Genome Sequence Resource of the Causal Agent of Persimmon Anthracnose, *Colletotrichum horii* Strain SD010 from China

Jie Wang, Xianmei Yu, Chengxiang Ai,[†] and Rui Gao[†]

Shandong Institute of Pomology, Taian, 271000, China

Abstract

Colletotrichum horii is a main causal agent of persimmon (*Diospyros kaki*) anthracnose and is distributed widely in persimmon-producing areas of the world. Here, we report the first high-quality draft genome sequence of *C. horii* strain SD010. This will provide a reference for understanding adaptive evolution of genome structure, genes, and population diversity among members of the *C. gloeosporioides* species complex, and also help in understanding the mechanisms of host–pathogen interactions and improve management strategies of anthracnose.

Persimmon (*Diospyros kaki* Thunb.), which presumably originated in China, is known as the “oriental product of beauty” due to its attractive fruit (Luo and Wang 2008). Based on Food and Agriculture Organization data (FAO 2019), the persimmon cultivated area in China in 2019 was 914,883 ha, with an estimated production of 3,207,516 tons of edible fruit, accounting for 75% of the world total production.

Anthracnose is a devastating disease found widely in the persimmon-producing countries of the world (Carraro et al. 2019; Hassan et al. 2018; Wang et al. 2016; Williamson and Sutton 2010). It causes defoliation, new shoot blight or stem-end wither, followed by premature fruit drop and plant death, which seriously affect fruit productivity and quality (Hassan et al. 2018; Wang et al. 2016; Xie et al. 2010). Thus far, to our knowledge, nine *Colletotrichum* spp. (*Colletotrichum acutatum*, *C. coccodes*, *C. fructicola*, *C. gloeosporioides* sensu stricto, *C. horii*, *C. karsti*, *C. melonis*, *C. nymphaeae*, and *C. siamense*) have been reported to cause persimmon anthracnose (Carraro et al. 2019; Hassan et al. 2018; Jang et al. 2019; Wang et al. 2016; Williamson and Sutton 2010; Xie et al. 2010). Among them, whole or draft genome sequences of all species except *C. horii*, *C. melonis*, and *C. nymphaeae* are available in the GenBank (<https://www.ncbi.nlm.nih.gov/genome>). As the primary pathogen causing persimmon anthracnose, *C. horii*, initially assumed as *Gloeosporium kaki* (Hori 1910) and *C. gloeosporioides* (Von Arx 1970), was later identified as a distinct species of the *C. gloeosporioides* complex, which is composed of more than 20 closely related species, based on morphological characteristics and multilocus phylogenetic analysis (Fu et al. 2019; Weir and Johnston 2010; Weir et al. 2012). Currently, *C. horii* has been reported in different countries (Hassan et al. 2018; Weir and Johnston 2010; Xie et al. 2010) but little is known about the species at the genomic level. In this study, the genome of *C. horii* strain SD010 was sequenced, providing insight into the pathogenic mechanism and serving as a valuable resource for comparative genomics of fungal pathogens of persimmon anthracnose.

C. horii SD010 was isolated from the infected twigs (with necrotic lesions) of persimmon in Qingzhou, Shandong Province, China (36°68' N; 118°47' E) in 2017. The identification and pathogenicity of SD010 was confirmed by both the morphological and phylogenetic analyses of five loci (internal transcribed spacer, glyceraldehyde 3-phosphate dehydrogenase, actin,

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Keywords

Colletotrichum, *Diospyros kaki*, disease management, forest, fruit, fungi, tree fruits

[†]Corresponding authors: C. X. Ai; chengxiang_1975@163.com, and R. Gao; gaorui368@163.com

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Table 1. Complete genome summary of *Colletotrichum horii* strain SD010

Variables	Statistics
Assembly size (bp)	75,154,806
Number of contigs	53
GC content (%)	46.44
Contig N ₅₀ (bp)	2,568,590
Contig N ₉₀ (bp)	594,756
Longest sequence length (bp)	7,214,349
BUSCO completeness ^a	98.1%
Number of predicted genes	15,112
Average gene length (bp)	1651.77
Average protein length (aa)	463.09

^a Benchmarking universal single-copy orthologs.

β-tubulin 2, and chalcone synthase 1), and an inoculation test on healthy persimmon branches and reisolation test, respectively (unpublished). Total DNA was extracted by using an E.Z.N.A. High-Performance Fungal DNA Kit (Omega Bio-tek Inc., Guangzhou, China) from fungal mycelia collected from single-spore cultures. The genome of *C. horii* SD010 was sequenced using both PacBio Sequel II System (Pacific Biosciences [PacBio], Menlo Park, CA, U.S.A.) and Illumina HiSeq 4000 system (paired-end sequencing; 2 × 150 bp) (Illumina, San Diego, CA, U.S.A.). Genomic DNA was sheared into fragments (15 kb) using a g-TUBE (Covaris M220 Focused-ultrasonicator; Thermo Fisher Scientific, Inc. Woburn, MA, U.S.A.) and a SMRTbell Express Template Prep Kit 2.0 (PacBio) was used to construct the PacBio Sequel II library, while the Illumina TruSeq Nano DNA Sample Prep Kit (300 bp insert size) was used to generate a library for Illumina sequencing.

