Genome Sequence of *Fusarium oxysporum* f. sp. *matthiolae*, a Brassicaceae Pathogen

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Abstract

The filamentous fungus *Fusarium oxysporum* is a soilborne pathogen of many cultivated species and an opportunistic pathogen of humans. *F. oxysporum* f. sp. *matthiolae* is one of three formae speciales that are pathogenic to crucifers, including *Arabidopsis thaliana*, a premier model for plant molecular biology and genetics. Here, we report a genome assembly of *F. oxysporum* f. sp. *matthiolae* strain PHW726, generated using a combination of PacBio and Illumina sequencing technologies. The genome assembly presented here should facilitate in-depth investigation of *F. oxysporum*–*Arabidopsis* interactions and shed light on the genetics of fungal pathogenesis and plant immunity.

Genome Announcement

Strains of the filamentous fungus *Fusarium oxysporum*, a notorious plant pathogen, can infect hundreds of cultivated species and, at the same time, have distinct host specificity (Kistler 1997; Ma et al. 2013; Michielse and Rep 2009). This host specificity is used to classify *F. oxysporum* into formae speciales, and a forma specialis typically represents one to a few monophyletic clonal lineages that cause disease in a narrow range of taxonomically related plants (Kistler 1997). At the genomic level, host specificity corresponds to the presence of lineage-specific chromosomes (Ma et al. 2010). However, little is known about molecular mechanisms involved in these host-specific plant–fungus interactions. One of the three formae speciales that are pathogenic to the crucifer *Arabidopsis thaliana* (Diener and Ausubel 2005; Provart et al. 2016), the genome sequence of *F. oxysporum* f. sp. *matthiolae* will enable the genetic analysis of fungal pathogenesis and host immunity using the model plant *A. thaliana*.

Phylogenetic analyses indicate that *F. oxysporum* f. sp. *matthiolae* isolates form a single clonal lineage (Bosland and Williams 1987; Kistler and Benny 1989; Kistler et al. 1987, 1991; O'Donnell et al. 2009), although two races of *F. oxysporum* f. sp. *matthiolae* are distinguished by the differential susceptibility of varieties of *Matthiola incana* (Bosland and Williams 1988). Natural variation of immunity is observed among wild accessions or ecotypes of *A. thaliana* toward Fusarium wilt (Diener and Ausubel 2005). Quantitative trait loci mapping in offspring of

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Keywords

Arabidopsis thaliana, *Fusarium oxysporum* f. sp. *matthiolae*, genome, microbe–plant molecular interaction, model

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Table 1. Summary of the *Fusarium oxysporum* f. sp. *matthiolae* strain PHW726 genome assembly and a comparison with Fol4287 (Ayhan et al. 2018)

Variables	Statistics	
	PHW726	Fol4287
Assembly size (bp)	57,270,650	53,912,367
Core sequence size (bp)	43,818,233	42,239,438
Number of scaffolds	583	499
Number of core scaffolds	66	55
Size of largest scaffold (bp)	3,557,637	5,733,288
Interspersed repeat content	5.35%	4.21%
N ₅₀ (bp)	774,050	1,338,693
N ₉₀ (bp)	47,752	49,310
L ₅₀ (bp)	18	11
GC content	47.44%	47.68%

crosses between resistant and susceptible ecotypes has identified three *RESISTANCE TO F. OXYSPORUM* (*RFO*) genes—one receptor-like protein gene (*RFO2*) and two receptor-like kinase (RLK) genes (*RFO1* and *RFO3*)—from different RLK gene subfamilies (Cole and Diener 2013; Diener 2013; Diener and Ausubel 2005; Shen and Diener 2013). Because receptor-mediated immunity is reported to be the major determinant of disease resistance to *F. oxysporum* f. sp. *matthiolae* (Cole and Diener 2013), investigation of the interaction of *F. oxysporum* f. sp. *matthiolae* and *A. thaliana* should lead to a fundamental understanding of receptor-mediated plant immunity, especially against fungal pathogens. The genome sequence described here comes from DNA purified from *F. oxysporum* f. sp. *matthiolae* race 2, isolated from wilted garden stock (*M. incana*), a cultivated plant in the crucifer or mustard (Brassicaceae) family, prized for its colorful flowers (Baker 1948; Tatsuzawa et al. 2012). This strain was previously deposited in the American Type Culture Collection (ATCC 16603) by G. M. Armstrong and subsequently designated by P. H. Williams as PHW726 (Kistler et al. 1987).

