Genome Sequence Resource for *Colletotrichum scovillei*, the Cause of Anthracnose Disease of Chili

Jianfei Huo,¹ Yafei Wang,² Yongjuan Hao,¹ Yurong Yao,¹ Yong Wang,¹ Kai Zhang,² Xinqiu Tan,³ Zhiqiang Li,^{2,†} and Wanli Wang^{1,†}

¹ Institute of Plant Protection, Tianjin Academy of Agricultural Sciences, 300381 Tianjin, China

² State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, 100193 Beijing, China

³ Institute of Plant Protection, Hunan Academy of Agricultural Sciences, 410125 Changsha, Hunan Province, China

Abstract

Colletotrichum species cause anthracnose disease on the economically important spice crop chili. A total of 24 *Colletotrichum* species are known to infect chili and cause anthracnose. *C. scovillei* belongs to the *C. acutatum* species complex, and it shows greater aggressiveness than other species, particularly in the case of inoculation onto the nonwounded fruits of chili plants. The current work introduces an initial Illumina-Nanopore hybrid draft genome for *C. scovillei* TJNH1 together with the related annotations. Knowledge of this genome sequence provides an important reference genome of *C. scovillei* and will help further understand the pathogenic mechanism of *C. scovillei* to plant.

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Keywords

Colletotrichum scovillei, chili, genome, Nanopore

Genome Announcement

Chili is one of the widely consumed crops in the world. Anthracnose is a severe disease of chili affecting both fruit and seed quality. It can cause serious damage to the crop not only in the field but also during postharvest storage. *Colletotrichum* spp. are fungal pathogens that cause anthracnose. So far, 24 *Colletotrichum* species have been reported to cause chili anthracnose (de Silva et al. 2019; Diao et al. 2017; Liu et al. 2016; Mongkolporn and Taylor 2018). *C. scovillei* belongs to the *C. acutatum* species complex, which contains at least 34 species (Baroncelli et al. 2017; Bragança et al. 2016; Damm et al. 2012; De Silva et al. 2017, 2019; Jayawardena et al. 2016). In recent years, *C. scovillei* has been reported to cause anthracnose on chili in different countries (Caires et al. 2014; Kanto et al. 2014; Oo et al. 2017; Shin et al. 2019a). Liu et al. (2016) first isolated *C. scovillei* from pepper fruits in the Sichuan province of China and confirmed that *C. scovillei* was more pathogenic than six other *Colletotrichum* species (*C. gloeosporioides*, *C. simense*, *C. fruticola*, *C. truncatum*, *C. brevisporum*, and *C. sichunensis*). Other scholars subsequently isolated *C. scovillei* from chili fruits in different areas in China (Diao et al. 2017; Gao et al. 2017). Although *C. scovillei* is prevalent and causes severe damage to the chili crops in some countries (Kanto et al. 2014; Oo et al. 2017), there is no sufficient information on the genome of *C. scovillei*. Hence,

†Corresponding authors: W. Wang; wlwang122@163.com and Z. Li; zhiqiangdo_771@163.com

*The *e-Xtra* logo stands for “electronic extra” and indicates supplementary materials are published online.

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Table 1. Genome assembly statistics of *Colletotrichum scovillei* TJNH1

Variables	Statistics
Genome assembly size (Mb)	52.03
Number of scaffolds	16
Scaffold N ₅₀ (bp)	4,871,440
Largest scaffold (bp)	6,315,016
GC content (%)	51.8
BUSCO completeness	98.62%
Number of genes	13,419
Number of signal peptides	1,828
Number of carbohydrate-active enzymes	934
Number of secreted proteins	1,357

sequencing the genome is an important step toward understanding the pathogenic mechanism of *C. scovillei*.

The strain TJNH1 was isolated from infected chili fruits from Tianjin City, China. Based on morphological characteristics and multigene phylogenetic analyses of *ITS* (Gardes and Bruns 1993; White et al. 1990), *CAL* (O'Donnell et al. 2000), *CHS-1* (Carbone and Kohn 1999), *ACT* (Carbone and Kohn 1999), *GAPDH* (Guerber et al. 2003), and *TUB2* (Woudenberg et al. 2009), TJNH1 was identified as *C. scovillei*. Genomic DNA was extracted from fresh fungal tissue samples using an E.Z.N.A. HP fungal DNA kit (Omega Bio-Tek). The Qubit and Thermo Scientific NanoDrop 2000 systems were utilized to evaluate DNA purity and content, and 0.35% agarose gel electrophoresis was carried out to test the DNA integrity. We recycled large segments of DNA using the BluePippin system and prepared a library using the ligation sequencing kit (SQK-LSK109; Oxford Nanopore Technologies) and NEB Next FFPE DNA repair mix kit. The high-quality library was sequenced on the ONT PromethION platform (ONT) using the ligation sequencing kit (SQK-LSK109) and the flow cell priming kit (EXP-FLP002). Subreads that were of low quality and too short were removed, and the remaining subreads were used for genome assembly, using Canu v1.5 software (Koren et al. 2017). All settings were the default except for the following: genomeSize = 50 m, rawErrorRate = 0.5, correctedErrorRate = 0.12, corOutCoverage = 100. To obtain more accurate genome data, Illumina short reads obtained using the Nova-Seq 6000 system were used to correct the sequencing errors generated by Oxford Nanopore PromethION sequencing, using Pilon software (Walker et al. 2014) with default settings, except the mindepth and iteration parameters were set to 0.1 and 5, respectively.

