Genome Sequence of *Venturia carpophila*, the Causal Agent of Peach Scab

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Abstract

Venturia carpophila, the causal agent of scab disease on peach, is a host-specific fungus that is widely distributed around the world, including China. In our previous study, samples were collected from 14 provinces in China, and 750 isolates were obtained by single-spore separation. Here, we reported the first highly contiguous whole-genome sequence (35.87 Mb) of the *V. carpophila* isolate ZJHZ1-1-1, which included 33 contigs with N₅₀ value of 2.01 Mb and maximum contig length of 3.39 Mb. The high-quality genome sequence and annotation resource will be useful to study the fungal biology, pathogen-host interaction, fungicide resistance, characterization of important genes, population genetic diversity, and development of molecular markers for genotyping and species identification.

Genome Announcement

Peach scab, caused by *Venturia carpophila* (syn. *Cladosporium carpophilum*, *Fusicladosporium carpophilum*), is a common and widespread fungal disease in peach-growing regions in China (Chen et al. 2018; Schubert and Braun 2004). *V. carpophila* is a pathogenic fungus that belongs to the phylum Ascomycota, order Pleosporales in family Venturiaceae. In general, *Venturia* species are host-specific pathogens and each can only infect one plant species or close relatives in a given host family (González-Domínguez et al. 2017). It was reported that *V. carpophila* could also cause apricot scab and plum scab (Bensch et al. 2003; Kim et al. 2017; Schnabel and Layne 2004). Although its sexual stage has been described on overwintered apricot leaves in Australia (Fisher 1961), it has not been found on peach. Peach scab generally produces dark brown to black freckles, spots, or scabs of variable size on the fruit surface. Lesions on fruits are oval or circular, approximately 2 to 5 mm in diameter (Chen et al. 2014; Scherm et al. 2008). Leaf symptoms are less common and are not obvious but occur as yellowish blotches that turn grayish as spores are produced, with lesions most often on the back of the leaf (Chen et al. 2014). The disease is particularly

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*The e-Xtra logo stands for “electronic extra” and indicates there is supplementary material published online.

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severe in temperate climate regions characterized by high humidity, cool spring weather, and poor air circulation. Fruit infection by *V. carpophila* can significantly reduce both fruit yield and quality and result in considerable economic losses (Schnabel and Layne 2004).

To date, eight members of the genus *Venturia* have been sequenced and there are more than 102 genome assemblies available for *Venturia* spp., including *V. effusa* (GCA_007735645.1), *V. inaequalis* (QFBF000000000), *V. asperata* (QWWL000000000), *V. pirina* (JEMP000000000), *V. nashicola* (VCHV000000000), *V. aucupariae* (QWZN000000000), *V. oleaginea* (JABMLP000000000), *V. carpophila* (MECS000000000), the causal agents of pecan scab (*V. effusa*), apple scab (*V. inaequalis* and *V. asperata*), pear scab (*V. pirina* and *V. nashicola*), sorbus scab (*V. aucupariae*), olive leaf scab (*V. oleaginea*), and peach scab (*V. carpophila*) (Bock et al. 2016; Chen et al. 2017; Cooke et al. 2014; Jaber et al. 2020; Johnson et al. 2019; Le Cam et al. 2019; Lichtner et al. 2020; Shiller et al. 2015). Among them, the best studied and most economically important species is *V. inaequalis*, with 88 genome assemblies reported. *V. effusa* genomic sequence is of highest stitching quality and is also the first chromosomal-level assembly among *Venturia* species (Winter et al. 2020). In contrast, fewer studies have been performed for other related *Venturia* species. In *V. carpophila*, only one United States isolate genome was deposited in the GenBank database (accession MECS000000000). The complete genome sequences of *V. carpophila* isolates from China remain to be determined and the genomic differences between Chinese isolates and other isolates are still unclear. In addition, the first reported genome assembly from the United States seems to be highly fragmented (657 scaffolds) (Chen et al. 2017). Here, we report the complete whole-genome sequence for the Chinese *V. carpophila* isolate ZJHZ1-1-1, aiming to provide better *V. carpophila* genome assembly and annotations.

