



# Draft Genome Sequencing of the Highly Halotolerant and Allopolyploid Yeast *Zygosaccharomyces rouxii* NBRC 1876

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**ABSTRACT** The highly halotolerant and allopolyploid yeast *Zygosaccharomyces rouxii* is industrially used for the food production in high concentrations of salt, such as brewing soy sauce and miso paste. Here, we report the draft genome sequence of *Z. rouxii* NBRC 1876 isolated from miso paste.

*Zygosaccharomyces rouxii* is an osmotolerant and halotolerant yeast that grows in high concentrations of salt and/or sugar (1). This yeast is industrially used for brewing soy sauce and miso paste in high concentrations of salt (2).

It is supposed that there are at least two different genomic types in *Z. rouxii*. One is haploid type with one copy of each gene, which includes CBS 732<sup>T</sup> isolated from concentrated black grape must (3). Another is allopolyploid hybrids, which includes ATCC 42981 isolated from miso paste, with two copies of functional genes involved in the production of glycerol as a compatible solute to protect the cell against lysis and efflux of Na<sup>+</sup> from cells in high concentrations of salt (4–7). These redundant genes in an allopolyploid strain can contribute to survival under high-osmotic conditions, such as the process of brewing soy sauce. In soy sauce brewing, *Z. rouxii* contributes to the production of its distinctive aroma (8).

The genomic DNA of the allopolyploid type has been partially sequenced (7) but not enough to analyze the genome in detail, while that of the haploid type strain was completely sequenced (9). Therefore, we conducted genome sequencing of *Z. rouxii* NBRC 1876, isolated from miso paste, as a model strain of osmotolerant/halotolerant yeast with an allopolyploid genome.

Cultivation of NBRC 1876 and genomic DNA extraction that followed were performed according to the methods described in our previous report (10). The genomic DNA was sequenced using Roche 454 GS FLX+ single-end and titanium paired-end sequencing. To remove artificial replicates from emulsion PCR, all single-end reads were identified using CD-HIT-454 (11). All paired-end reads were confirmed longer than 45-mer sequences. After removing all artifacts, the obtained reads were assembled using the GS *de novo* assembler (12). The assembly generated 62 nonredundant scaffolds composed of 482 contigs, and the total genome size was estimated to be 19.4 Mb, which was twice that of CBS 732 (9.8 Mb) (9). This result suggests that NBRC 1876 is allopolyploid. This genome information contributes to further studies on food science and of its physiology and taxonomy.

**Accession number(s).** This whole genome shotgun project has been deposited in DDBJ/ENA/GenBank. Accession numbers for the 62 scaffold sequences are [DF983528](#) to [DF983589](#). Accession numbers for the 482 contig sequences are [BBQV01000001](#) to [BBQV01000482](#). The version described in this paper is the first version, BBQV01000000.

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## REFERENCES

1. Solieri L, Giudici P. 2008. Yeasts associated to traditional balsamic vinegar: ecological and technological features. *Int J Food Microbiol* 125:36–45. <https://doi.org/10.1016/j.ijfoodmicro.2007.06.022>.
2. Onishi H. 1963. Osmophilic yeasts, p 53–94. *In* Chichester CO, Mraz EM, Stewart GF (ed), *Advances in food research*. Academic Press, New York, NY.
3. Kinclová O, Potier S, Sychrová H. 2001. The *Zygosaccharomyces rouxii* strain CBS 732 contains only one copy of the HOG1 and the SOD2 genes. *J Biotechnol* 88:151–158. [https://doi.org/10.1016/S0168-1656\(01\)00274-7](https://doi.org/10.1016/S0168-1656(01)00274-7).
4. James SA, Bond CJ, Stratford M, Roberts IN. 2005. Molecular evidence for the existence of natural hybrids in the genus *Zygosaccharomyces*. *FEMS Yeast Res* 5:747–755. <https://doi.org/10.1016/j.femsyr.2005.02.004>.
5. Solieri L, Cassanelli S, Giudici P. 2007. A new putative *Zygosaccharomyces* yeast species isolated from traditional balsamic vinegar. *Yeast* 24: 403–417. <https://doi.org/10.1002/yea.1471>.
6. Solieri L, Landi S, De Vero L, Giudici P. 2006. Molecular assessment of indigenous yeast population from traditional balsamic vinegar. *J Appl Microbiol* 101:63–71. <https://doi.org/10.1111/j.1365-2672.2006.02906.x>.
7. Gordon JL, Wolfe KH. 2008. Recent allopolyploid origin of *Zygosaccharomyces rouxii* strain ATCC 42981. *Yeast* 25:449–456. <https://doi.org/10.1002/yea.1598>.
8. Van Der Sluis C, Tramper J, Wijffels RH. 2001. Enhancing and accelerating flavour formation by salt-tolerant yeasts in Japanese soy-sauce processes. *Trends Food Sci Technol* 12:322–327. [https://doi.org/10.1016/S0924-2244\(01\)00094-2](https://doi.org/10.1016/S0924-2244(01)00094-2).
9. Génolevures Consortium, Souciet JL, Dujon B, Gaillardin C, Johnston M, Baret PV, Cliften P, Sherman DJ, Weissenbach J, Westhof E, Wincker P, Jubin C, Poulain J, Barbe V, Ségurens B, Artiguenave F, Anthouard V, Vacherie B, Val ME, Fulton RS, Minx P, Wilson R, Durrrens P, Jean G, Marck C, Martin T, Nikolski M, Rolland T, Seret ML, Casarégola S, Despons L, Fairhead C, Fischer G, Lafontaine I, Leh V, Lemaire M, de Montigny J, Neuvéglise C, Thierry A, Blanc-Lenfle I. 2009. Comparative genomics of protoploid *Saccharomycetaceae*. *Genome Res* 19:1696–1709. <https://doi.org/10.1101/gr.091546.109>.
10. Sato A, Oshima K, Noguchi H, Ogawa M, Takahashi T, Oguma T, Koyama Y, Itoh T, Hattori M, Hanya Y. 2011. Draft genome sequencing and comparative analysis of *Aspergillus sojae* NBRC 4239. *DNA Res* 18: 165–176. <https://doi.org/10.1093/dnares/dsr009>.
11. Niu B, Fu L, Sun S, Li W. 2010. Artificial and natural duplicates in pyrosequencing reads of metagenomic data. *BMC Bioinformatics* 11:187. <https://doi.org/10.1186/1471-2105-11-187>.
12. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Ho CH, Irzyk GP, Jando SC, Alenquer ML, Jarvie TP, Jirage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437:376–380. <https://doi.org/10.1038/nature03959>.