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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% β-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 β-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 μl of diethyl pyrocarbonate (DEPC)-treated water and then dissolved in 50 μl 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 μg of DNA

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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.

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We declare no conflicts of interest.

REFERENCES

1. Andrews SR, Gill LS. 1943. Western red rot in immature Ponderosa pine in the Southwest. J Forest 41:565–573.
2. Blanchette RA, Otjen L, Carlson MC. 1987. Lignin distribution in cell walls of birch wood decayed by white rot basidiomycetes. Phytopathology 77:684–690. https://doi.org/10.1094/Phyto-77-684.
3. Renvall P, Renvall T, Niemelä T. 1991. Basidiomycetes at the timberline in Lapland 2. An annotated checklist of the polypores of northeastern Finland. Karstenia 31:13–28. https://doi.org/10.29203/ka.1991.282.
4. Daly P, Casado López S, Peng M, Lancefield CS, Purvine SO, Kim YM, Zink EM, Dohnalkova A, Singan VR, Lipzen A, Dilworth D, Wang M, Ng V, Robinson E, Orr G, Baker SE, Bruijnincx PCA, Hildén KS, Grigoriev IV, Mäkelä MR, de Vries RP. 2018. *Dichomitus squalens* partially tailors its molecular responses to the composition of solid wood. Environ Microbiol 20:4141–4156. https://doi.org/10.1111/1462-2920.14416.
5. Floudas D, Binder M, Riley R, Barry K, Blanchette RA, Henrissat B, Martínez AT, Otillar R, Spatafora JW, Yadav JS, Aerts A, Benoit I, Boyd A, Carlson A, Copeland A, Coutinho PM, de Vries RP, Ferreira P, Findley K, Foster B, Gaskell J, Glotzer D, Górecki P, Heitman J, Hesse C, Hori C, Igarashi K, Jurgens JA, Kallen N, Kersten P, Kohler A, Kües U, Kumar TKA, Kuo A, LaButti K, Larrondo LF, Lindquist E, Ling A, Lombard V, Lucas S, Lundell T, Martin R, McLaughlin DJ, Morgenstern I, Morin E, Murat C, Nagy LG, Nolan M, Ohm RA, Patyshakuliyeva A, et al. 2012. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336:1715–1719. https://doi.org/10.1126/science.1221748.
6. Rytioja J, Hildén K, Di Falco M, Zhou MM, Aguilar-Pontes MV, Sietiö O-M, Tsang A, de Vries RP, Mäkelä MR. 2017. The molecular response of the white-rot fungus *Dichomitus squalens* to wood and non-woody biomass as examined by transcriptome and exoproteome analyses. Environ Microbiol 19:1237–1250. https://doi.org/10.1111/1462-2920.13652.
7. Casado López S, Peng M, Issak TY, Daly P, de Vries RP, Mäkelä MR. 2018. Induction of plant cell wall degrading CAZyme encoding genes by lignocellulose-derived monosaccharides and cellobiose in the white-rot fungus *Dichomitus squalens*. Appl Environ Microbiol 84:e00403-18. https://doi.org/10.1128/AEM.00403-18.
8. Casado López S, Theelen B, Manserra S, Issak TY, Rytioja J, Mäkelä MR, de Vries RP. 2017. Functional diversity in *Dichomitus squalens* monokaryons. IMA Fungus 8:17–25.
9. Mäkelä MR, Sietiö O-M, de Vries RP, Timonen S, Hildén K. 2014. Oxalate-metabolising genes of the white-rot fungus *Dichomitus squalens* are differentially induced on wood and at high proton concentration. PLoS One 9:e87959. https://doi.org/10.1371/journal.pone.0087959.
10. Rytioja J, Hildén K, Hatakka A, Mäkelä MR. 2014. Transcriptional analysis of selected cellulose-acting enzymes encoding genes of the white-rot fungus *Dichomitus squalens* on spruce wood and microcrystalline cellulose. Fungal Genet Biol 72:91–98. https://doi.org/10.1016/j.fgb.2013.12.008.
11. Rytioja J, Hildén K, Mäkinen S, Vehmaanperä J, Hatakka A, Mäkelä MR. 2015. Saccharification of lignocelluloses by carbohydrate active enzymes of the white rot fungus *Dichomitus squalens*. PLoS One 10:e0145166. https://doi.org/10.1371/journal.pone.0145166.
12. Hildén K, Martínez AT, Hatakka A, Lundell T. 2005. The two manganese peroxidases Pr-MnP2 and Pr-MnP3 of *Phlebia radiata*, a lignin-degrading basidiomycete, are phylogenetically and structurally divergent. Fungal Genet Biol 42:403–419. https://doi.org/10.1016/j.fgb.2005.01.008.
13. Patyshakuliyeva A, Mäkelä MR, Sietiö O-M, de Vries RP, Hildén KS. 2014. An improved and reproducible protocol for the extraction of high quality fungal RNA from plant biomass substrates. Fungal Genet Biol 72:201–206. https://doi.org/10.1016/j.fgb.2014.06.001.
14. Walker C, Ryu S, Na H, Zane M, LaButti K, Lipzen A, Haridas S, Barry K, Grigoriev IV, Quarterman J, Slininger P, Dien B, Trinh CT. 2018. Draft genome assemblies of five robust *Yarrowia lipolytica* strains exhibiting high lipid production, pentose sugar utilization, and sugar alcohol secretion from undetoxified lignocellulosic biomass hydrolysates. Microbiol Resour Announc 7:e01040-18. https://doi.org/10.1128/MRA.01040-18.
15. Gnerre S, MacCallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, Berlin AM, Aird D, Costello M, Daza R, Williams L, Nicol R, Gnirke A, Nusbaum C, Lander ES, Jaffe DB. 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc Natl Acad Sci U S A 108:1513–1518. https://doi.org/10.1073/pnas.1017351108.
16. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res 18:821–829. https://doi.org/10.1101/gr.074492.107.
17. Martin J, Bruno VM, Fang Z, Meng X, Blow M, Zhang T, Sherlock G, Snyder M, Wang Z. 2010. Rnnotator: an automated *de novo* transcriptome assembly pipeline from stranded RNA-Seq reads. BMC Genomics 11:663. https://doi.org/10.1186/1471-2164-11-663.
18. Grigoriev IV, Nikitin R, Haridas S, Kuo A, Ohm R, Otillar R, Riley R, Salamov A, Zhao X, Korzeniewski F, Smirnova T, Nordberg H, Dubchak I, Shabalov I. 2014. MycoCosm portal: gearing up for 1000 fungal genomes. Nucleic Acids Res 42:D699–D704. https://doi.org/10.1093/nar/gkt1183.
19. Haridas S, Salamov A, Grigoriev IV. 2018. Fungal genome annotation. Methods Mol Biol 1775:171–184. https://doi.org/10.1007/978-1-4939-7804-5_15.