Genome Sequence Resource and Annotation of *Calonectria montana*, an Economically Important Pathogen with a Broad Host Range

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Genome Announcement

Calonectria montana is an important soilborne fungal pathogen with a broad host range which causes serious diseases on forestry, agricultural, and horticultural plants. Here, we report a complete genome assembly of *C. montana* PCam007 (assembly = 59.3 Mb and contig N₅₀: 6.36 Mb), using a combination of Illumina paired-end and Oxford Nanopore Technologies (ONT) long-read sequencing data. In total, 14,771 protein-coding genes and 477 noncoding RNAs were identified, among which 14,365 genes were annotated. Benchmarking universal single-copy orthology (BUSCO) analysis indicated that 98.50% of the BUSCOs were complete whereas 98.20, 0.40, and 0.10% were single copy, duplicated, and fragmented, respectively. The availability of this high-quality genome resource is expected to facilitate research on the evolution, host adaption, and pathogenicity of *C. montana* and to provide new insight into the infection mechanisms of *C. montana* and disease management.

Calonectria represents a genus of phytopathogenic ascomycetous fungi with a world-wide distribution. Species of this genus have a broad host range that includes more than 335 plant species, distributed across nearly 100 plant families (Wu and Chen 2021). *Calonectria* spp. cause important diseases on various forestry, horticultural, and agricultural crops (Crous 2002; Liu et al. 2020). Symptoms associated with infection by these fungi include stem cankers and leaf and shoot blight, as well as root rot.

The significance of *Calonectria* spp. has justified many taxonomic studies on these fungi in recent years. Thus far, 169 species have been identified based on a combination of DNA sequence data and morphological characteristics (Wu and Chen 2021). China has relatively high *Calonectria* spp. diversity. Up to now, 23 species of *Calonectria* have been described (Wu and Chen 2021). Of these, most species were isolated from *Eucalyptus* plants or soils. *C. montana* is a newly described species which was first isolated from natural forest soil in central China in 2016 (Liu and Chen 2017). It was later described in Poland to cause seedling damping-off on *Pinus sylvestris* and *Picea abies* (Stepniewska et al. 2020). A recent study reported that the species can cause root rot in sugar beet (Shao and Li 2021). Additionally, *C. montana* can also cause brown spot disease on tree peony, a well-known Chinese traditional flower widely cultivated all over the world (Fig. 1A) (Cheng 2007) (unpublished data).

Keywords

Calonectria montana, brown spot disease, fungus–plant interactions, genomics, metabolomics, Oxford Nanopore Technology, peony (*Paeonia Suffruticosa*), proteomics, whole-genome sequencing

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Fig. 1. A, Disease phenotype of *Calonectria montana* on leaf of tree peony and **B**, phylogenetic tree of the genus *Calonectria* based on maximum likelihood (ML) analyses of combined DNA dataset of *act*, *cmdA*, *his3*, *ITS*, *LSU*, *rpb2*, *tef1*, *tub2*, *MAT1-1-1*, and *MAT1-2-1* gene sequences. Sequences of the other *Calonectria* spp. were downloaded from NCBI. Bootstrap values for maximum-likelihood analyses are presented at the branches.

The isolate PCam007 in this study was first isolated from infected tree peony leaves collected from Sui and Tang Dynasty Town Botanic Gardens, located in Luoyang City, China. Based on morphological characteristics and multigene phylogenetic analyses of the internal transcribed spacer (Liu et al. 2020), PCam007 was identified as *C. montana*. A pathogenicity test on 15 tree peony varieties showed that the pathogen is highly virulent toward all tested varieties, even under no-wounding inoculation (unpublished data). Whole-genome sequencing of the *C. montana* isolate PCam007 was conducted by ONT long-read sequencing (for de novo assembly) and Illumina short-read sequencing (for polishing). The overall aim was to facilitate future research regarding this important fungus, especially relating to its taxonomy, population genetics, and pathogenicity.

High-quality genomic DNA of PCam007 was extracted and purified from 3-day-old fresh mycelium using the cetyltrimethylammonium bromide method, and the DNA quality and concentration were determined by 0.75% agarose gel electrophoresis on a NanoDrop One spectrophotometer (Thermo Fisher Scientific) and a Qubit v3.0 Fluorometer (Life Technologies, Carlsbad, CA, U.S.A.) respectively. It was then randomly sheared by a Covaris ultrasonic disruptor. Illumina sequencing pair-end libraries with insert size of 300 bp were prepared using a Nextera DNA Flex Library Prep Kit (Illumina, San Diego, CA, U.S.A.). Sequencing was performed using the Illumina NovaSeq platform (Illumina). Raw reads were cleaned to discard low-quality reads (reads with adaptors or unknown nucleotides or reads with more than 20% low-quality bases) using the SOAPnuke (v2.1.4) tool and, after data filtering, clean data were used for subsequent analyses.

For ONT sequencing, the libraries were prepared using the SQK-LSK109 ligation kit and the standard protocol. The purified library was loaded onto primed R9.4 Spot-On Flow Cells and sequenced using a PromethION sequencer (Oxford Nanopore Technologies, Oxford, U.K.) with 48-h runs. Base-calling analyses of raw data were performed using the ONT GUPPY software (v0.3.0).

