Long-Read Genome Sequence Resource of *Ascochyta versabilis* Causing Leaf Spot Disease in *Pseudostellaria heterophylla*

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Abstract

Ascochyta versabilis is the fungal pathogen that causes the severe leaf spot disease of *Pseudostellaria heterophylla* (Miq.) Pax, a vital Chinese herbal plant. Here, we deployed PacBio single-molecule real-time long-read sequencing technology to generate a near-complete genome assembly for the *A. versabilis* KC1 strain and obtained a total of 9.80 Gb raw reads. These reads were processed into a 41.05 Mb genome assembly containing 95 contigs with N50 of 1.70 Mb and a maximum length of 3.93 Mb. A total of 10,457 gene models, of which 1,004 encode putatively secreted proteins, were identified in the genome. This high-quality genome assembly and gene annotation resource will facilitate the institution of functional genetic studies aimed at providing a better insight into the infection mechanisms of *A. versabilis* to support the development of effective control strategies for leaf spot disease of *P. heterophylla*.

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Keywords

Ascochyta versabilis, fungal pathogen, genome, leaf spot disease

Genome Announcement

Ascochyta versabilis is the pathogen causing severe leaf spot disease of *Pseudostellaria heterophylla* (Miq.) Pax, an important traditional Chinese herb with several health benefits, including antitussive activity (Pang et al. 2011), ability to alleviate fatigue (Sheng et al. 2011), and as an antioxidant (Ng et al. 2004). Annual yield losses caused by this pathogen are consistently high due to the absence of *A. versabilis*-resistant plant cultivars (Li et al. 2013; Wang et al. 1997).

The pathogenic fungus of leaf spot disease of *P. heterophylla* was first isolated in 1997 and was subsequently classified as a member of the genus *Phoma* (Wang et al. 1997). However, it was recently reclassified as *A. versabilis* based on morphological features and phylogenetic characterization of internal transcribed spacer regions 1 and 2, including the 5.8S nuclear ribosomal DNA gene (Li et al. 2018).

Although the leaf spot fungus represents a significant threat to the commercial cultivation of *P. heterophylla*, the molecular mechanisms regulating the pathogenesis of *A. versabilis* are

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still unknown, owing to the absence of genome resources to support the identification and elucidation of pathogenesis mechanisms of *A. versabilis*.

To generate a high-quality genome assembly resource data for *A. versabilis*, the *A. versabilis* KC1 strain was isolated from a fungus-infected leaf spot on *P. heterophylla* from the Fujian province of China. High-quality genomic DNA of the strain KC1 was extracted from mycelia, following the CTAB method (Weiland 1997), and was sequenced using single-molecule real-time (SMRT) sequencing technology developed by PacBio at the Novogene Bioinformatics Technology Co. Ltd. (Beijing). A single SMRT cell (1M V3) was used to sequence a 20-kb-long insert genomic library by the PacBio Sequel System. After filtering the low-quality raw reads, we obtained 9.80 Gb of raw reads with an average length of 10.23 kb and N50 of 14.37 kb. The maximum read length was 64.70 kb, and the average read accuracy was 86.24% (Table 1).

The genome of *A. versabilis* KC1 strain was de novo assembled with the HGAP4 pipeline of SMRT Link (v5.1.0, default parameters except expected genome length = 45 Mb). Initially, the longest reads (30% of the total, length cutoff = 25 kb) were selected as seed reads, were corrected with the remaining long reads, and were then used to generate a draft assembly. To improve the continuity of assembly and fix base errors and gaps, 95.71% of the raw reads (229× coverage) were mapped to the draft assembly and generated a polished genome assembly with the Arrow algorithm. Removing organelle-originating contigs, we obtained a 41.05 Mb assembly consisting of 95 contigs with N50 of 1.70 Mb and maximum contig length of 3.93 Mb (Table 1). Genome completeness was assessed with BUSCO v4.05 (Seppey et al. 2019). The result showed that the assembly contains 752 (99.21%) and 1,693 (99.24%) complete orthologs in fungi ($n = 758$) and Ascomycota ($n = 1,706$), respectively (Table 1).

Repeats were identified by RepeatModeler v1.08 (available online) and the genome sequence was masked by RepeatMasker v4.0.9 (available online). Notably, 13.93% of the genomic sequences are repetitive sequences. The soft-masking (with repeat regions into lower case letters) assembly was used to perform gene prediction using BRAKER2 (available online) (Hoff et al. 2019). Integrating ab initio and close homologous protein-based methods, a total of 10,457 protein-coding gene models were identified (Table 1). BUSCO assessment of gene predictions showed 98.28 and 96.42% completeness with 745 and 1,642 complete

Table 1. Genome characteristics^a

Features	Strain KC1 ^b
Sequencing platform	PacBio Sequel
Total reads (Gb)	9.80
Average read length (kb)	10.23
Reads N50 (kb)	14.37
Maximum read length (kb)	64.70
Average read accuracy	86.24%
Coverage	229×
Assembly size (Mb)	41.05
GC content	49.62%
Contig number	95
Contig N50 (Mb)	1.70
Contig L50	9
Average contig length (Mb)	0.43
Maximum contig length (Mb)	3.93
Protein-coding genes	10,457
Candidate secreted proteins	1,004
Repeat sequence	13.93%
Genes annotated by Pfam, GO, KEGG, CAZy and eggNOG	8,368
Genome BUSCO assessment in fungi ($n = 758$)	C: 752 [S: 750, D: 2], F: 1, M: 5
Genome BUSCO assessment in Ascomycota ($n = 1,706$)	C: 1,693 [S: 1,690, D: 3], F: 2, M: 11
Gene BUSCO assessment in fungi ($n = 758$)	C: 745 [S: 745, D: 0], F: 6, M: 7
Gene BUSCO assessment in Ascomycota ($n = 1,706$)	C: 1,642 [S: 1,637, D: 5], F: 26, M: 38

^a The N50 is defined as the shortest sequence length at 50% of the genome, while the L50 is defined as the smallest number of contigs whose length sum produces N50.

^b C = complete BUSCOs, S = complete and single-copy BUSCOs, D = complete and duplicated BUSCOs, F = fragmented BUSCOs, and M = missing BUSCOs.

orthologs in fungi ($n = 758$) and Ascomycota ($n = 1,706$), respectively (Table 1). Classically secreted proteins (with a signal peptide in the N terminus) were predicted per our previous pipeline (Bao et al. 2017), and 1,004 candidate secreted proteins were identified (Table 1). Also, a total of 8,368 (80%) genes were annotated using Pfam, Gene Ontology, CAZy, Kyoto Encyclopedia of Genes and Genomes, and eggNOG databases (Table 1).

The nearly complete genome assembly and gene annotations data have been deposited at GenBank under the accession number JAAROD000000000 (BioProject: PRJNA613783).

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Author-Recommended Internet Resources

BRAKER2: <https://github.com/Gaius-Augustus/BRAKER>

CAZy database: <http://www.cazy.org>

eggNOG: <http://eggNOG5.embl.de>

Gene Ontology resource: <http://geneontology.org>

Kyoto Encyclopedia of Genes and Genomes database: <https://www.kegg.jp>

RepeatModeler v1.08: <http://www.repeatmasker.org/RepeatModeler>

RepeatMasker v4.0.9: <http://www.repeatmasker.org>

Pfam: <http://pfam.xfam.org>

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