



Complete Genome Sequence of *Malassezia restricta* CBS 7877, an Opportunist Pathogen Involved in Dandruff and Seborrheic Dermatitis

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ABSTRACT *Malassezia restricta*, one of the predominant basidiomycetous yeasts present on human skin, is involved in scalp disorders. Here, we report the complete genome sequence of the lipophilic *Malassezia restricta* CBS 7877 strain, which will facilitate the study of the mechanisms underlying its commensal and pathogenic roles within the skin microbiome.

Malassezia restricta, one of the most abundant *Malassezia* species of the human skin microbiota (1–3), is considered an opportunistic pathogen associated with skin disorders such as seborrheic dermatitis and dandruff (4–6). Due to the absence of fatty acid synthase, most *Malassezia* yeast growth depends on the presence of host lipids and the expression of an extensive number of lipases (7–10). However, other mechanisms are likely involved in its interactions with skin and surrounding bacteria (11, 12). Currently, public databases contain three *M. restricta* genomes identified at either the contig or scaffold level (whole-genome sequences [WGS] of LFCZ01, AAXK01, and LFDA01) and unassembled next-generation sequencing (NGS) reads of the *M. restricta* KCTC 27527 strain (8), which limits comparative genomic and *in vivo* metagenomics studies. We succeeded in completely resequencing and assembling the *M. restricta* genome at the chromosomal level.

Genomic DNA of *M. restricta* CBS 7877, a strain isolated from normal human skin (13), was purchased from the ATCC (reference MYA4611D5). Sequencing was performed at BaseClear on both the HiSeq 2500 (Illumina) and PacBio RS II (Pacific Biosciences) platforms. The Illumina library was obtained following the Nextera protocol (Illumina). The Illumina 125-base paired-end short reads (4,849,647 reads after quality control with FastQC version 0.10.0; 1.222 Mbp in total; coverage, 167×) were trimmed and *de novo* assembled using CLC Genomics Workbench version 7.5.1 (CLC bio, Denmark). The optimal *k*-mer size was automatically determined using KmerGenie version 1.6213 with default parameters (14). For PacBio, the library was prepared using the standard procedure for the PacBio RS II instrument. PacBio reads were processed and filtered using the SMRT Analysis software suite version 2.3.0, leading to 858,347 continuous long reads (1.721 Mbp in total; coverage, 235×). Illumina contigs were then aligned with the PacBio CLR reads using BLASR (15). From the alignment, the orientation, order, and distances between contigs were estimated via SSPACE-LongRead Scaffold version 1.0 (16) and gaps filled with GapFiller version 1.10 (17). The final assembly consisted of a mitochondrial plasmid (33.6 kbp, 22 tRNA genes, 31.5% GC content) and 9 scaffolds. The longest scaffold was 1,419,096 bp. The genome size was 7.26 Mbp, with a G+C content of 56.8%. Structural annotation was performed using Augustus version 3.3 (18) with the training model organism *M. restricta* strain KCTC

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27527 (GenBank accession numbers [CP030251](#) to [CP030259](#)). Functional annotation was completed using a combination of BLAST-p and Blast2GO (19, 20) against Pfam (version 31) and the UniProt/Swiss-Prot (release 2017-04) databases, as included in the Prokka annotation framework version 1.12. A total of 4,096 protein-coding genes cover 86.8% of the genome.

