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Authors

Shea, Terrance
Mohabir, Jason T
Kurbessoian, Tania
[et al.](#)

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Genome Sequence of *Lichtheimia ornata*, an Emerging Opportunistic Mucorales Pathogen

Terrance Shea,^a Jason T. Mohabir,^a Tania Kurbessoian,^b Brittany Berdy,^a James Fontaine,^a Andreas Gnirke,^a Jonathan Livny,^a
 Jason E. Stajich,^b  Christina A. Cuomo^a

^aInfectious Disease and Microbiome Program, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA

^bDepartment of Microbiology and Plant Pathology, University of California, Riverside, Riverside, California, USA

ABSTRACT *Lichtheimia ornata* is an emerging opportunistic Mucorales pathogen that is associated with fatal infections in immunocompromised individuals. While these environmentally acquired infections have rarely been reported to date, cases were noted in a recent analysis of coronavirus disease 2019 (COVID-19)-associated mucormycosis in India. Here, we report the annotated genome sequence of the environmental isolate CBS 291.66.

Lichtheimia ornata is an opportunistic fungal pathogen that is an emerging cause of invasive disease. *L. ornata* is taxonomically classified in the order Mucorales, which includes other *Lichtheimia* and *Rhizopus* species (1). These species live in the environment as saprotrophs, and many can grow at high temperatures, which is an advantage for growth at human body temperature (1). Infections by these species can be very difficult to treat and consequently have high mortality rates (2). *L. ornata* was recently detected among cases of coronavirus disease 2019 (COVID-19)-associated mucormycosis (CAM) in India in 2021 (3).

The CBS 291.66 isolate of *L. ornata* was obtained from the Westerdijk Fungal Biodiversity Institute. The website entry for this isolate reports that it was collected from a bird dung sample in India in 1961. This isolate was cultured in malt extract-yeast extract (MEYE) agar at 20°C for >1 week until full hyphal growth reached the edges of the plate. Genomic DNA was isolated using a standard fungal cetyltrimethylammonium bromide (CTAB) extraction protocol (4) with collected fungal hyphal material, which yielded 136 ng/ μ L genomic DNA dissolved in water.

From genomic DNA, two Oxford Nanopore Technologies (ONT) libraries were constructed using the 1D ligation kit (catalog number SQK-LSK109), and each was loaded in a FLO-MIN106D flow cell on a GridION instrument. Base calling was performed using Guppy v6.0.7 (for SRA accession number [SRR23855900](https://www.ncbi.nlm.nih.gov/sra/SRR23855900)) and Guppy v6.1.5 (for SRA accession number [SRR23855899](https://www.ncbi.nlm.nih.gov/sra/SRR23855899)). Illumina sequencing was performed on the sample using two different library construction methods. One library was prepared using a standard NEBNext Ultra II protocol and sequenced on a HiSeq X Ten system with 300 cycles. The other, which was a set of four transposase enzyme-linked long-read sequencing (TELL-Seq) libraries for which reads from the same bead-bound genomic DNA molecule shared the same barcode (5), was constructed from genomic DNA depleted of <10-kb fragments using the Pacific Biosciences (PacBio) short-read eliminator (SRE) XS kit. Libraries from 0.33 and 0.67 ng of input DNA were constructed using the TELL-Seq kit (Universal Sequencing Technologies). Five- or 10- μ L aliquots of the 20- μ L bead-bound library were amplified by 13 to 15 PCR cycles and sequenced on a NovaSeq SP system with 300 cycles. Total coverage of 4 \times was generated with ONT reads (106,228 reads [read N_{50} 5.2 kb]), 25 \times with Illumina 150-bp paired-end reads, and 3,800 \times with TELL-Seq reads.

The TELL-Seq libraries were processed with TELL-Read analysis pipeline software (5), and the four libraries were merged into single index I1 read, R1 read, and R2 read fastq files

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Address correspondence to Christina A. Cuomo, cuomo@broadinstitute.org.

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and then converted into 10×-compatible format using the *ust10x* tool (<https://www.universalsequencing.com/software/>). Reads were assembled using Supernova v2.1.1 (6) with the following parameters: `-accept-extreme-coverage, -maxreads=300000000 scaffold >=500` bases. Gaps were closed by running three iterations of *LR_Gapcloser* (unversioned) (7) using ONT reads, followed by one iteration of *Pilon* v1.23 (8) using Illumina (NEBNext Ultra II library) reads and then three more iterations of *LR_Gapcloser* with ONT reads. Finally, the assembly was polished through three iterations of *Pilon* v1.23 using Illumina (NEBNext Ultra II library) reads. Scaffolds were aligned with the NCBI nucleotide database using *BLAST* v2.12.0+ (`-task blastn`); along with GC content and read coverage analyses, 93 scaffolds were identified as bacterial sequences and removed from the final assembly. The assembly of CBS 291.66 consists of 603 scaffolds (795 contigs), with a scaffold N_{50} value of 386 kb (contig N_{50} 151 kb) and a total length of 37.5 Mb.

The genome was annotated using a data set of 35,966 proteins from three *Lichtheimia* species (*Lichtheimia corymbifera* JMRC:FSU:9682 [9], *Lichtheimia ramosa* JMRC:FSU:6197 [10], and *Lichtheimia hyalospora* FSU:10163 [11]), which were used with BRAKER2 (12) to identify candidate gene structures in the assembly that had been masked using RepeatMasker v4.1 (13). Genes containing Pfam domains found in repetitive elements or overlapping tRNA/rRNA features were removed. Genes were named and numbered sequentially. Protein-coding genes were named from Pfam/TIGRFAM database (searched with HMMER) and Swiss-Prot and KEGG database (searched with BLASTp) products (14–16). A total of 13,172 genes were predicted, and BUSCO v3 (17) identified 99.3% of the mucorales_odb10 gene set.

Data availability. The sequence, assembly, and annotation reported here are available in GenBank under BioProject accession number [PRJNA909826](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA909826). The Illumina reads from the TELL-Seq libraries are available under SRA accession numbers [SRR23705169](https://www.ncbi.nlm.nih.gov/sra/SRR23705169) to [SRR23705174](https://www.ncbi.nlm.nih.gov/sra/SRR23705174). The Illumina reads from the NEBNext Ultra II library are available under SRA accession number [SRR23705175](https://www.ncbi.nlm.nih.gov/sra/SRR23705175). The ONT reads are available under SRA accession numbers [SRR23855899](https://www.ncbi.nlm.nih.gov/sra/SRR23855899) and [SRR23855900](https://www.ncbi.nlm.nih.gov/sra/SRR23855900). The genome assembly is available under GenBank accession number [JARTCD000000000](https://www.ncbi.nlm.nih.gov/genbank/JARTCD000000000).

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