




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/46/11/1982)) 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/46/11/1982) to [BJFO01000008](https://doi.org/10.1093/nar/46/11/1982). The raw sequencing reads were submitted to the DRA under accession no. [DRA008229](https://doi.org/10.1093/nar/46/11/1982).

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