Draft Genome Resources for Brassicaceae Pathogens *Fusarium oxysporum* f. sp. *raphani* and *Fusarium oxysporum* f. sp. *rapae*

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Abstract

The soilborne filamentous fungus *Fusarium oxysporum* causes devastating diseases of many cultivated plant species. *F. oxysporum* f. sp. *raphani* and f. sp. *rapae* are two of four formae speciales that are pathogenic to Brassicaceae plants. Here, we present high-quality genome sequences of *F. oxysporum* f. sp. *raphani* strain Tf1262 and *F. oxysporum* f. sp. *rapae* strain Tf1208 that were isolated from radish (*Raphanus sativus*) and turnip (*Brassica rapa* var. *rapa*), respectively. These genome resources should facilitate in-depth investigation of interactions between *F. oxysporum* and Brassicaceae plants, and enable comparative genomics of the *F. oxysporum* species complex to uncover how pathogenicity evolved within *F. oxysporum*.

Genome Announcement

Fusarium oxysporum is a ubiquitous soilborne pathogen that causes vascular wilt and root diseases on a broad range of economically important crops such as banana, tomato, melon, and cruciferous vegetables. Individual pathogenic strains of *F. oxysporum* are restricted to one or a few host species, and are divided into more than 100 forms (formae speciales) based on their host specificity (Edel-Hermann and Lecomte 2019). However, little is known about what molecular mechanisms determine its host specificity. High-quality genome sequences are a critical tool that can be used to elucidate pathogenic mechanisms and host specificity. Already, the genetic analysis of *F. oxysporum* f. sp. *conglutinans* (Wolterw.) W. C. Snyder & H. N. Hansen (Snyder and Hansen 1940), which infects Brassicaceae plants, including *Arabidopsis thaliana* and cabbage (*Brassica oleracea* var. *capitata*), uncovered specific conditionally dispensable chromosomes that are required for virulence on *A. thaliana*, cabbage, or both, and also revealed two adjacent effector genes required for suppression of *A. thaliana*-specific immunity (Ayukawa et al. 2021). In addition to *F. oxysporum* f. sp. *conglutinans*, there are three formae speciales that cause disease on Brassicaceae plants: *F. oxysporum* f. sp. *matthiolae* K. F. Baker (Baker 1948), *F. oxysporum* f. sp. *raphani* J. B. Kendr. & W. C. Snyder (Kendrick and Snyder 1942), and *F. oxysporum* f. sp. *rapae* Enya, Togawa, T. Takeuchi & Arie (Enya et al. 2008). There is some host overlap among these four formae speciales but the main hosts are different: *F. oxysporum* f. sp. *conglutinans* for cabbage, *F. oxysporum* f. sp. *matthiolae* for hoary stock (gilly-flower, common stock; *Matthiola incana*), *F. oxysporum* f. sp. *raphani* for radish (*Raphanus sativus*), and *F.*

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Table 1. Genome statistics of *Fusarium oxysporum* strains

Parameters	Organism, strain ^a					
	<i>Forpi</i> Tf1262	<i>Forpe</i> Tf1208	<i>F. oxysporum</i> f. sp. <i>conglutinans</i>			<i>Fomt</i> PHW726
			Fo5176	Cong:1-1	IVC-1	
Assembly size (Mb)	66.8	59.8	68.0	68.8 (72.2) ^b	71.2	57.3
Number of contigs/scaffolds	59	25	19	22 (125) ^c	55	583
Max contig/scaffold length (kb)	6,689	6,462	6,729	7,006	7,512	3,558
N ₅₀ contig/scaffold length (kb)	2,955	4,228	4,091	4,865	4,998	774
Number of genes	18,973	17,241	17,912	21,781	23,374	–
Number of proteins	19,208	17,423	17,912	22,094	–	–
GC content (%)	48.12	47.78	48.2	48.4	48.19	47.44
BUSCO coverage (%) ^d	98.6	99.0	99.1	99.1	99.1	99.0
Technology ^e	RSII	Sequel	Sequel + Hi-C	RSII + OM	Sequel + HiSeq	RSII + MiSeq
Reference	This study	This study	Fokkens et al. 2020	Ayukawa et al. 2021	Yu et al. 2021	Yu et al. 2020

^a Abbreviations: *Forpi* = *F. oxysporum* f. sp. *raphani*, *Forpe* = *F. oxysporum* f. sp. *rapae*, and *Fomt* = *F. oxysporum* f. sp. *matthiolae*.

^b The number in parentheses is total assembly size, including nonscaffolded contigs.

^c The number in parentheses is the number of nonscaffolded contigs.

^d Results using the Sordariomyceta data set (3,725 genes).