PacBio sequencing generated a total of 1,426,915 filtered subreads, with an average length of 8.37 kb, an N₅₀ of 9.72 kb, and coverage of 160×. Illumina sequencing generated approximately 18,600,018 clean reads (99.84% of the total reads), with 40× coverage. PacBio subreads were assembled using FALCON v0.3.0 (Chin et al. 2016) into contigs. The contigs were further corrected by the Illumina short reads using Pilon v1.16 (Walker et al. 2014). Finally, a 75,154,806-bp assembled genome containing 53 scaffolds was obtained, with a GC content of 46.4%, N₅₀ of 2,568,590 bp, L₅₀ of 8, and the largest scaffold size of 7,214,349 bp (Table 1). Among the published genomes of the *C. gloeosporioides* species complex, the present genome is the largest one compared with the *C. gloeosporioides*, *C. fructicola*, *C. siamense*, and *C. asianum* genomes (Alkan et al. 2013; Gan et al. 2013; Liang et al. 2018; Meng et al. 2019, 2020). Furthermore, the completeness of the genome assembly was evaluated using benchmarking universal single-copy orthologs (BUSCO) v3.0.2 based on the lineage-specific profile library sordariomyceta_odb9 (Simão et al. 2015). The analysis revealed that the assembly had a BUSCO completeness of 98.1% (97.9% complete and single-copy BUSCOs, 0.2% complete and duplicated BUSCOs) and 0.8% missing BUSCOs from a total of 3,725 BUSCO groups.

Analysis of repeat sequences was performed by using Repeat Masker v4.0.5 (<http://www.repeatmasker.org>) based on the filtered custom RepeatModeler library generated by de novo-based method, and de novo repeats detection was performed using the Repeat Modeler v1.0.7 pipeline with default settings (Saha et al. 2008). Approximately 21.42% of sequences were identified as repeat sequences. Then, the protein-coding genes were predicted using the homology-based prediction program Exonerate v2.4.7 (Slater and Birney 2005) and two ab initio gene annotation programs, Augustus v3.3.3 (Stanke et al. 2008) and GeneMark-ES v4.38 (Lomsadze et al. 2005). A self-training algorithm was used to predict genes by GeneMark-ES and the gene models of the *C. horii* SD010 predicted by Exonerate and GeneMark-ES were used to train Augustus. The predicted results were further integrated using EvidenceModeler v1.1.1 (Haas et al. 2008), and 15,112 protein-coding genes were identified. Among these, 2,101 secretory protein-coding genes, 26 laccases, 1,131 peptidases, and 518 transcription factors were identified with the databases FunseqKB2, LccED v6.4, MEROPS v12.1, and FTFD v1.2, respectively, by BLASTP (Meinken et al. 2014; Park et al. 2008a; Rawlings et al. 2014; Sirim et al. 2011). Among the secretomes, 536 proteins (25.5%, less than 300 amino acids) were small secreted proteins, which were considered as candidate secreted effectors (Kim et al. 2016). Other previously reported candidate virulence

genes, including putative cytochrome P450s, carbohydrate active enzymes (CAZymes), and secondary metabolite synthetases, were also identified from the genome of SD010 in this study (Huang et al. 2019; Kleemann et al. 2012; Liang et al. 2018; Meng et al. 2020). In all, 342 fungal cytochrome P450s and 986 CAZymes were identified using FCPD v1.2, and dbCAN v6.0 databases, with an *e*-value < 1e-15 (Park et al. 2008b; Yin et al. 2012). In total, 61 secondary metabolite clusters (23 polyketide synthase clusters, 20 nonribosomal peptide-synthetase clusters, 10 terpene clusters, 6 indole clusters, 1 β -lactone cluster, and 1 fungal-PiPP cluster) were identified in the *C. horii* SD010 genome using antiSMASH v4.0 (Blin et al. 2017). Furthermore, a whole-genome comparison with other species of the *C. gloeosporioides* complex using MUMmer v3.23 revealed a similar association of *C. horii* with *C. gloeosporioides* Cg-14 (77.7%), *C. fructicola* Nara gc5 (77.1%), and *C. fructicola* 1104-7 (75.7%) (Alkan et al. 2013; Gan et al. 2013; Liang et al. 2018). The ortholog clustering analysis using OrthoFinder v2.2.6 (Emms and Kelly 2015) showed 3,377 orthogroups specific to the species complex, including 1,168 shared among the closed four strains and 60 shared between *C. horii* SD010 and *C. gloeosporioides* Cg-14 (67 genes in SD010 and 65 genes in Cg-14). Meanwhile, 617 lineage-specific genes were identified in SD010 (unclustered or clustered only with proteins from the same genome). Among them, 549 were orphan genes, and the top three enriched Pfam domains were related to oxidoreductase (26 genes), cation binding (19 genes), and mycotoxin metabolic process (3 genes).

To conclude, this is the first report of a whole-genome sequence of *C. horii*, which provide a reference for the classification and population diversity analysis of the *C. gloeosporioides* species complex. The sequenced genome will also help in understanding the host-pathogen interaction. The genome assembly of *C. horii* SD010 was deposited at GenBank with the accession number JAGTUO000000000 (BioProject: PRJNA725041; BioSample: SAMN18868286). The version described in this article is version JAGTUO010000000. *C. horii* SD010 has been deposited in the China Forestry Culture Collection Center with the preservation number CFCC 55691.

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