Genomic Sequence Resource of *Alternaria alternata* Strain B3 Causing Leaf Blight on *Ophiopogon japonicus*

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Abstract

Black spot of *Ophiopogon japonicus* is an economically important disease, which can result in significant losses of both yield and quality of this traditional Chinese medicinal plant. The disease is caused by the small-spored fungal pathogen *Alternaria alternata*, a necrotrophic fungus that is ubiquitously distributed in the environment. Here, we present the draft whole-genome sequence of *A. alternata* strain B3 that caused black spot on *O. japonicus*. The assembly consists of 76 contigs with an estimated genome size of 33.8 Mb. Furthermore, we identified genes that may be associated with the pathogenicity, such as carbohydrate-active enzymes, secreted proteins, and secondary metabolite gene clusters. This genome resource will provide a useful source for future research on the evolution of pathogenicity of *A. alternata* and phylogenomic analysis in delineating phylogenetic lineages within the genus *Alternaria*.

Alternaria alternata, one of the small-spored *Alternaria* spp., can be ubiquitously found in soil, various plants, and decaying plant debris. Some strains in this species can cause diseases on more than 400 host plants and result in severe crop losses worldwide (Thomma 2003). However, the systematics of delimitation of *A. alternata* has been ambiguous. Some *Alternaria* species can be morphologically very similar but exhibit substantial pathological differences, and researchers failed to delineate significant variation among those species based on DNA sequences of popular marker genes (Woudenberg et al. 2013, 2015). Therefore, the concept of *formae speciales* and pathotypes were introduced into the systematics of *A. alternata* (Tsuge et al. 2013; Woudenberg et al. 2015). For example, the pathogen that causes citrus brown spots on tangerines and tangerine hybrids was defined as the tangerine pathotype of *A. alternata* (Wang et al. 2019).

Ophiopogon japonicus (Thunb.) Ker-Gawl (Liliaceae) is a traditional Chinese medicinal plant widely cultured in China, and its root, which was admitted as one of the functional food ingredients by the Ministry of Health of the People's Republic of China, is used as the main ingredient in many prescriptions. Over thousands of years, *O. japonicus* has shown curative effects on symptoms such as cough and phlegm (Chen et al. 2016). However, black spot caused by *A. alternata* is an important leaf disease during *O. japonicus* cultivation. At the early stage of infection, yellow or brown patches form at the apex of leaves, which then proceed toward the leaf base; at later stages, the spots turn black and putrescent. The disease rate can reach up to 90% in years with high temperatures and heavy rainfall, which seriously affects the yield and quality of *O. japonicus*.

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Keywords

black spot, *Ophiopogon japonicus*, *Alternaria alternata*, genomics, pathogenicity

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Table 1. Genome statistics of *Alternaria alternata* strain B3.

Features	<i>Alternaria alternata</i> B3
Genome size (Mb)	33.8
BUSCOs (%)	99.7
Number of contigs	76
N50 (Mb)	1.48
N90 (Mb)	0.48
GC content (%)	51.0
Protein-coding genes	12,680
Gene density (number of genes per Mb)	374
Average intron number	2
Average intron length (bp)	76
Average exon number	3
Average exon length (bp)	500
Mean gene length (bp)	1,663
Number of genes with intron	10,121
tRNA genes	141
Repeat content (%)	1.10
Carbohydrate-active enzymes	538
Secreted proteins	1,049
Small secreted cysteine-rich proteins	186
Secondary metabolites gene clusters	26

The mechanisms underlying the ability of *A. alternata* to effectively infect *O. japonicus* leaves and its interaction with host cells remain completely unknown. The genes involved in sporulation, stress response, nutrient absorption, and toxin biosynthesis of this pathogen are yet to be identified. For these reasons, we have fully sequenced the genome of the B3 strain of *A. alternata* that caused black spots on *O. japonicus*.

Genome sequencing and assembly. A total of eight *A. alternata* strains were isolated from blight infected leaves from Zhejiang Province, China. These isolates showed identical morphological characteristics and we randomly chose strain B3 as a representative strain. This strain was ascertained as the causal agent of leaf blight of *Ophiopogon japonicus* using Koch's postulates by inoculating it on healthy *O. japonicus* leaves and reisolating it from the inoculated leaves. Cultures of this fungal strain were maintained in liquid potato dextrose broth (PDB, extract of 200 g potato-boiled water and 20 g dextrose per liter) on a rotary shaker at 160 rpm at 25°C for 3 days. The genomic DNA of *A. alternata* strain B3 was extracted from the mycelia with the CTAB method (Möller et al. 1992). Short insert library (350 bp) was constructed and sequenced on an Illumina HiSeq 4000 platform (Nanjing Personalbio Co., Ltd.), generating 150 bp paired-end reads. Raw reads were trimmed of adapter sequences and low-quality reads using Trimmomatic v. 0.38 (Bolger et al. 2014). In total, 3.8 Gb clean reads were generated in the sequencing process, which corresponds to more than 100-fold of sequence depth. The genome was de novo assembled into 76 contigs (>1 kb) using SPAdes v 3.14.1 (Bankevich et al. 2012) and resulted in a genome size of 33.8 Mb with an N50 and N90 of 1.48 Mb and 0.48 Mb, respectively (Table 1). The completeness of the genome assembly was estimated by BUSCO v4.0.2 based on lineage-specific profile library dothi-deomycetes_odb10 (Simão et al. 2015). We found 3,773 out of 3,786 (99.7%) BUSCO groups in the *A. alternata* strain B3 genome (Table 1), indicating a very high degree of completeness.

