Annotated Genome Sequence of *Aspergillus tanneri* NIH1004

Stephanie Mounaud,a Pratap Venepally,a Indresh Singh,a Liliana Losada,a,* Seyedmojtaba Seyedmousavi,b* Kyung J. Kwon-Chung,b William C. Niermana

aDepartment of Infectious Disease, J. Craig Venter Institute, Rockville, Maryland, USA
bMolecular Microbiology Section, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

Kyung J. Kwon-Chung and William C. Nierman contributed equally to this work.

ABSTRACT The annotated genome of *Aspergillus tanneri*, a recently discovered drug-resistant pathogen, was determined by employing the Oxford Nanopore MiniON platform and the Funannotate pipeline. The genome size and the number of protein-coding genes are notably larger than those of the most common etiological agent of aspergillosis, *Aspergillus fumigatus*.

Two fatal invasive aspergillosis (IA) cases in chronic granulomatous disease (CGD) patients who failed to respond to aggressive antifungal therapies were caused by a newly discovered *Aspergillus* species, *Aspergillus tanneri* (1), in the *Aspergillus* taxonomic section Tanneri (2). Mycological characterization and targeted gene identification of the clinical *A. tanneri* strains were performed (1). *A. tanneri* strain NIH1004 was isolated from a 19-year-old CGD patient who suffered from fatal aspergillosis. Primary clinical cultures of *A. tanneri* NIH1004 were grown on Sabouraud dextrose agar. Isolates were subcultured and incubated for 2 to 3 weeks at 37°C. DNA was isolated from lyophilized mycelium that had been grown in liquid yeast glucose medium for 36 h at 37°C and was extracted using the cetyltrimethylammonium bromide (CTAB) method (3) after vigorous mixing with glass beads. Genomic DNA was sheared and sized for preparation of libraries to sequence on three sequencing platforms and was quality checked using an Agilent 2100 Bioanalyzer (Santa Clara, CA), as well as by quantitative PCR (catalog number KK4835; Kapa library quantification kit). Initial sequencing was performed on the Illumina HiSeq 2500 platform using 100-bp paired-end reads and 8-kb paired-end 454 reads. The Illumina sequence reads provided 30X genome coverage, consisting of 37,410,025 bp (G+C content, 47.4%). Reads were assembled de novo using the Celer Assembler (4), which resulted in 870 contigs with an N50 of 134,193 bp. An improved assembly was obtained using a long-read sequencing technology. An *A. tanneri* library was prepared using a ligation sequencing kit (product number SQK-LSK108; Oxford Nanopore) and was analyzed in an Oxford 9.4.1 flow cell with a MiniION device. Assembly was performed using Minimap2 (5) and miniasm (6), with default parameters. The sequences within the assembled contigs were error corrected using Racon v1.3.1 (7) and Pilon (v1.22; four rounds) (8). The reads used for error correcting were generated using wgsim (https://github.com/lh3/wgsim) to simulate reads based on the Celer Assembler-assembled contigs. We generated 4 million simulated 150-bp paired-end reads with a quality score of 40. The wgsim tool was modified from the MAQ read simulator by dropping dependencies; wgsim was originally released in the SAMtools software package. The resulting MiniION assembly consisted of 38,719,388 bp (G+C content, 47.3%). This improved *A. tanneri* assembly resulted in 14 contigs, with an N50 of 4,499,170 bp, and is the first published *A. tanneri* sequence.

Whole-genome annotation of the *A. tanneri* assembly was performed using the
Funannotate pipeline (v1.5.1-93c317b) (9). Initially, following the masking of repeats identified by RepeatMasker (v1.332) (10) and RepeatModeler (v1.0.11) (10), ab initio gene models for the contig sequences were predicted using the GeneMark-ES (v4.36) (11) and AUGUSTUS (v3.2.3) (12) programs. Evidence-based gene models were generated by aligning the contig sequences from the A. tanneri genome with the combined protein sequence (UniProtKB) database using DIAMOND (v0.9.21.122) (13) and later polishing using Exonerate (v2.4.0) (14). EVidenceModeler (v0.1.30) (15) with its weighting algorithm, as implemented in the Funannotate pipeline, was used to select the consensus models from among the ab initio and evidence-based gene models. Functional annotation of the consensus models was performed after removal of those with short lengths, gaps, and transposable elements. A total of 11,846 genes were associated with 64,436 annotations by performing sequence similarity searches against the Pfam (v32.0) (16), InterPro (v71.0) (17), BUSCO (v2.0) (18), EggNOG (v4.5) (19), MEROPS (v12.0) (19), and CAZyme (v7.0) (20) databases and using the SignalP secretome prediction program (v4.1) (21). The tRNA genes were identified by using trNAScan-SE (v1.23) (22).

The biosynthetic gene cluster (BGC) mining program antiSMASH (v4.1.0) (23), with its Minimum Information on Biosynthetic Gene cluster (MIBiG) repository of experimentally characterized BGCs (24), was utilized to identify 95 distinct secondary metabolite BGCs. This number of clusters is considerably higher than those in a set of eight related aspergilli, with a range from the highest at 68 for A. niger to the lowest at 39 for A. fumigatus at 39 (25).

Data availability. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number QUQM00000000. Raw sequence reads have been deposited in the SRA under accession number SRX4502713.

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