Whole Genome Sequence Resource for *Fusarium oxysporum* f. sp. *capsici* 14003, the Causative Agent of Pepper Wilt

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Abstract

Pepper wilt, caused by the fungal pathogen *Fusarium oxysporum* f. sp. *capsici*, is one of the main diseases on pepper. In this study, we obtained the whole genome sequence of the highly virulent *F. oxysporum* f. sp. *capsici* strain 14003 sequenced using the Illumina HiSeq 2000 platform. In total, 3.87 million paired-end reads were obtained and assembled into 796 scaffolds with a genome-wide length of 47.6 Mb. In addition, we filtered genes that may be related to specific virulence factors and performed a comparative analysis between the genome of strain 14003 and the sequenced genomes of 36 isolates. The genome-sequencing results for strain 14003 will enhance the theoretical basis for pepper wilt prevention and control.

Genome Announcement

Pepper is an important vegetable and spice crop belonging to the nightshade family Solanaceae, which is cultivated in approximately 130 countries. The plant is a rich source of vitamin C- and vitamin A-containing β -carotenoids, which are potent antioxidants (Serra et al. 2002; USDA-NASS 2003). Pepper wilt is the main disease on pepper, which is caused by *Fusarium oxysporum* f. sp. *capsici*, a highly virulent fungal pathogen of peppers (Rivelli 1989). Generally, the characteristic symptoms of *Fusarium* wilt of pepper include wilting, stunting, leaf chlorosis, slight vein clearing on the outer leaflets, vascular necrosis, and, ultimately, death (Altinok et al. 2019); and, under damp conditions, white mold may grow on the diseased parts of the plant. *Fusarium* wilt readily establishes and can spread rapidly in warm humid environments and when plants are grown under monoculture conditions (Velarde-Félix et al. 2018). Chemical and biological control of pepper wilt are of limited efficacy, and there are currently no pepper varieties showing durable resistance to this disease (Mao and Mo 2019). Consequently, at present, there are no completely effective measures available for the control of pepper wilt. From a long-term control perspective, for the effective management of *Fusarium* wilt, it will be essential to examine the pathogenic mechanisms of *F. oxysporum* f. sp. *capsici* and to identify the associated virulence genes and their functions. Currently, however, there is a conspicuous lack of information relating to the genomic features and mechanisms of interaction underlying the pathogenicity of strain 14003 against pepper. Therefore, sequencing and analyzing the genome of this pathogen has become an urgent prerequisite.

In this study, we determined the genome sequence of *F. oxysporum* f. sp. *capsici* strain 14003, which was isolated from Guangdong Province, China. *F. oxysporum* f. sp. *capsici* 14003 was cultured for 5 days on potato dextrose medium at 25°C. Genomic DNA was

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Keywords

Fusarium oxysporum f. sp. *capsici*, pepper wilt, whole genome sequence

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Table 1. Information on the sequenced genomes of 37 *Fusarium* isolates

