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Draft Genome Sequence of the Basidiomycete White-Rot Fungus *Phlebia centrifuga*

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ABSTRACT Here, we report the genome sequence of wood-decaying white-rot fungus *Phlebia centrifuga* strain FBCC195, isolated from Norway spruce (*Picea abies*) in Finnish Lapland. The 34.66-Mb genome containing 13,785 gene models is similar to the genome length reported for other saprobic white-rot species.

The basidiomycete *Phlebia centrifuga* is a corticioid wood-decaying white-rot fungus which belongs to the genus *Phlebia* (order *Aphyllophorales*, family *Corticiaceae*). It is a typical inhabitant of fallen decomposing trunks in unmanaged forests, and it has been used as an indicator species of old-growth forests in Nordic countries (1). Dikaryotic *P. centrifuga* FBCC195 has been isolated from Norway spruce (*Picea abies*) in Finnish Lapland (Sodankylä), and it has been classified as near-threatened species (2).

P. centrifuga FBCC195 was maintained on 2% malt agar plates, from which four plugs (diameter, 7 mm) were used to inoculate 100-ml 2% malt extract liquid cultures. Stationary cultures were incubated at 25°C for 21 days. Genomic DNA was extracted from the cultures using a cetyltrimethylammonium bromide (CTAB)-based buffer (3). For RNA extraction, the fungus was cultivated on solid-state cultures containing 2 g (dry weight) of Norway spruce (*Picea abies*) wood sticks (approximately 2.5 by 0.3 by 0.2 cm) or wheat (*Triticum aestivum*) straw pieces (2 cm in length) on top of 1% water agar at 25°C for 21 days. The moisture content of the cultures was adjusted to 60% with sterile H_2O. The stationary cultures were inoculated with 4 ml of homogenized *P. centrifuga* mycelium (4) from low-nitrogen asparagine medium (pH 4.5) (5), supplemented with 1% glycerol and incubated at 25°C for 21 days. RNA was extracted using CsCl ultracentrifugation (6) and checked using a fragment analyzer (Advanced Analytical Technologies). The concentration and quality of the DNA were determined using a Qubit fluorometer (Life Technologies) and 0.6% agarose gel, respectively. Genome and transcriptome sequencing were performed at GenomeScan.

The DNA was fragmented using a Focused-ultrasonicator (Covaris). The NEBNext Ultra DNA library prep kit and NEBNext Ultra directional RNA library prep kit for Illumina (catalog numbers E7370S/L and E7420S/L, respectively) were used according to the manufacturer's instructions. The quality and yield after sample preparation were measured with Lab-on-a-Chip analysis or a fragment analyzer. Illumina cBot and HiSeq 2500 standard Illumina primers and the HiSeq control software HCS version 2.2.58 were used according to the manufacturer's protocols for clustering and DNA sequencing, with concentrations of 8.0 pM DNA and 16.0 pM cDNA. The Illumina data analysis pipeline

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RTA version 1.18.64 and bcl2fastq version 1.8.4 were used for image analysis, base calling, and quality checking. fastqFilter version 2.05, a GenomeScan in-house pipeline, was used for adapter removal and quality checking; bases with a Phred score above Q22 and reads longer than 36 bp passed the filtering. For the assembly, ABySS version 1.3.7 (7), with a k-mer length of 64, was used. Scaffolds shorter than 500 bp were removed. A total of 1,367 contigs were used for the assembly of the 34.66-Mb genome. The GC content was 48.91%, as assessed by QUAST (8). The genome of *Phlebia brevispora* was used as a gene-finding trainer for the HMM-based algorithm Glimmer (version 3.02) (9). Mapped mRNA-Seq reads were used by the CodingQuarry software tool (10) for an evidence-based method of gene finding. Using the two methods combined, gene models for 13,785 genes were obtained. Gene models encoding lignin degradation-related proteins are present in multiple copies (four manganese peroxidases, four lignin peroxidases, and four laccases).

Accession number(s). This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number MLYV00000000. The version described in this paper is version MLYV02000000 and is also available through MycoCosm (11).

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REFERENCES

1. Eriksson J, Hjortstam K, Ryvarden L. 1981. The Corticiaceae of North Europe, vol 6, *Phlebia—Sarcodontia* Fungiflora, Oslo, Norway.
2. Rassi P, Hyvärinen E, Juslen A, Mannerkoski I. 2010. The 2010 Red List of Finnish species Ministry of the Environment & Finnish Environment Institute, Helsinki, Finland.
3. Hildén K, Martinez 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.
4. Mäkelä MR, Galkin S, Hatakka A, Lundell TK. 2002. Production of organic acids and oxalate decarboxylase in lignin-degrading white rot fungi. Enzyme Microbial Technol 30:542–549. https://doi.org/10.1016/S0141-0229(02)00012-1.
5. Hatakka A, Uusi-Rauva A. 1983. Degradation of ^{14}C-labelled poplar wood lignin by selected white-rot fungi. Eur J Appl Microbiol Biotechnol 17:235–242. https://doi.org/10.1007/BF00510422.
6. 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.
7. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I. 2009. ABySS: a parallel assembler for short read sequence data. Genome Res 19:1117–1123. https://doi.org/10.1101/gr.089532.108.
8. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
9. Majoros WH, Pertea M, Salzberg SL. 2004. TigrScan and GlimmerHMM: two open source ab initio eukaryotic gene-finders. Bioinformatics 20:2878–2879. https://doi.org/10.1093/bioinformatics/bth315.
10. Testa AC, Hane JK, Ellwood SR, Oliver RP. 2015. CodingQuarry: highly accurate hidden Markov model gene prediction in fungal genomes using RNA-seq transcripts. BMC Genomics 16:170. https://doi.org/10.1186/s12864-015-1344-4.
11. 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.