Genome Sequence of *Fusarium oxysporum* f. sp. *conglutinans*, the Etiological Agent of Cabbage Fusarium Wilt

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Abstract

Fusarium oxysporum f. sp. *conglutinans* is the causal agent of Fusarium wilt of cabbage (*Brassica oleracea* var. *capitata* L.), which results in severe yield loss. Here, we report a high-quality genome sequence of a race 1 strain (IVC-1) of *F. oxysporum* f. sp. *conglutinans*, which was assembled using a combination of PacBio long-read and Illumina short-read sequences. The assembled IVC-1 genome has a total size of 71.18 Mb, with a contig N50 length of 4.59 Mb, and encodes 23,374 predicted protein-coding genes. The high-quality genome of IVC-1 provides a valuable resource for facilitating our understanding of *F. oxysporum* f. sp. *conglutinans*–cabbage interaction.

Genome Announcement

Fusarium oxysporum is a ubiquitous soil-inhabiting fungus that infects more than 100 different plant species worldwide (Ma et al. 2013; Michielse and Rep 2009). *F. oxysporum* is also notorious for causing opportunistic infections in immunocompromised patients (Nucci and Anaissie 2007). Due to its great scientific and economic importance, *F. oxysporum* has been nominated as one of the top 10 fungal pathogens (Dean et al. 2012). The infection of plants by pathogenic *F. oxysporum* involves several steps, including root recognition, root surface attachment and colonization, penetration and colonization of the root cortex, and hyphal growth within the xylem vessels (Di Pietro et al. 2003). The key to successful invasion by *F. oxysporum* depends on its ability to properly recognize the host roots by sensing secreted plant peroxidases through the mitogen-activated protein kinase cascade and the seven-pass transmembrane protein Ste2 (Turrà et al. 2015). Differing in host specificity, *F. oxysporum* can be classified into more than 120 *formae speciales*, some of which can be further divided into several physiological races (Edel-Hermann and Lecomte 2019; Gordon and Martyn 1997; Michielse and Rep 2009).

F. oxysporum f. sp. *conglutinans* is the causal agent of cabbage Fusarium wilt (CFW), which poses a great threat to global cabbage production. *F. oxysporum* f. sp. *conglutinans* populations with high pathogenicity have been emerging in China in recent years (Liu et al. 2017). To date, only two races have been documented in *F. oxysporum* f. sp. *conglutinans* (Morrison et al. 1994; Ramirez-Villupadua et al. 1985), and race 1 is the dominant strain in

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Keywords

Brassica oleracea var. *capitata* L.,
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microbe-plant interaction

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Fig. 1. K-mer analysis of *Fusarium oxysporum* f. sp. *conglutinans* genome. **A**, The 17-mer depth distribution of the Illumina paired-end reads. A total of 3,325,251,032 distinct 17-mers were recovered, with a peak depth of 45. The genome size was estimated based on the following formula: genome size = (total number of k-mers)/(position of peak depth) = 3,325,251,032/45 = 73.89 Mb. **B**, Comparison of 17-mer spectra between the Illumina paired-end reads and the genome assembly.

Table 1. Genome statistics of *Fusarium oxysporum* f. sp. *conglutinans* race 1 (IVC-1 and FGL03-6), race 2 (54008), and *F. oxysporum* f. sp. *lycopersici* 4287 (Fol4287)

Strain	IVC-1 (race 1)	FGL03-6 (race 1)	54008 (race 2)	Fol4287
Genome size (Mb)	71.18	61.66	53.58	61.39
Number of scaffolds	55	414	2,552	114
Scaffold N50 (Mb)	5.00	1.21	0.16	1.98
Number of contigs	64	1,079	3,350	1,362
Contig N50 (Mb)	4.59	0.34	0.09	0.10
Number of genes	23,374	9,790	26,246	27,347
GC content	48.19%	48.00%	47.70%	48.43%
BUSCO completeness	99.4%	99.2%	99.2%	96.4%

China (Liu et al. 2019). Improving our current knowledge of the molecular mechanisms underlying pathogenesis and host specificity of *F. oxysporum* f. sp. *conglutinans* races is fundamental for CFW management. To this end, it would be very valuable to obtain high-quality genome sequences for *F. oxysporum* f. sp. *conglutinans* races.

