High-Quality Genome Assembly and Annotation Resource of *Botryosphaeria dothidea* Strain BDLA16-7, Causing Trunk Canker Disease on Chinese Hickory

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Genome Announcement

Botryosphaeria dothidea is a latent pathogen with global importance to woody plant health, which causes serious tree trunk cankers on Chinese hickory. To date, only one Illumina short-read-based genome assembly of strain CK16 is available for host Chinese hickory. To address this problem, we report a near telomere-to-telomere genome assembly of strain BDLA16-7 using Oxford Nanopore Technologies (ONT) sequencing. The almost chromosomal-level and well-annotated genome assembly will provide a valuable genetic resource for understanding of the infection mechanisms of *B. dothidea* in future.

Chinese hickory (*Carya cathayensis* Sarg.) is an endemic subtropical tree species of commercial importance, which is known for its distinctive fragrance and the high nutritional value of its nuts. However, nearly 90% of the Chinese hickory trees have been severely threatened by trunk cankers caused by *B. dothidea* (Zhang and Xu 2011). This disease occurs during late March to September with obvious latent infections, for which the pathogen primarily overwinters in the diseased trunks (Dai et al. 2017; Wang and Zhang 2019). Canker symptoms generally occur on trunks of hickory less than 2 m above the ground, and few lesions are observed on other parts such as twigs, branches, and nuts. *B. dothidea* is a worldwide fungal pathogen with global importance: damaging woody plants; infecting through wounds; breaching the outer bark and colonizing the phloem, vascular cambium, and xylem; and causing the weakening and decay of the woody hosts (Marsberg et al. 2017; Slippers et al. 2007).

Currently, the genome sequences of six *B. dothidea* strains with host plants including apple (Liu et al. 2016; Wang et al. 2018; Yu et al. 2021), pear (Hu et al. 2019), kiwifruit (Liang et al. 2021), and Chinese hickory (Rao et al. 2021) are publicly available in the NCBI Assembly Database. The assembly sizes of the six strains were similar, ranging from 44.17 to 51.76 Mb (Table 1). Of these strains, only one strain, named CK16, was an agent for trunk canker disease on Chinese hickory (Rao et al. 2021). The genome assembly of CK16 was assembled based on Illumina short-read sequencing technology, and has the highest contig numbers among these strains.

Here, we isolated *B. dothidea* strain BDLA16-7 from the trunk of a Chinese 'Linan' hickory tree with canker disease in Linan, Zhejiang Province, China. Its colony was initially pale white on potato dextrose agar (PDA) plates, then became black. The pycnidia were typically

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Keywords

Botryosphaeria dothidea, Chinese hickory, genome, trunk canker disease

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Table 1. Genome features of the *Botryosphaeria dothidea* strains^a

Features	BDLA16-7	CK16	sdau11-99	LW030101	PG45	LW-Hubei	PTZ1
Host	Chinese hickory	Chinese hickory	Apple	Apple	Apple	Pear	Kiwifruit
Sequencing technology	ONT + Illumina	Illumina	PB + Illum	Illumina	Illumina	PB + Illum	PB + Illum
Reference (PubMed ID)	This study	33761770	33258431	27789635	30622881	32647612	Liang et al. 2021
Assembly accession ^b	GWHBEO00000000	ASM1515935	ASM1150312	ASM171744	ASM401626	ASM1106463	ASM1334390
Assembly size (Mb)	46.05	44.39	51.76	47.39	44.17	46.35	44.45
Contig number	15	1,303	88	1,251	423	68	28
Contig N ₅₀ (Mb)	3.87	0.52	4.04	0.21	0.35	2.72	2.68
Contig L ₅₀	5	26	6	65	37	7	7
Average contig length (Mb)	3.07	0.03	0.59	0.04	0.11	0.95	1.59
Maximum contig length (Mb)	6.19	1.48	6.15	1.26	1.15	4.86	3.62
GC content (%)	54.45	54.60	52.93	53.09	54.60	54.30	54.59
Repeat sequence (%) ^c	7.96	5.29	13.96	12.58	5.64	9.40	6.68
BUSCO completeness (%) ^d	99.00	97.83	97.13	97.83	97.89	97.71	97.82
Protein-coding genes	12,812	13,856	14,118	10,411	15,661	14,091	10,415
BDLA16-7 unique genes	–	261	1,745	1,539	194	357	246

^a Abbreviations: ONT = Oxford Nanopore Technologies, PB + Illum = PacBio + Illumina, and BUSCO = benchmarking universal single-copy orthologs.

