

Draft Genome Sequence of the Beer Spoilage Bacterium *Megasphaera cerevisiae* Strain PAT 1^T

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The genus *Megasphaera* harbors important spoilage organisms that cause beer spoilage by producing off flavors, undesirable aroma, and turbidity. *Megasphaera cerevisiae* is mainly found in nonpasteurized low-alcohol beer. In this study, we report the draft genome of the type strain of the genus, *M. cerevisiae* strain PAT 1^T.

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Improvements in modern brewing technology have resulted in beer with significantly reduced oxygen content, allowing growth of strictly anaerobic microorganisms such as *Megasphaera* sp. and *Pectinatus* sp. (1, 2). *M. cerevisiae* alone was found responsible for 3 to 7% of beer spoilage cases in Europe between 1980 and 2002 (3, 4). Contamination of unpasteurized beer by *M. cerevisiae* and *Pectinatus* sp. causes high turbidity and off taste due to a considerable accumulation of butyric acid along with smaller amounts of acetic, isovaleric, valeric, and caproic acid, as well as acetoin (5, 6). The type strain of the genus, *M. cerevisiae* strain PAT 1^T, was isolated from spoiled bottled beer in 1985 (7).

M. cerevisiae strain PAT 1^T (DSM 20462 = ATCC-43254) was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). The strain was grown in modified PYG medium (DSMZ) anaerobically at 30°C until early stationary phase. Genomic DNA was isolated for whole-genome sequencing using the bacterial DNA purification kit (Pi Biologique, Seattle, WA, USA). The genome library was then prepared using the Nextera XT DNA sample prep kit (Illumina, San Diego, CA, USA). Genome sequencing was performed using an Illumina MiSeq desktop sequencer (Illumina) with a paired-end 2 × 250 cycle MiSeq reagent kit. The 1,548,102 generated shotgun reads were assembled using the A5-miseq assembly software (8), resulting in 203 contigs with 90-fold coverage. The draft genome was annotated using Rapid Annotations using Subsystems Technology version 2.0 (RAST) (9, 10) and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (11). The draft sequence has a total length of 3,238,021 bp, an average GC content of 44.8%, an *N*₅₀ length of 49,680 bp, and a maximum contig size of 154,499 bp. Based on PGAP, the genome contains 17 rRNAs, 54 tRNAs, and 3,017 protein-coding genes. Using the PHAge search tool (PHAST) (12), we found seven prophage regions, of which two were intact (23.2 kb and 55 kb) and five were incomplete (28.1 kb, 11.9 kb, 38.7 kb, 20.2 kb, and 9.8 kb). RAST annotation identified CRISPRs and its associated elements on multiple scaffolds.

In well-studied beer spoilage bacteria, such as *Lactobacillus bre-*

vis, catabolism of arginine and resistance to antimicrobial compounds from hops have been identified as key factors contributing to growth in beer and wine (13, 14). Arginine is catabolized using the arginine deiminase pathway through a series of metabolic reactions performed by arginine deiminase (*arcA*), ornithine transcarbamoylase (*arcB*), and carbamate kinase (*arcC*), leading to putrescine accumulation. The *M. cerevisiae* draft genome encodes an intact *arcABC* operon, suggesting that it can catabolize arginine. Hop compounds found in beer exhibit antimicrobial effects because of their ability to lower the pH and sequester divalent metal cations such as iron and manganese (15). The *M. cerevisiae* draft genome encodes several operons implicated in iron homeostasis, such as the *fur* and *suf* operons, as well as an ABC-type transporter (*mntH*) that is critical for manganese uptake. Taken together, these observations support that *M. cerevisiae* is well equipped to replicate and grow in beer and cause its spoilage.

Nucleotide sequence accession number. The *M. cerevisiae* strain PAT 1^T genome sequence was deposited in GenBank under the accession number [LEKT000000000](https://www.ncbi.nlm.nih.gov/nuccore/LEKT000000000).

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REFERENCES

- Jespersen L, Jakobsen M. 1996. Specific spoilage organisms in breweries and laboratory media for their detection. *Int J Food Microbiol* 33: 139–155. [http://dx.doi.org/10.1016/0168-1605\(96\)01154-3](http://dx.doi.org/10.1016/0168-1605(96)01154-3).
- Lee SY. 1994. SMMP-A medium for selective isolation of *Megasphaera* and *Pectinatus* from the brewery. *J Am Soc Brew Chem* 52:115–119.
- Back W. 1994. Secondary contamination in the filling area. *Brauwelt International* 4:326–328.
- Back W. 2003. Biofilme in der brauerei und getränkeindustrie—15 jahre praxiserfahrung. *Brauwelt Online* 24/25:1–5.
- Haikara A. 1985. Detection of anaerobic, gram-negative bacteria in beer. *Monatsschr Brauwissenschaft* 38:239–243.
- Seidel H, Back W, Weiss N. 1979. Isolierung und systematische zuordnung bierschädlicher Gramnegativer bakterien III: welche gefahr stellen die in den beiden vorausgegangenen mitteilungen vorgestellten Gramnegativen. Kokken und Stäbchen für Das Bier dar *Brauwiss* 32:262–270.

7. Engelmann U, Weiss N. 1985. *Megasphaera cerevisiae* sp. nov.: a new Gram-negative obligately anaerobic coccus isolated from spoiled beer. *Syst Appl Microbiol* 6:287–290. [http://dx.doi.org/10.1016/S0723-2020\(85\)80033-3](http://dx.doi.org/10.1016/S0723-2020(85)80033-3).
8. Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. *Bioinformatics* 31:587–589. <http://dx.doi.org/10.1093/bioinformatics/btu661>.
9. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.
10. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res* 42:D206–D214. <http://dx.doi.org/10.1093/nar/gkt1226>.
11. Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of Standard operating procedures (SOPs) for (meta)genomic annotation. *Omics* 12:137–141. <http://dx.doi.org/10.1089/omi.2008.0017>.
12. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. *Nucleic Acids Res* 39:W347–W352. <http://dx.doi.org/10.1093/nar/gkr485>.
13. Sakamoto K, Konings WN. 2003. Beer spoilage bacteria and hop resistance. *Int J Food Microbiol* 89:105–124. [http://dx.doi.org/10.1016/S0168-1605\(03\)00153-3](http://dx.doi.org/10.1016/S0168-1605(03)00153-3).
14. Araque I, Bordons A, Reguant C. 2013. Effect of ethanol and low pH on citrulline and ornithine excretion and arc gene expression by strains of *Lactobacillus brevis* and *Pediococcus pentosaceus*. *Food Microbiol* 33:107–113. <http://dx.doi.org/10.1016/j.fm.2012.09.005>.
15. Schurr BC, Hahne H, Kuster B, Behr J, Vogel RF. 2015. Molecular mechanisms behind the antimicrobial activity of hop iso-alpha-acids in *Lactobacillus brevis*. *Food Microbiol* 46:553–563. <http://dx.doi.org/10.1016/j.fm.2014.09.017>.