Peach scab samples were collected from 14 provinces in China and 750 isolates were obtained, as described previously (Zhou et al. 2021). Among them, the isolate ZJHZ1-1-1 grew fast on oatmeal agar medium and showed high virulence in field inoculation experiments and was thus used for sequencing. Briefly, asexually produced conidia were scraped from a single lesion on the fruit surface using a scalpel and was then spread on the water agar (20 g of agar per liter) plates. Using a modified microscope (Goh 1999), a single spore was picked with a glass needle from water agar plates and was then transferred to potato dextrose agar (200 g of potato, 20 g of dextrose, and 20 g of agar per liter) amended with lactic acid (0.50 ml per liter), streptomycin (0.20 g per liter), tetracycline (0.05 g per liter), and chloramphenicol (0.05 g per liter) (Chen et al. 2018). Mycelial suspension of *V. carpophila* was transferred to 50 ml of antibiotic-amended malt extract broth (30 g of malt extract and 5 g of peptone per liter) in an Erlenmeyer flask in the dark, with shaking at 21°C for 10 days, to culture sufficient quantities of mycelium for DNA extraction. Genomic DNA was extracted with the modified sodium dodecyl sulfate method (Hu et al. 2004) and was quantified with a Qubit 2.0 Fluorometer (Thermo Scientific). For isolate identification, we used primer pairs ITS4/ITS5 and LROR/LR5 to amplify the internal transcribed spacer (ITS) region and the complete large subunit ribosomal RNA gene (*LSU*), respectively (Vilgalys and Hester 1990; White et al. 1990). The ITS and *LSU* sequences of ZJHZ1-1-1 have been deposited in GenBank (MN958609 and MT772296). The whole genome of ZJHZ1-1-1 was sequenced using PacBio Sequel and Illumina NovaSeq PE150 platforms at the Beijing Novogene Bioinformatics Technology Co., Ltd. PacBio Sequel platform libraries for single-molecule real-time (SMRT) sequencing was constructed with an insert size of 20 kb, using the SMRTbell™ Template kit, version 1.0. Illumina NovaSeq platform libraries for sequencing were generated with an insert size of 350 kb, using a NEBNext Ultra DNA Library Prep kit.

By the variant Caller module of the SMRT Link software, the arrow algorithm was used to correct and count the variant sites in the preliminary assembly results. SMRT cell data were generated and low-quality reads were filtered with SMRT link software (v5.0.1) (Ardui et al. 2018; Reiner et al. 2018). The preassembly was polished, using the arrow algorithm, by mapping raw long reads back to the preassembled genome. As a result, 14.92 Gb of long-read data were obtained, and the preliminary assembled genome length was 36 Mb. After the preassembled genome was polished, a genome sequence of 35.87 Mb (416× genome coverage) was assembled into 33 contigs (N_{50} value of 2.01 Mb) with maximum and average contig lengths of 3.39 and 1.09 Mb, respectively, and GC content of 47.75% (Table 1). Based on the reference of *V. effusa* isolate FRT5LL7 (Winter et al. 2020), we anchored and sorted our contigs to chromosomes by Chromosomer software (Tamazian