Genome assemblies have been released for *F. oxysporum* f. sp. *conglutinans* race 1 FGL03-6 (NR1A00000000.2) and race 2 54008 (AGNF00000000.1). However, both genomes were assembled using Illumina short reads and were thus highly fragmented, making it challenging to perform in-depth analyses of genomic features that associate with variations and virulence among *F. oxysporum* f. sp. *conglutinans* races. Here, we report the high-quality genome sequence of a race 1 strain of *F. oxysporum* f. sp. *conglutinans*, IVC-1, which was initially isolated from roots of *F. oxysporum* f. sp. *conglutinans*-infected cabbage collected in North China. Genomic DNA was extracted from the mycelia of IVC-1 and was used to construct an Illumina paired-end library with an insert size of approximately 270 bp, using the TruSeq Nano DNA high throughput library prep kit (Illumina) and a PacBio single-molecule real-time (SMRT) library, with an insert size of approximately 20 kb, using the SMRTbell template prep kit 1.0 (PacBio), following manufacturer instructions. The Illumina paired-end library was sequenced on an Illumina HiSeq X Ten platform, yielding a total of 3.7 Gb of high-quality sequences, with a read length of 150 bp, which represented approximately 52× coverage of the IVC-1 genome that had an estimated size of 73.89 Mb, based on the k-mer depth distribution analysis of the Illumina short reads (Fig. 1A). The PacBio SMRT library was sequenced on four SMRT cells of a PacBio Sequel system, which yielded a total of 18.9 Gb

(approximately 265× coverage of the IVC-1 genome) long-read data with read N50 and mean lengths of 22,160 and 16,911 bp, respectively.

The PacBio long reads were de novo assembled using Falcon (v0.3.0) (Chin et al. 2016), with parameters '-v -dal8 -t32 -h60 -e.96 -l500 -s100 -H3000'. The resulting assembled contigs were corrected for base errors using the Illumina reads with GATK software (available online). The corrected contigs were further connected into scaffolds, using SSPACE (v2.0) (Boetzer et al. 2011) with the Illumina reads, and gap filling of scaffolds was then performed, using PBJelly2 (v15.8.24) (English et al. 2012). The final assembled genome of IVC-1 had a total length of 71,184,270 bp and a GC content of 48.19% (Table 1). The assembly comprised 64 contigs with an N50 length of 4.59 Mb and 55 scaffolds with an N50 length of 5.00 Mb. The assembled genome of IVC-1 (71.18 Mb) was much larger than those of *F. oxysporum* f. sp. *conglutinans* race 1 FGL03-6 (NR1A00000000.2, 61.66 Mb), *F. oxysporum* f. sp. *conglutinans* race 2 54008 (AGNF00000000.1, 53.58 Mb), and *F. oxysporum* f. sp. *lycopersici* 4287 (AAXH00000000.1, 61.39 Mb) and also had much better continuity than the other three *F. oxysporum* assemblies, indicated by a 13- to 50-fold improvement of contig N50 length in IVC-1 assembly compared with other three (Table 1). The completeness of the IVC-1 assembly was assessed using BUSCO (v3.0.2) (Simão et al. 2015). Of the 1,315 single-copy orthologs in the ascomycota data set, 1,306 (99.4%) were found complete in the IVC-1 assembly (Table 1).

Comparison of k-mers in Illumina paired-end reads and those in the IVC-1 assembly revealed that almost the entire assembly was covered by single-copy 17-mers (1×), while a very small portion of k-mers with ≥2× coverage represented the repetitive sequences in the assembly (Fig. 1B), confirming that there were no redundant sequences in the assembly. Blast analysis of the assembly against the GenBank nucleotide database showed that all hits were from fungi, with 99% of the hits from *Fusarium*, suggesting there was no contamination in the assembly. Alignment of the assembly to other *Fusarium* genomes showed that the aligned scaffolds exhibited high collinearity with published *Fusarium* genomes, while, as expected, some scaffolds failed to align. In summary, the size of this genome assembly is larger than other published *Fusarium* genomes, but there is no evidence of contamination or redundancy in this assembly, and the assembled genome size of IVC-1 (71.18 Mb) is close to the estimated genome size (73.89 Mb). Fungal genomes evolve very fast, especially on the dispensable chromosomes (Peng et al. 2019; Sperschneider et al. 2015), which are normally divergent between species or even strains. In addition, this genome was assembled using the long-read technology and, therefore, could include some regions that were not assembled in other assemblies with short-read sequences.

A total of 23,374 protein-coding genes were predicted in the genome of IVC-1 by integrating homology predictions, using GeneWise (v2.20) (Birney et al. 2004), and de novo predictions using SNAP (v2010-07-28) (Korf 2004), Augustus (v3.2.1) (Stanke et al. 2008), and GeneMark-ES (v4.21) (Ter-Hovhannisyan et al. 2008). The number of predicted genes in IVC-1 was comparable to those in 54008 and Fol4287 but more than that in FGL03-6 (Table 1).

The high-quality genome assembly of the race 1 strain of *F. oxysporum* f. sp. *conglutinans*, IVC-1, provides a valuable resource that will enable in-depth analysis of *Fusarium* pathogenesis and interaction between *F. oxysporum* f. sp. *conglutinans* and cabbage and will facilitate the development of better control approaches against CFW.

This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JABTBS000000000. Raw PacBio and Illumina sequencing reads have been deposited into National Center for Biotechnology Information Short Read Archive under the accessions SRR11823423 and SRR11823424, respectively.

Author-Recommended Internet Resource

The Genome Analysis Toolkit (GATK): <https://www.broadinstitute.org/gatk>

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