The mitochondrial sequence is identical to the previously released *M. restricta* CBS 7877 mitochondrion sequence (GenBank accession number [KY911093](#)), with the exception of one repeated region. A previous karyotype study found nine chromosomes for the *M. restricta* CBS 7877 strain (21). Here, the nine scaffolds exhibit short tandem repeats (TKA GTG, >60 bp) considered to be telomeres, as previously reported (9). Six scaffolds show telomere repeats at both ends, suggesting that these are complete chromosomes, and these hexamer-repeated motifs were not found elsewhere in the assembly. Six and three lipase coding sequences (CDS) have been identified, harboring the Pfam PF01764 and PF03583 signatures, respectively. Among the cell wall biosynthesis-related proteins analyzed, 20 chitin-chitosan-processing genes were identified by BLAST using a *Malassezia globosa* protein data set (19). These activities are of main importance since the *M. restricta* cell wall contains a very high percentage (20%) of chitosan (22), and chitosan is reported to be required for fungal virulence and persistence in mammals (23). Further investigation on specific factors and functions (such as proteases and glycosyl hydrolases [9, 12]) will be essential to a better understanding of the physiopathology of *M. restricta*.

Data availability. The complete genome sequence of CBS 7877 is available in GenBank under the accession numbers [CP033148](#) to [CP033157](#). The version described in this paper is the first version. Raw data have been deposited in the SRA under the accession numbers [SRX5004588](#) and [SRX5004589](#) and BioProject number [PRJNA474956](#).

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REFERENCES

- Byrd AL, Belkaid Y, Segre JA. 2018. The human skin microbiome. *Nat Rev Microbiol* 16:143–155. <https://doi.org/10.1038/nrmicro.2017.157>.
- Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, Schoenfeld D, Nomicos E, Park M, Kong HH, Segre JA. 2013. Topographic diversity of fungal and bacterial communities in human skin. *Nature* 498:367–370. <https://doi.org/10.1038/nature12171>.
- Grice EA, Dawson TL. 2017. Host–microbe interactions: *Malassezia* and human skin. *Curr Opin Microbiol* 40:81–87. <https://doi.org/10.1016/j.mib.2017.10.024>.
- Clavaud C, Jourdain R, Bar-Hen A, Tichit M, Bouchier C, Pouradier F, El Rawadi C, Guillot J, Ménard-Szczebara F, Breton L, Latgé JP, Mouyna I. 2013. Dandruff is associated with disequilibrium in the proportion of the major bacterial and fungal populations colonizing the scalp. *PLoS One* 8:e58203. <https://doi.org/10.1371/journal.pone.0058203>.
- Hiruma M, Cho O, Hiruma M, Kurakado S, Sugita T, Ikeda S. 2014. Genotype analyses of human commensal scalp fungi, *Malassezia globosa*, and *Malassezia restricta* on the scalps of patients with dandruff and healthy subjects. *Mycopathologia* 177:263–269. <https://doi.org/10.1007/s11046-014-9748-2>.
- Theelen B, Cafarchia C, Gaitanis G, Bassukas ID, Boekhout T, Dawson TL, Jr. 2018. *Malassezia* ecology, pathophysiology, and treatment. *Med Mycol* 56:10–25. <https://doi.org/10.1093/mmy/myx134>.
- Gioti A, Nystedt B, Li W, Xu J, Andersson A, Averette AF, Münch K, Wang X, Kappauf C, Kingsbury JM, Kraak B, Walker LA, Johansson HJ, Holm T, Lehtiö J, Stajich JE, Mieczkowski P, Kahmann R, Kennell JC, Cardenas ME, Lundeberg J, Saunders CW, Boekhout T, Dawson TL, Munro CA, de Groot PW, Butler G, Heitman J, Scheynius A. 2013. Genomic insights into the atopic eczema-associated skin commensal yeast *Malassezia sympodialis*. *mBio* 4:e00572-12. <https://doi.org/10.1128/mBio.00572-12>.
- Park M, Cho YJ, Lee YW, Jung WH. 2017. Whole genome sequencing analysis of the cutaneous pathogenic yeast *Malassezia restricta* and identification of the major lipase expressed on the scalp of patients with dandruff. *Mycoses* 60:188–197. <https://doi.org/10.1111/myc.12586>.
- Wu G, Zhao H, Li C, Rajapakse MP, Wong WC, Xu J, Saunders CW, Reeder NL, Reilman RA, Scheynius A, Sun S, Billmyre BR, Li W, Averette AF, Mieczkowski P, Heitman J, Theelen B, Schröder MS, De Sessions PF, Butler G, Maurer-Stroh S, Boekhout T, Nagarajan N, Dawson TL. 2015. Genus-wide comparative genomics of *Malassezia* delineates its phylogeny, physiology, and niche adaptation on human skin. *PLoS Genet* 11:e1005614. <https://doi.org/10.1371/journal.pgen.1005614>.
- Xu J, Saunders CW, Hu P, Grant RA, Boekhout T, Kuramae EE, Kronstad JW, Deangelis YM, Reeder NL, Johnstone KR, Leland M, Fieno AM, Begley WM, Sun Y, Lacey MP, Chaudhary T, Keough T, Chu L, Sears R, Yuan B, Dawson TL, Jr. 2007. Dandruff-associated *Malassezia* genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens. *Proc Natl Acad Sci USA* 104:18730–18735. <https://doi.org/10.1073/pnas.0706756104>.
- Gaitanis G, Velegriaki A, Maysor P, Bassukas ID. 2013. Skin diseases associated with *Malassezia* yeasts: facts and controversies. *Clin Dermatol* 31:455–463. <https://doi.org/10.1016/j.clindermatol.2013.01.012>.
- Li H, Goh BN, Teh WK, Jiang Z, Goh JPZ, Goh A, Wu G, Hoon SS, Raida M, Camattari A, Yang L, O'Donoghue AJ, Dawson TL, Jr. 2018. Skin commensal *Malassezia globosa* secreted protease attenuates *Staphylococcus aureus* biofilm formation. *J Invest Dermatol* 138:1137–1145. <https://doi.org/10.1016/j.jid.2017.11.034>.
- Guého E, Midgley G, Guillot J. 1996. The genus *Malassezia* with description of four new species. *Antonie Van Leeuwenhoek* 69:337. <https://doi.org/10.1007/BF00399623>.
- Chikhi R, Medvedev P. 2014. Informed and automated k-mer size selection for genome assembly. *Bioinformatics* 30:31–37. <https://doi.org/10.1093/bioinformatics/btt310>.
- Chaisson MJ, Tesler G. 2012. Mapping single molecule sequencing reads using basic local alignment with successive refinement (BLASR): appli-