Name	Assembly	Total sequence length
<i>Fusarium beomiforme</i>	GCA_002980475.2	46,521,357
<i>F. fujikuroi</i> IMI 58289	GCA_900079805.1	43,832,314
<i>F. mangiferae</i>	GCA_900044065.1	46,292,722
<i>F. verticillioides</i> 7600	GCA_000149555.1	41,844,914
<i>F. coffeatum</i>	GCA_003316985.1	37,769,732
<i>F. oxysporum</i> f. sp. <i>cubense</i> race 1	GCA_000350365.1	52,926,277
<i>F. oxysporum</i> f. sp. <i>cubense</i> tropical race 4 54006	GCA_000350345.1	47,657,417
<i>F. oxysporum</i> f. sp. <i>cubense</i> race 4	GCA_000260195.2	46,553,780
<i>F. oxysporum</i> f. sp. <i>cubense</i>	GCA_007994515.1	48,588,396
<i>F. oxysporum</i> f. sp. <i>lycopersici</i> 4287	GCA_000149955.2	61,386,934
<i>F. oxysporum</i> f. sp. <i>conglutinans</i> race 2 54008	GCA_000260215.2	53,575,352
<i>F. oxysporum</i> f. sp. <i>cepae</i>	GCA_003615085.1	53,425,788
<i>F. oxysporum</i> f. sp. <i>cepae</i>	GCA_003615095.1	51,407,732
<i>F. oxysporum</i> f. sp. <i>cepae</i>	GCA_003615075.1	51,009,701
<i>F. oxysporum</i> f. sp. <i>lycopersici</i> MN25	GCA_000259975.2	48,637,398
<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i> 26381	GCA_000260155.3	49,359,289
<i>F. oxysporum</i> f. sp. <i>raphani</i> 54005	GCA_000260235.2	53,499,362
<i>F. oxysporum</i> f. sp. <i>radicis-cucumerinum</i>	GCA_001702695.2	52,908,293
<i>F. oxysporum</i> f. sp. <i>vasinfectum</i> 25433	GCA_000260175.2	52,914,414
<i>F. oxysporum</i> f. sp. <i>narcissi</i>	GCA_004141715.1	57,517,562
<i>F. oxysporum</i> f. sp. <i>pisi</i> HDV247	GCA_000260075.2	55,188,216
<i>F. oxysporum</i> f. sp. <i>melonis</i> 26406	GCA_000260495.2	54,034,280
<i>F. oxysporum</i>	GCA_003615165.1	50,515,499
<i>F. oxysporum</i>	GCA_003615155.1	53,038,185
<i>F. oxysporum</i>	GCA_003615185.1	50,292,220
<i>F. oxysporum</i> Fo47	GCA_000271705.2	49,664,628
<i>F. oxysporum</i> NRRL 32931	GCA_000271745.2	47,906,303
<i>F. oxysporum</i> Fo5176	GCA_000222805.1	54,767,602
<i>F. oxysporum</i>	GCA_900096695.1	49,085,590
<i>F. oxysporum</i>	GCA_003615115.1	54,787,818
<i>F. langsethiae</i>	GCA_001292635.1	37,543,021
<i>F. poae</i>	GCA_001675295.1	46,476,831
<i>F. graminearum</i> PH-1	GCA_000240135.3	36,458,046
<i>F. pseudograminearum</i> CS3096	GCA_000303195.2	36,973,259
<i>F. sporotrichioides</i>	GCA_003012315.1	37,429,079
<i>F. venenatum</i>	GCA_900007375.1	38,660,186
<i>F. oxysporum</i> f. sp. <i>capsici</i>	GCA_014770115.1	47,555,656

extracted from fresh mycelium using the cetyltrimethylammonium bromide method (Kim et al. 2010). DNA sequencing was carried out using an Illumina HiSeq 2000 Sequencer operating in 100-bp paired-end mode, with library insert sizes of 300 bp. In total, 3.87 million raw reads were generated, from which low-quality data were filtered using fastqc and Trimmomatic. After quality filtering, high-quality reads were de novo assembled using Abyss, Velvet, and SOAPdenovo. In total, we constructed 796 scaffolds for a genome size of 47,555,656 bp, with an N₅₀ value of 209,562 bp. The overall G+C content of the strain 14003 assembly was 51.0% and the genome coverage was 59.0×. The total length of strain 14003 is within the size range of previously published *Fusarium* genomes (Table 1). BUSCO (Simão et al. 2015) analysis showed that the gene models predicted that the three genomes covered 99% of the single-copy conserved fungal genes, indicating the near-completeness of the assemblies. We used AUGUSTUS v3.3.3 (Stanke et al. 2004) to annotate the genome, and 15,762 protein-encoding genes were predicted, with an average length of 510 amino acids.

Genes were then functionally assigned using BLAST (version 2. 7. 1+) (Kent 2002) against the following databases: SwissProt, NR, KOG, KEGG, MEROPS, DFFV, LIPABASE, Cytochrome P450, PHI Database, and CAZymes, for E-values $\leq 1e^{-5}$. Additionally, within the *F. oxysporum* f. sp. *capsici* 14003 genome, we identified potential pathogenicity and virulence-related genes, including 3,389, 863, and 6,187 genes encoding P450 enzymes, CAZymes, and PHI proteins, respectively.