^b Assembly of BDLA16-7 is available at the Genome Warehouse (GWH) (<http://ngdc.cncb.ac.cn/gwh/>) while other assemblies are available at the NCBI Assembly database.

^c Repeats were conducted by RepeatMasker V4.1.2 with the de novo repeat library generated from assembly of BDLA16-7.

^d The completeness of genome assemblies were assessed by BUSCO v5.1.2 at the Ascomycota level ($n = 1,706$).

solitary, gray-black, globular, and covered by mycelia. Its genomic DNA and messenger RNA were extracted from 10-day-old mycelium cultivated on PDA media. Its genome and transcriptome were sequenced by the ONT PromethION sequencing platform and Illumina HiSeq4000 sequencing platform, respectively, at Biomarker Technologies Co., Ltd. (Beijing, China). Finally, we obtained 5.89-Gb ONT long reads (approximately 108×, N₅₀ 20.41 kb) for genome assembling and 7.06-Gb Illumina short reads (approximately 156×, 2 × 150 bp) for genome assembly polishing and gene annotation (Table 1).

The de novo genome assembler suit NextDenovo v2.4.0 and NextPolish v1.3.1 (both developed by NextOmics; <https://github.com/Nextomics>) were employed to generate a genome assembly of high accuracy and continuity. First, a draft genome assembly was assembled by NextDenovo using ONT long reads (seed reads ≥ 29,488 bp); then, it was polished by NextPolish using both ONT long reads and Illumina short reads. Finally, we obtained a 46.05-Mb genome assembly (GC content 54.45%) consisting of 15 contigs with a contig N₅₀ of 3.87 Mb (L₅₀ = 5). The average contig length was 3.07 Mb and the maximum contig length was 6.19 Mb (Table 1). Compared with the previously reported genome assembly of strain CK16, genome assembly of BDLA16-7 was a little bigger in size (46.05 versus 44.39 Mb), reduced 98.85% (15 versus 1,302) in number of contigs, and, thus, increased approximately 100-fold in average contig length (3.07 versus 0.03 Mb), approximately 7-fold (3.87 versus 0.52 Mb) in contig N₅₀, and approximately 4-fold (6.19 versus 1.48 Mb) in the maximum contig length (Table 1). Compared with the genome assemblies of other host strains, the genome assembly of BDLA16-7 had the minimum contig number and the largest contig N₅₀, as well as the maximum contig length (Table 1).

The completeness of genome assembly BDLA16-7 was assessed by benchmarking universal single-copy orthologs (BUSCO v5.12; <https://busco.ezlab.org/>) at Fungi and Ascomycota levels. It contained 753 (99.34%) complete (749 single and 4 duplicated), 3 fragmented, and 2 missing orthologs at the Fungi level ($n = 758$), and 1,689 (99.00%) complete (1,678 single and 11 duplicated), 4 fragmented, and 13 missing orthologs at Ascomycota level ($n = 1,706$) (Fig. 1; Table 1). Compared with the BUSCO assessment results of other reported genome assemblies at the Ascomycota level, the completeness of genome assembly BDLA16-7 was the best one (Table 1). Also, the genome assembly completeness was valued by telomere repeats (5'-TTAGGG-3' and 5'-CCCTAA-3') at the start and end of contigs. Of 15 contigs, 8 and 6 contigs were found at the start or end, respectively, with telomere repeats, and 3 contigs had both ends with telomere repeats, which indicated that these contigs were reached at the telomere-to-telomere (Miga et al. 2020) chromosomal level.