We obtained 12.78 Gb of ONT reads, with N_{50} length of 20.79 kb, and 4.64 Gb of paired-end Illumina reads (Table 1). The sequencing depth of ONT reads and Illumina reads represent 215× and 81× genome coverage, respectively (Table 1). To obtain genome assembly, we exploited NextDenovo v2.3.1 to generate a draft assembly, using pure ONT reads, and NextPolish v1.3.1 to polish the assembly using Illumina reads. The final genome

Table 1. Genome features of *Calonectria montana* PCam007

Feature	Isolate PCam007
Oxford Nanopore Technologies (ONT) long reads (Gb) ^a	12.78 (approximately 215×)
N ₅₀ of ONT reads (kb)	20.79
Mean ONT read length (kb)	11.88
Maximum ONT read length (kb)	195.00
Illumina short reads (Gb) ^a	4.64 (approximately 81×)
Estimated genome size (Mb)	57.16
Estimated genome repeats (Mb)	11.04
Estimated genome heterozygosity (%)	0.096
Assembly size (Mb)	59.30
Contig number	13
Contig N ₅₀ (Mb)	6.36
Contig N ₉₀ (Mb)	14.00
Average contig length (Mb)	4.56
Maximum contig length (Mb)	9.32
GC content (%)	47.25
Illumina reads mapping rate	97.17
Benchmarking universal single-copy orthology (BUSCO) complete (%)	98.50
Fragmented BUSCOs (%)	0.10
Missing BUSCOs (%)	1.30
Noncoding RNA	477
Protein-coding genes ^b	14,771
Genes annotated by Pfam	12,056
Genes annotated by Gene Ontology	8,734
Genes annotated by Kyoto Encyclopedia of Genes and Genomes	4,868
Genes annotated by Carbohydrate Active Enzymes	539
Genes annotated by Clusters of Orthologous Groups	1,380
Genes annotated by the Transporter Classification Database	2,442
Cytochrome P450s	1,721
Transmembrane proteins	3,058
Secreted proteins	1,555

^a Genome heterozygosity, size, and repeat content were estimated by kmer-based method GenomeScope (version 1.0.0) using Illumina short reads.

^b Predictions were based on a previously described pipeline (Liang et al. 2018).

assembly was 59.30 Mb in length and contained 13 scaffolds (Table 1). The assembly size is within the range of published *Calonectria* genomes (*C. aciculata*, *C. crousiana*, *C. fujianensis*, *C. henricotiae*, *C. honghensis*, *C. leucothoes*, *C. naviculata*, *C. pseudonaviculata*, *C. pseudoreteauidii*, *C. pseudoturangelicola*, and *C. illicicola*), ranging from 47.50 to 68.97 Mb. The phylogenetic tree, based on maximum-likelihood analyses of a 10-gene (*act*, *cmdA*, *his3*, *ITS*, *LSU*, *rpb2*, *tef1*, *tub2*, *MAT1-1-1*, and *MAT1-2-1*) combined DNA dataset, showed that the *C. montana* isolate PCam007 constitute a single branch (Fig. 1B) (Liu et al. 2019; Liu et al. 2020; Zhang et al. 2020). The N₅₀ scaffold length and the maximum scaffold length were 4.56 and 9.32 Mb, respectively. The assembly GC content was 47.25%, which is close to the reported *Calonectria* genomes (Liu et al. 2019; Liu et al. 2020). The completeness of PCam007 genome assembly was evaluated through benchmarking universal single-copy orthologs (BUSCO) v4.1.4 with 'eukaryota_odb10' (*n* = 255) as reference dataset (Seppey et al. 2019). This analysis indicates that genome assembly of isolate PCam007 contains 98.50% completed BUSCOs, 0.10% fragmented BUSCOs, and 1.30% missing BUSCOs (Table 1).

Protein-encoding gene prediction was performed by BRAKER2 (Brůna et al. 2021). In total, 14,771 protein-encoding genes were predicted (Table 1). To gain a functional gene annotation, we annotated whole-genome protein-encoding genes with Pfam, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), Clusters of Orthologous Groups (COG), and carbohydrate active enzymes (CAZymes) databases (Guo et al. 2021). We identified a total of 12,056 proteins with Pfam domains, 8,734 genes with GO items, 4,868 genes involved in different KEGG pathways, and 1,380 COG genes. In total, 759 genes were mapped to the CAZyme database and encoded carbohydrate-active enzymes and 3,058 genes encoded transmembrane proteins as performed by Tmhmm v5.0. INFERNAL v1.1.2 analysis predicted 477 noncoding RNAs, including 341 transfer RNAs, 89 ribosomal RNAs, 43 small nuclear RNAs, and four small RNA.

Data Availability

The availability of this high-quality genome resource will accelerate studies with *C. montana* regarding comparative genomics, evolution, and population genetics. This advanced understanding on *C. montana* will further improve strategies for disease management. The assembled genome has been deposited at GenBank under the accession number JAIZZI000000000.

Author-Recommended Internet Resource

SOAPnuke (v2.1.4) tool: <https://github.com/BGI-flexlab/SOAPnuke>

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