We predicted 15,762 protein-encoding genes in the *F. oxysporum* f. sp. *capsici* 14003 genome, which is considered a moderate number. Gene families among 37 other sequenced genomes of *Fusarium* isolates were classified based on orthologous clustering using the

Orthofinder platform (Emms and Kelly 2015), and yielded 1,615 single-copy orthologs. The whole genomes of the type isolates representing the other *Fusarium* isolates have previously been sequenced and are available in the NCBI database (Table 1).

We compared the 37 *Fusarium* genomes and identified 232 genes specific to strain 14003. SignalP (version 4.1), TMHMM V2.0 TargetP (V1.1), and ProtComp predicted that 23 of these 232 specific genes are secreted proteins. We used the PHI database to annotate these 232 proteins and, among these, the deletion of 90 proteins has been shown to result in reduced virulence. Following deletion, the pathogenicity 10 proteins, classified as effectors, was completely lost, and we accordingly speculate that these might be pathogenic factors. In total, 7 proteins have been shown to increase virulence, whereas 77 proteins were annotated as unaffected, and a further 6 proteins were classified as lethal. The other 23 proteins were annotated as mixed results. We subsequently used PFAM (Finn et al. 2008) to predict the domains of the 232 specific proteins. In total, we annotated 104 genes in the *F. oxysporum* f. sp. *capsici* 14003 genome, among which 35 genes collectively belonged to the polysaccharide lyases (EC 4.2.2.-) (PLs) based on their CAZymes identifications. PLs play significant roles in breaching plant cell wall defenses after degrading cellulose and pectin and, among the 37 *Fusarium* genomes we assessed, that of strain 14003 was found to have the highest number of PL genes. PL1 and PL3 are the most common pectin lyases found in plant pathogens and, in the present study, we detected 14 and 11 copies of PL1 and PL3, respectively, in *F. oxysporum capsici* 14003. Among six other PL members (PL11, PL20, PL22, PL4, PL7, and PL9), it has previously been hypothesized that PL9, PL 20, and PL22 are specific to *F. virguliforme* and *Nectria hematococca* (teleomorph: *F. solani*), and are not found in other plant fungal pathogens or oomycetes (Fei et al. 2019). However, we detected both PL9 and PL22 domains in the strain 14003 genome. In other *Fusarium* strains, PL11 and PL4 have been observed. Notably, we identified a novel β -elimination (PL7) in the strain 14003 genome. To analyze the secondary metabolite synthesis potential of strain 14003, we used antiSMASH 3.0 (fungal version) (Weber et al. 2015) to identify secondary metabolite biosynthesis gene clusters in the genome of strain 14003, and performed similar analyses for the other 36 *Fusarium* genomes for comparative purposes. We accordingly identified a total of 44 key biosynthetic genes in the strain 14003 genome that are potentially associated with secondary metabolites, among which there are 21 genes encoding nonribosomal peptide synthetases (NRPS), 16 encoding polyketide synthases (PKS), 5 genes encoding PKS-NRPS, and 1 gene encoding terpene synthase. The remainder included single biosynthesis genes for other secondary metabolites. On the basis of the comparison of the secondary metabolite-associated genes identified in the 37 *Fusarium* genomes, it is clear that the number of genes annotated as NRPS and PKS in the *F. oxysporum* f. sp. *capsici* 14003 genome is considerably higher than that in most *Fusarium* strains, although similar to that in the *F. beomiforme* genome. In contrast, we detected fewer PKS-NRPS and other secondary metabolite-associated genes in the *F. oxysporum* f. sp. *capsici* 14003 genome than in the other 36 *Fusarium* genomes.

Data availability. The whole genome data presented in this paper have been deposited in GenBank under the accession number JACYOB000000000 (BioProject PRJNA664524).

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