nmeth.1923>.
- 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>.
- Waterhouse RM, Seppey M, Simão FA, Manni M, Ioannidis P, Kliuchnikov G, Kriventseva EV, Zdobnov EM. 2018. BUSCO applications from quality assessments to gene prediction and phylogenomics. *Mol Biol Evol* 35:543–548. <https://doi.org/10.1093/molbev/msx319>.
- 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/btv319>.