Repeats were masked before gene prediction by RepeatMasker v4.1.2 (<http://www.repeatmasker.org/>) with a de novo repeat library generated by RepeatModeler v2.01 (<http://www.repeatmasker.org/RepeatModeler/>). In total, 7.96% of repeats (mainly including approximately 3% long terminal repeat and approximately 2% long interspersed retrotransposable

Fig. 1. Completeness of genome assembly and predicted genes were assessed by benchmarking universal single-copy orthologs (BUSCO v5.1.2) at the Fungi and Ascomycota levels.

element) were identified in the genome assembly of BDLA16-7. It is a little higher than repeats of strains CK16 (5.29%), PG45 (5.64%), and PTZ1 (6.68%) but lower than repeats of strains sdau11-99 (13.96%), LW030101 (12.58%), and LW-Hubei (9.40%) (Table 1). The repeat-masked genome assembly of BDLA16-7 was used for gene prediction, and identified 12,812 protein-coding genes by BRAKER2 (Brůna et al. 2021), which integrated ab initio gene prediction from AUGUSTUS v3.4.0 (Stanke et al. 2008), as well as evidence from RNA-sequencing data and fungal homologous proteins (fungi_odb10) (<https://busco-data.ezlab.org/v5/data/lineages/>). Compared with other strains, the number of protein-coding genes of BDLA16-7 was in the middle (12,815 versus approximately 10,411 to 15,661), which may have been caused by different gene prediction methods. Using BLASTn search against other genome assemblies, the number of isolate-specific genes in BDLA16-7 ranged from 194 to 1,745 (Table 1). The BUSCO gene completeness assessment showed that the predicted gene set of BDLA16-7 included 739 (97.50%) and 1,650 (96.72%) complete orthologs at the Fungi and Ascomycota levels, respectively (Fig. 1).

The gene functional annotation was conducted by InterProScan v5.51-85.0 (<https://github.com/ebi-pf-team/interproscan>) and eggNOG-mapper v2 (<http://eggno-mapper.embl.de/>). In total, 10,272 (80.16%) genes were assigned a annotation from either Pfam (9,231), gene ontology (4,027), Kyoto Encyclopedia of Genes and Genomes (4,741), or eukaryotic orthologous groups (9,998). We identified a set of pathogenicity-related genes, including 3,642 pathogen–host interaction genes (PHI-base v4.12; <http://www.phi-base.org/>), 250 carbohydrate active enzymes (dbCAN2; <https://bcb.unl.edu/dbCAN2/>), and 252 cytochrome P450 enzymes. Furthermore, 752 putative secreted proteins were identified by SignalP v5.0 (Almagro Armenteros et al. 2019) and TMHMM v2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>), following our previous method (with signal peptide but without any transmembrane domain in mature protein) (Bao et al. 2017).

Fungal secondary metabolites such as fumonisins and deoxynivalenol were toxic to plants and, thus, played an important role in fungal infection and plant disease development (Diamond et al. 2013; Iqbal et al. 2021). The genes related to products of any secondary metabolite were fused as secondary metabolite biosynthesis gene clusters (SMBGCs) (Keller 2019). In total, 63 SMBGCs, including 18 nonribosomal peptide synthetases (NRPSs), 13 NRPS-like, 11 terpenes, 19 type I polyketide synthases, and 2 β -lactones were identified by the fungal version of antiSMASH v5.2.0 (Blin et al. 2019).

The whole-genome sequence data reported in this article have been deposited in the Genome Warehouse (GWH) (<https://ngdc.cnbc.ac.cn/gwh/>) in the National Genomics Data Center, China National Center for Bioinformation (CNCB-NGDC Members and Partners 2021), under accession number GWHBEO00000000. The raw sequence reads also are publicly accessible at Genome Sequence Archive (<https://ngdc.cnbc.ac.cn/gsa/>) in CNCB-NGDC under accession number CRA004612 (BioProject PRJCA005744).

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