Thereafter, the completeness of the genome assembly was assessed using the BUSCO v3.0 program (Simão et al. 2015). This analysis indicated that the completeness of the TJNH1 genome assembly was 98.62% and there were 286 complete BUSCOs, two missing BUSCOs, two fragmented BUSCOs, and 290 total lineage BUSCOs. The genome had a total assembly length of 52,032,179 bp and contained 16 scaffolds, with an N₅₀ value of 4,871,440 bp and a GC content of 51.8%. The largest scaffold was 6,315,016 bp and other characteristics of this genome were summarized in Table 1. The mitochondrial genome was identified by performing blast searches in the National Center for Biotechnology Information (NCBI) database. The sequences on scaffold 16 showed high similarity (99.99%) to mitochondrial genes from *Colletotrichum acutatum* KC05, indicating that this scaffold is the mitochondrial genome, which is about 30.9 kb in size. The fungal genome repeat sequence database was constructed based on the principle of structure prediction and *ab initio* prediction with the help of four software programs, namely, LTR_FINDER v1.05 (Xu and Wang 2007), MITE-Hunter (Han and Wessler 2010), RepeatScout v1.0.5 (Price et al. 2005), and PILER-DF v2.4 (Edgar and Myers 2005), was classified using PASTECClassifier (Wicker et al. 2007), and was then merged with the Repbase (Jurka et al. 2005) database to generate the final repeat sequence database. The repeat sequences of the fungus were predicted based on the constructed repeat sequence database, using RepeatMasker v4.0.6 (Chen 2004) software, and a repetitive sequence of 1,325,448 bp was obtained, with a repetitive sequence ratio of 2.55%. By analyzing the repeats on both ends of each assembled scaffold, telomeric repeats

(CCCTAA, which is the reverse complement of TTAGGG, the telomere repeat sequence reported for other *Colletotrichum* species [Liang et al. 2020]) were identified on the 5' ends of scaffolds 9 and 11.

To better predict protein-coding genes, we obtained RNA-seq data for strain TJNH1 using the Nova-Seq 6000 system. The RNA-seq data were submitted to GenBank under accession number SRR12362003. We obtained 24,035,109 read pairs with a final total length of 7,152,034,788 bp. The percentages of Q20 and Q30 bases were 98.05 and 94.65%, respectively. The genome was annotated based on three different prediction strategies: *ab initio* prediction, homology-based prediction, and RNA-seq-based prediction. For RNA-seq-based prediction, the RNA-seq reads were aligned against our genome using HISAT2 (v2.0.4) (Pertea et al. 2016) to generate spliced alignments. Next, the RNA-seq reads were assembled using Trinity software (v2.1.1). Subsequently, the assembled transcripts were used for gene model prediction with TransDecoder v2.0 (B. J. Haas and A. Papanicolaou *personal communication*) and PASA v2.4.1 (Campbell et al. 2006), separately. The *ab initio* gene predictions were performed with Augustus v2.4 (Stanke and Waack 2003), Genscan (Burge and Karlin 1997), and GlimmerHMM v3.0.4 (Majoros et al. 2004), with parameters trained on the PASA predictions. For the homology-based prediction, three sequenced genomes of the *Colletotrichum* genus (*C. higginsianum*, *C. fructicola*, *C. gloeosporioides*) from NCBI were used to predict gene models with GeMoMa v1.3.1 (Keilwagen et al. 2016). Finally, RNA-seq-based gene models, *ab initio*-based gene models, and homology-based gene models were combined using EvidenceModeler (EVM) (Haas et al. 2008) to integrate a final gene set. For EVM, the default settings were used for all parameters except the weight file; we set the weight of the RNA-seq-based predictions to 20, the weight of the homology based predictions to 10, and the weight of *ab initio*-based predictions to 1. For PASA the default settings were used, except for maxIntronLength, which was set to 20,000. Default settings were used for all the other programs (i.e., HISAT2, Trinity, TransDecoder, Augustus v 2.4, Genscan, and GeMoMa). In total, 13,419 protein-coding genes were predicted. By analyzing the predicted protein sequences, 1,828 signal peptides were predicted, using SignalP 4.0 software (with default parameters) (Petersen et al. 2011). A total of 1,357 secreted proteins were predicted using EffectorP 2.0 software (Sperschneider et al. 2018). In the secretome, 258 proteins were identified as potential effectors, which may play vital roles in inactivating the host defense system. In addition, 2,957 transmembrane proteins were predicted by TMHMM software (with default parameters) (Krogh et al. 2001), and 934 carbohydrate-active enzymes, 137 transport proteins, and 4,473 pathogen-host interaction proteins were identified in searches of the CAZy, TCDB, and PHI databases (*E* value cutoff = 1e-05 for all searches), respectively (Altschul et al. 1997; Cantarel et al. 2009; Eddy 1998; Saier et al. 2006; Winnenburg et al. 2006).

Moreover, we compared the strain TJNH1 assembly with other *C. caudatum* species complex assemblies, including those of *C. fiorinae*, *C. nymphaeae*, and *C. salicis* (Baroncelli et al. 2014, 2016). Based on these comparisons (Supplementary Table S1), the TJNH1 assembly is markedly better than the assemblies from other species in the species complex in terms of the number of scaffolds, scaffold N₅₀, genome coverage, and sequencing technology. Our result provided a high-quality genome annotation reference of *C. scovillei* and an advanced research method on the genome sequencing of plant pathogen.

The genome described here is the first available for *C. scovillei*. This reference genome will provide insight into the pathogenic mechanism of *C. scovillei* and molecular plant-pathogen interactions. The Nanopore and Illumina read data generated in this study have been submitted to GenBank under accession numbers SRR11060983 and SRR11091834, respectively. This whole-genome shotgun project has been deposited in GenBank under the accession number JAAJBS000000000. The version described in this paper is version JAAJBS010000000.

Author-Recommended Internet Resource

TransDecoder: <http://transdecoder.github.io>

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