The overall G + C content of the *A. alternata* strain B3 assembly was 51.0%. The tRNA and its secondary structure were predicted by tRNAscan-SE v 2.0 (Chan and Lowe 2019), revealing 141 tRNA genes (Table 1). Simple repeats, transposable elements, low-complexity sequences, and satellites were detected and annotated using RepeatMasker open 4.1.0 (<http://www.repeatmasker.org>). Results showed that repetitive sequences represented 1.1% of the genome (Table 1). The actual repeat content is expected to be larger as small contigs shorter than 1 kb (we filtered them out) may contain many repetitive sequences.

Genome annotation. To obtain high-quality gene calls, RNA-Seq was conducted with the same sample (mycelia harvested from PDB) and 6.0 Gb Illumina paired-end reads were obtained. RNA-seq data were then aligned to the genome using HISAT v 2.0.9 (Kim et al. 2019). Genome annotation was performed using the BRAKER version 2.0 pipeline (Hoff et al. 2019) based on GeneMark-ES v 4.48 (Borodovsky and Lomsadze 2011) and Augustus v

2.5.5 (Stanke et al. 2008), combining the RNA-seq-based gene prediction and ab initio gene prediction. A total of 12,680 protein-coding genes were predicted in the *A. alternata* strain B3 genome, with the average gene length 1,663 bp (Table 1). To functionally annotate the 12,680 genes in the *A. alternata* strain B3 genome, we classified their protein products into protein families using the Pfam, v 32.0 databases (El-Gebali et al. 2018). Results showed that 8,903 genes contained Pfam protein domains and they were assigned to 3,916 protein families.

Carbohydrate-active enzymes (CAZymes) play important roles in the breakdown of complex carbohydrates and are responsible for the acquisition of nutrients from plants for plant-associated fungi (Kubicek et al. 2014). The carbohydrate-active enzymes were annotated by the web-based dbCAN2 meta server (supported by ≥ 2 tools) (Zhang et al. 2018). A total of 538 putative CAZyme genes were identified in *A. alternata* strain B3, which included 270 glycoside hydrolases (GHs), 108 auxiliary activities (AAs), 90 glycosyl transferases (GTs), 39 carbohydrate esterases (CEs), 24 polysaccharide lyases (PLs), and 7 carbohydrate-binding modules (CBMs).

Pathogens can secrete a series of proteins that are deployed to the host-pathogen interface during infection, and secretome proteins play an important role in pathogenicity (Lo Presti et al. 2015). Putative extracellular proteins were identified as secreted proteins if they contained a signal peptide, located at the extracellular subcellular, and lacked transmembrane domains and glycosylphosphatidylinositol (GPI)-anchor signals. To identify secreted proteins, we used SignalP 5.0 (Armenteros et al. 2019) and TMHMM v 2.0 (Krogh et al. 2001) to predict the signal peptide and transmembrane domains (PredHel <2) and we excluded non-extracellular and GPI-anchored proteins by using targetP 1.1 (Emanuelsson et al. 2000) and GPI-SOM (Fankhauser and Mäser 2005). Approximately 8% of the total proteins (1,049) of *A. alternata* strain B3 were predicted to be secreted. The GO enrichment in molecular functions of the secreted proteins was then produced with the dcGO database (Fang and Gough 2013). “Hydrolase activity” was the most abundant molecular function of the secretome; other GO terms over-represented among the secreted proteins included “exopeptidase activity,” “transferase activity, transferring hexosyl groups,” “lipase activity,” “isomerase activity,” “exopeptidase activity,” and “UDP-glycosyltransferase activity.” All resulting secreted proteins that were shorter than 200 amino acids in length and contained at least four cysteine residues were considered as small secreted cysteine-rich proteins (SSCPs) (Wang et al. 2019). According to this standard, 186 of them were SSCP and their functions were largely unknown.

Secondary metabolites, especially fungal toxins, are believed to be involved in the pathogenicity of many plant pathogenic fungal species and can be described as potential virulence factors. Fungal secondary metabolite pathways were predicted using the online tool antiSMASH 4.0 (Blin et al. 2017). A total of 26 secondary metabolites biosynthesis clusters were identified in the *A. alternata* strain B3. These clusters comprise seven PKS clusters (annotated as T1PKS), three NRPS clusters, seven NRPS-like clusters, four terpene clusters, three PKS-NRPS cluster, one fungal-RiPP cluster, and one NRPS-indole cluster.

In the present study, we provide the draft genome sequence of *A. alternata* strain B3, a fungal species belonging to the family Pleosporaceae in the class Dothideomycetes. The sequence represents a useful source for future research on fungal comparative genomics studies.

Data availability. The whole genome shotgun project of *A. alternata* strain B3 has been deposited at DDBJ/ENA/GenBank under accession number JACBJF000000000 (BioProject PRJNA644072; BioSample SAMN15447420). The version described here is version JACBJF010000000. All the annotation data generated in this study have been deposited in the figshare repository at <https://doi.org/10.6084/m9.figshare.12612797>.

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