et al. 2016). We concluded that *V. carpophila* had at least 19 chromosomes, which was similar to the number of identified for *V. effusa* isolate FRT5LL7 (20 chromosomes) (Winter et al. 2020). The completeness of the ZJHZ1-1-1 assembly was assessed using BUSCO v3.0 (Waterhouse et al. 2018). Genomic evaluation with the ascomycota ($n = 1,315$) and fungal dataset ($n = 290$) showed 97.5 and 97.6% completeness, respectively (Simão et al. 2015). Genomic annotation predicted the coding genes, repetitive sequences, and noncoding RNAs. We used the Augustus 2.7 program (Stanke et al. 2008) to retrieve the related coding genes, integrating the RNA-seq data of the isolate ZJHZ1-1-1. A total of 10,080 protein-coding genes were found by combining RNA-seq and de novo strategies, which is similar to the 10,820 genes predicted for *V. effusa*, of which 8,508 have at least one functional annotation (Winter et al. 2020). The circular representation of the complete genome of *V. carpophila* isolate ZJHZ1-1-1 is shown in Supplementary Figure S1. The interspersed repetitive sequences were predicted using RepeatMasker (Saha et al. 2008). SINE and LINE (short and long interspersed nuclear elements) repetitive sequences were 0.09%. The tandem repeats were analyzed by the TRF (tandem repeats finder) (Benson 1999), which were 1%. Seventy-five transfer RNA (tRNA) genes were predicted by the tRNAscan-SE (Lowe and Eddy 1997), having a total length of 6,928 bp. We used seven databases to predict gene functions, including Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, Clusters of Orthologous Groups, Non-Redundant Protein Database databases, Transporter Classification Database, P450, and Swiss-Prot. Following a previous pipeline (Bao et al. 2017), gene annotation analysis revealed that 894 proteins had putative secretion signals and 699 candidate secreted proteins were identified. In addition, 214 proteins were associated with production of secondary metabolites (Table 1).

The draft genome sequence and annotation of *V. carpophila* isolate JP3-5 from the United States has been reported previously (MECS00000000) (Chen et al. 2017), the genome was sequenced with Illumina MiSeq platform, and the genome sequence of 36.92 Mb ($263\times$ genome coverage) was assembled into 657 contigs (N_{50} value of 0.31 Mb) with maximum and average contig lengths of 1.45 and 0.06 Mb, respectively. Based on the development of bioinformatics and the deeper sequencing compared with the *V. carpophila* JP3-5 genome assembly, *V. carpophila* ZJHZ1-1-1 genome assembly resulted in a higher-quality genome assembly and annotations. For example, the ZJHZ1-1-1 genome was assembled into 33 contigs (N_{50} value of 2.01 Mb) with maximum and average contig lengths of 3.39 and 1.09 Mb, respectively. The details of comparison are shown in Table 1. Although, other *Venturia* genomes, including *V. carpophila*, have been completely sequenced, ZJHZ1-1-1 represents the first fully sequenced *V. carpophila* genome from China. The outcome of this study will be a valuable resource for scientists working in the comparative genomics of scab pathogens. This will provide insights into the epidemiology, evolution, and population biology of the genus *Venturia*. Further, it will also be a useful resource for functional analysis of many important genes associated with fungicide resistance and pathogenicity.

Table 1. Whole-genome assembly statistics for <i>Venturia carpophila</i> isolates ZJHZ1-1-1 and JP3-5		
Assembly parameters	ZJHZ1-1-1	JP3-5
Sequencing platform	PacBio Sequel and Illumina NovaSeq PE150	Illumina MiSeq
Assembly method	SMRT Link v5.0.1	A5-MiSeq v.1.0
Assembly level	Contig	Scaffold
Genome size	35.87 Mb	36.92 Mb
Sequencing coverage	$416\times$	$263\times$
Number of contigs/scaffold	33	657
Average contig/scaffold length	1.09 Mb	0.06 Mb
Contig N_{50}	2.01 Mb	0.31 Mb
Maximum contig length	3.39 Mb	1.45 Mb
GC content %	47.75	47.40
Genes	10,080	8,352
Candidate secreted proteins	699	390
Annotated genes	8,508	4,632
GenBank ID	JACTAK000000000	MECS00000000

Data Availability

The ITS and *LSU* sequences of ZJHZ1-1-1 have been submitted to the GenBank nucleotide database under accession numbers MN958609 and MT772296, respectively. Full genome-assembly data and genome annotations were deposited in the National Center for Biotechnology Information database under BioProject PRJNA642010, BioSample SAMN15375461, and SRA accessions SRR12095801 and SRR12095802 for Illumina and PacBio data, respectively. This whole-genome shotgun project has been deposited in the DDBJ/ENA/GenBank database under accession JACTAK000000000.

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Author-Recommended Internet Resource

Arrow algorithm: <https://github.com/pacificbiosciences/genomicconsensus>

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