May 2, 2019

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AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

# Draft Genome Sequences of Three Monokaryotic Isolates of the White-Rot Basidiomycete Fungus *Dichomitus squalens*

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**ABSTRACT** Here, we report the draft genome sequences of three isolates of the wood-decaying white-rot basidiomycete fungus *Dichomitus squalens*. The genomes of these monokaryons were sequenced to provide more information on the intraspecies genomic diversity of this fungus and were compared to the previously sequenced genome of *D. squalens* LYAD-421 SS1.

*Dichomitus squalens* is a wood-decaying white-rot fungus commonly found in Europe, Asia, and North America (1). It is mainly found on softwoods (2, 3) and has an extensive repertoire of lignocellulose-degrading enzymes (4–6). Two of the genome-sequenced monokaryons, CBS463.89 and CBS464.89, are derived from the well-studied Polish dikaryon FBCC312 (CBS432.34) (4, 6–11), while OM18370.1 is derived from the Finnish dikaryon OM18370 (CBS139088).

Strains were maintained on 2% (wt/vol) malt extract (ME) and 1.5% (wt/vol) agar plates, from which four plugs (ø 5 mm) were used to inoculate stationary 50-ml 2% (wt/vol) ME liquid cultures, which were incubated at 28°C for 5 days. Genomic DNA was extracted from homogenized mycelium with extraction buffer (2% *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide [CTAB], 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, and 0.2% $\beta$-mercaptoethanol) and purified with chloroform-isoamyl alcohol (24:1) (12). For RNA extraction, the isolates were precultured on glycerol for 7 days (28°C) and transferred to solid-state cultures containing 2 g (dry weight) of Norway spruce wood sticks (2 cm by 0.2 cm by 0.2 cm) on top of 1% (wt/vol) water agar at 28°C for 2 and 4 weeks (4). RNA extracts were layered over a 2-ml CsCl solution (5.7 M CsCl [Serva, Germany], 25 mM sodium citrate [pH 7.0], 0.5% *N*-lauroylsarcosine [Sigma, USA], and 0.1 M $\beta$-mercaptoethanol [Sigma]) in 13.2-ml polyallomer ultracentrifuge tubes (Beckman Coulter, Brea, CA, USA) and centrifuged at 33,000 rpm for 21 h at 4°C in an Optima L-90 K ultracentrifuge, using the SW-41 Ti swinging bucket rotor (Beckman Coulter). After centrifugation, the supernatant was removed, the tube was inverted, and all but the bottom 1 cm was sheared off. The RNA in the clear pellet was rinsed with 100 $\mu$l of diethyl pyrocarbonate (DEPC)-treated water and then dissolved in 50 $\mu$l of DEPC-treated water and stored at −80°C (13). The genomes were sequenced using the Illumina platform and pairs of standard fragments (300 bp) and 4-kbp long mate pair (LMP) libraries. Fragment libraries were produced from 100 ng genomic DNA (gDNA) sheared to 300 bp using the Covaris LE220 instrument and size selected using solid-phase reversible immobilization (SPRI) beads (Beckman Coulter). The fragments were treated with end repair, A-tailing, and ligation of Illumina-compatible adapters (IDT, Inc.) using the Illumina library creation kit (Kapa Biosystems). For LMP, 5 $\mu$g of DNA

**Citation** Casado López S, Peng M, Daly P, Andreopoulos B, Pangilinan J, Lipzen A, Riley R, Ahrendt S, Ng V, Barry K, Daum C, Grigoriev IV, Hildén KS, Mäkelä MR, de Vries RP. 2019. Draft genome sequences of three monokaryotic isolates of the white-rot basidiomycete fungus *Dichomitus squalens*. Microbiol Resour Announc 8:e00264-19. https://doi.org/10.1128/MRA.00264-19.

**Editor** Jason E. Stajich, University of California, Riverside



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**Received** 5 March 2019
**Accepted** 5 April 2019
**Published** 2 May 2019