The pipeline for genome assembly was adapted from Ayhan et al. (2018). Genomic DNA was purified from the mycelium of PHW726, then sequenced by Illumina MiSeq and PacBio RS II platforms with ×119 and ×21 coverage, respectively. We used MiSeq paired-end sequencing with 150 cycles. The maximum size of the PacBio RS II reads was 59 kb while the mean size was 8.5 kb. Trimmomatic, version 0.32 (Bolger et al. 2014), was used to remove adaptors and trim ends of Illumina reads (parameters: ILLUMINACLIP:TruSeq3-PE.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36). FastQC, version 0.11.5, was used to check the quality of all reads. SPAdes, version 3.9.1 (Antipov et al. 2016), was used to combine PacBio subreads and trimmed Illumina reads into an initial hybrid assembly with default parameters. BWA, version 0.7.12 (Li and Durbin 2009), was used to map the Illumina reads to the assembly. Further cleaning, fixing, and sorting of mapping reads was done with Picard, version 2.0.1, and Samtools, version 1.3 (Li et al. 2009). A structural variant caller, GRIDSS, version 1.4.1 (Cameron et al. 2017), was used to identify links between scaffolds in the initial assembly. A custom script (scaffolding.m) was used for scaffolding. Minimap2, version 2.17 (Li 2018), was used to map PacBio subreads to new scaffolds, and links were manually inspected and, if necessary, fixed. Further polishing was performed by remapping Illumina reads to the assembly, during which FreeBayes, v0.9.10-3-g47a713e (Garrison and Marth 2012), was used to identify base variants between reads and the assembly (specially 70% support of minimal 10 alternate counts, with a minimal base mapping-quality greater than q30). Identified variants were used to correct the assembly by a custom script (FASTAeditWithVCF.m). RepeatMasker 4.0.5 (Tarailo-Graovac and Chen 2009) was used to screen the repeats. Mummer 3.22 (Kurtz et al. 2004) was used to align the assembly with the reference genome assembly for the tomato pathogen *F. oxysporum* f. sp. *lycopersici* Fol4287 (Ma et al. 2010).

The final assembly was 57.3 Mb in total length and comprised 583 scaffolds with an N₅₀ value of 0.77 Mb (Table 1). The largest scaffold size was 3.6 Mb. The GC content was 47.4%. The size of total interspersed repeats was 3.1 Mb, which accounted for 5.4% of the assembly. A comparison with Fol4287 assembly (Ayhan et al. 2018) suggested a larger assembly size and higher interspersed repeat content of PHW726. The size of sequence

mapped to the core chromosomes of F04287 which, including 66 scaffolds (defined as core scaffolds), was 43.8 Mb. The assembly also included a scaffold of 52,365 bp that captured the entire mitochondrial DNA. This Whole Genome Shotgun project has been deposited at DNA Data Bank of Japan/European Nucleotide Archive/GenBank under the accession WJXY00000000. The version described in this article is version WJXY01000000. This assembly for the genome of PHW726 should facilitate future molecular genetics and genomic studies. Candidate *F. oxysporum* f. sp. *matthiolae* genes that promote pathogenesis or elicit immune response in *A. thaliana* and *M. incana* can now be predicted, subcloned, and genetically characterized.

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Author-Recommended Internet Resources

FastQC: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

Picard: <http://broadinstitute.github.io/picard/>

Custom script scaffolding.m: <https://github.com/d-ayhan/tools/blob/master/scaffolding.m>

Custom script FASTAeditWithVCF.m: <https://github.com/d-ayhan/tools/blob/master/FASTAeditWithVCF.m>

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