- cation and theory. *BMC Bioinformatics* 13:238. <https://doi.org/10.1186/1471-2105-13-238>.
16. Boetzer M, Pirovano W. 2014. SSPACE-LongRead: scaffolding bacterial draft genomes using long read sequence information. *BMC Bioinformatics* 15:211. <https://doi.org/10.1186/1471-2105-15-211>.
 17. Boetzer M, Pirovano W. 2012. Toward almost closed genomes with Gap-Filler. *Genome Biol* 13:R56. <https://doi.org/10.1186/gb-2012-13-6-r56>.
 18. 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>.
 19. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
 20. Conesa A, Götz S. 2008. Blast2GO: a comprehensive suite for functional analysis in plant genomics. *Int J Plant Genomics* 2008:619832. <https://doi.org/10.1155/2008/619832>.
 21. Boekhout T, Kamp M, Gueho E. 1998. Molecular typing of *Malassezia* species with PFGE and RAPD. *Med Mycol* 36:365–372. <https://doi.org/10.1080/02681219880000581>.
 22. Stalhberger T, Simenel C, Clavaud C, Eijsink VG, Jourdain R, Delepierre M, Latgé JP, Breton L, Fontaine T. 2014. Chemical organization of the cell wall polysaccharide core of *Malassezia restricta*. *J Biol Chem* 289:12647–12656. <https://doi.org/10.1074/jbc.M113.547034>.
 23. Baker LG, Specht CA, Lodge JK. 2011. Cell wall chitosan is necessary for virulence in the opportunistic pathogen *Cryptococcus neoformans*. *Eukaryot Cell* 10:1264–1268. <https://doi.org/10.1128/EC.05138-11>.