**TABLE 1** Genome characteristics of the three *D. squalens* genomes in this study compared with the previously sequenced genome of LYAD-421 SS1[a]

| Characteristic | Data for strain: | | | |
|---|---|---|---|---|
| | CBS463.89 | CBS464.89 | OM18370.1 | LYAD-421 SS1 |
| Genome assembly size (Mbp) | 36.87 | 39.60 | 39.32 | 42.75 |
| Read coverage depth (×) | 145 | 118.7 | 100.8 | 50.63 |
| No. of reads sequenced (millions) | 42.6 | 32.7 | 38.3 | 7.7 |
| No. of contigs | 1,373 | 1,147 | 1,126 | 2,852 |
| No. of scaffolds | 1,259 | 467 | 439 | 542 |
| Scaffold $N_{50}$ value (Mbp) | 134 | 44 | 39 | 16 |
| Scaffold $L_{50}$ (Mbp) | 0.08 | 0.22 | 0.27 | 0.64 |
| No. of gaps | 114 | 680 | 687 | 1,155 |
| % scaffold length in gaps | 0.2 | 2.5 | 2.6 | 7.7 |
| Gene length (avg/median) (bp) | 1,691/1,437 | 1,678/1,425 | 1,694/1,449 | 1,890/1,562 |
| Transcript length (avg/median) (bp) | 1,370/1,140 | 1,358/1,128 | 1,365/1,150 | 1,484/1,213 |
| Exon length (avg/median) (bp) | 259/158 | 259/158 | 256/157 | 254/152 |
| Intron length (avg/median) (bp) | 76/61 | 77/60 | 78/61 | 86/61 |
| Protein length (avg/median) (aa[b]) | 387/314 | 382/311 | 388/319 | 419/345 |
| No. of exons per gene (avg/median) | 5.3/4 | 5.25/4 | 5.34/4 | 5.84/4 |
| No. of gene models | 14,946 | 15,295 | 14,950 | 12,290 |
| G+C content (%) | 55.7 | 55.6 | 55.6 | 55.6 |

[a] From reference 5.
[b] aa, amino acids.

was sheared using the g-TUBE (Covaris), and the gel size was selected for 4 kb. The sheared DNA was treated with end repair, ligated with biotinylated LoxP adapters, and circularized by a Cre excision reaction (New England BioLabs [NEB]). The products were randomly sheared, treated as indicated for the fragment library, and enriched using eight PCR cycles for the final library.

For the transcriptomes, which were used for genome annotations, stranded cDNA libraries were generated using the Illumina TruSeq stranded RNA low-throughput (LT) kit. mRNA was purified using magnetic beads containing poly(T) oligonucleotides, fragmented and reverse transcribed using random hexamers and SSII (Invitrogen), followed by second-strand synthesis, and then treated with end repair, A-tailing, adapter ligation, and eight PCR cycles.

The prepared libraries were quantified using the Kapa Biosystems next-generation sequencing library quantitative PCR (qPCR) kit and run on a Roche LightCycler 480 real-time PCR instrument. The quantified libraries were then multiplexed with other libraries, and the pool of libraries was then prepared for sequencing on the Illumina HiSeq sequencing platform utilizing a TruSeq paired-end cluster kit, v.4, and the Illumina cBot instrument to generate a clustered flow cell for sequencing. Sequencing of the flow cell was performed on an Illumina HiSeq 2500 sequencer using HiSeq TruSeq sequencing by synthesis (SBS) kits, v.4, following a 2 × 150-bp (2 × 100-bp for LMP) indexed run recipe (14).

Illumina FASTQ files were quality control (QC) filtered for artifact/process contamination. DNA reads were assembled with AllPaths-LG v.R49403 (15). For CBS463.89 lacking LMP, the initial assemblies of fragment data with Velvet v.1.2.07 (16) were used to create *in silico* long mate pair libraries with insert sizes of 3,000 ± 300 bp. RNA reads were assembled using Rnnotator v.3.4.0 (17). All three genomes were annotated using the JGI annotation pipeline v.1.9, which combines several *ab initio*, homology-based, and transcriptome-based gene predictors, as well as tools and databases for functional annotation (18, 19).

All four genomes are highly similar in genome size and characteristics (Table 1). The improvement in sequencing methodology is reflected in the lower contig and gap numbers of the three new genomes compared with those of the older genome (LYAD-421 SS1). These data are highly useful to evaluate intraspecies genome variation in *D. squalens*.

**Data availability.** Genome assemblies and annotations are available via MycoCosm (http://jgi.doe.gov/fungi [18]). The data are deposited at DDBJ/EMBL/GenBank under

BioProject/GenBank accession numbers PRJNA334679/SELY00000000, PRJNA334680/SELZ00000000, and PRJNA334681/SELX00000000.

## ACKNOWLEDGMENTS

P.D. and M.P. were supported by a grant from the Netherlands Scientific Organization, NWO 824.15.023, to R.P.D.V. The Academy of Finland grants 308284 and 297847 to M.R.M. and K.S.H., respectively, are acknowledged. The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, was supported by the Office of Science of the U.S. Department of Energy under contract number DE-AC02-05CH11231.

We thank Ad Wiebenga for his assistance with the gDNA isolation.

We declare no conflicts of interest.

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