^e RSII = PacBio RSII, Sequel = PacBio Sequel, Hi-C = high-throughput chromosome conformation capture, OM = optical genome mapping, HiSeq = Illumina HiSeq, and MiSeq = Illumina MiSeq.

oxysporum f. sp. *rapae* for *Brassica rapa*. Recently, highly contiguous genome assemblies of three *F. oxysporum* f. sp. *conglutinans* strains (Fo5176, Cong:1-1, and IVC-1) and one *F. oxysporum* f. sp. *matthiolae* strain (PHW726) were reported (Ayukawa et al. 2021; Fokkens et al. 2020; Yu et al. 2020; Yu et al. 2021) (Table 1). Here, we report high-quality genome sequences of *F. oxysporum* f. sp. *raphani* strain Tf1262 (*Forpi*Tf1262) and *F. oxysporum* f. sp. *rapae* strain Tf1208 (*Forpe*Tf1208). Comparative genomics among *Fusarium* spp. has revealed that horizontal chromosome transfer seems to have contributed to host specificity within the *F. oxysporum* species complex (Ayukawa et al. 2021; Li et al. 2020; Ma et al. 2010; van Dam et al. 2017). Therefore, these genome resources will contribute to research and management efforts for Fusarium wilt disease, and will also serve as reference genomes for comparative analysis of the species complex to unravel how pathogenicity evolved within *F. oxysporum*.

*Forpi*Tf1262 and *Forpe*Tf1208 were initially isolated from radish in Miyazaki, Japan and turnip (*B. rapa* var. *rapa*) in Ibaraki, Japan, respectively. Nucleotide sequences of the ribosomal DNA intergenic spacer region from *Forpi*Tf1262 and *Forpe*Tf1208 showed 100% identity to those of *F. oxysporum* f. sp. *raphani* strain MAFF731043 and *F. oxysporum* f. sp. *rapae* strain 100011, respectively (Fig. 1). Genomic DNAs were extracted from hyphae grown in NO₃-medium (0.17% yeast nitrogen base, 3% sucrose, and 100 mM KNO₃) using cetyltrimethylammonium bromide and the Genomic-tip 100/G kit (QIAGEN) as described for the 1,000 Fungal Genomes project. The genomes of *Forpi*Tf1262 and *Forpe*Tf1208 were sequenced on five PacBio RSII SMRT cells and one PacBio Sequel SMRT cell, respectively. PacBio library preparation and genome sequencing were performed by Quantitative Biosciences at the University of California–Berkeley (QB3-Berkeley) Genomics. In total, 450,881 filtered subreads (N₅₀ filtered subread length, 11,919 bp) for *Forpi*Tf1262 and 429,987 filtered subreads (N₅₀ filtered subread length, 22,849 bp) for *Forpe*Tf1208 were assembled with HGAP4 within SMRT Link v5.1.0. Default values were kept, with the expected genome size set to 66 Mb for *Forpi*Tf1262 and 60 Mb for *Forpe*Tf1208. The final assembly of *Forpi*Tf1262 yielded 59 contigs with a total assembly size of 66,822,448 bp (48.12% GC content, an N₅₀ value of 2,955,369 bp, and a maximum contig size of 6,689,312 bp with 41× coverage), whereas the assembly of *Forpe*Tf1208 comprised 25 contigs with a total assembly size of 59,760,091 bp (47.78% GC content, an N₅₀ value of 4,227,812 bp, and a maximum contig size of 6,462,777 bp with 96× coverage) (Table 1). Completeness of gene space within the assembly was assessed through the presence of conserved single-copy genes using BUSCO version 3.0.2 (Simão et al. 2015; Waterhouse et al. 2018). The analysis with the Sordariomyceta data set (3,725 genes) showed the presence of 3,673 genes (98.6%) and 3,689 genes (99.0%) in the assemblies of *Forpi*Tf1262 and *Forpe*Tf1208, respectively (Table 1).

Fig. 1. Phylogenetic tree of *Fusarium oxysporum* strains. A phylogenetic tree was generated based on sequences of approximately 580 bp of the ribosomal DNA intergenic spacer region using the maximum-likelihood method. All sequences were aligned using the multiple alignment program of Genetyx 15.0. Numbers at the nodes represent the percentage of replicate trees in which the associated taxa clustered together in a bootstrap test of 1,000 replicates. *F. fujikuroi* was used as an outgroup. The scale bar indicates the mean number of nucleotide substitutions per site. Accession numbers for the sequences are shown in parentheses.

For gene prediction, RNA sequencing data from axenic culture and plant infections were aligned to the genome using HISAT2 v.2.1.0 (Kim et al. 2015) and used to guide gene model prediction using the BRAKER2.1.5 pipeline (Brůna et al. 2021). BRAKER2 was run using the repeat-softmasked genome created by RepeatMasker v.4.0.7 (with `–engine, ncbi –species, “ascomycota” –xsmall`) and hints from RNA sequencing mapped reads to train Augustus 3.4.0 and genemark-ES v4.62 with the fungal and softmasking options. In total, 18,973 genes encoding 19,208 proteins for *Forpi*Tf1262 and 17,241 genes encoding 17,423 proteins for *Forpe*Tf1208 were predicted (Table 1), with 200 and 160 genes predicted to encode multiple transcripts in the *Forpi*Tf1262 and *Forpe*Tf1208 genomes, respectively. To assess protein conservation in the species, the predicted proteomes were compared among *Forpi*Tf1262, *Forpe*Tf1208, and *F. oxysporum* f. sp. *conglutinans* Fo5176 as a representative *F. oxysporum* f. sp. *conglutinans* strain, using OrthoFinder v2.5.4 (Emms and Kelly 2019). This comparison indicated 1,457 (7.6%), 1,319 (7.6%), and 1,992 (11.1%) of predicted proteins were specific to *Forpi*Tf1262, *Forpe*Tf1208, and Fo5176, respectively. These protein-encoding genes might be forma specialis-specific genes contributing to host specificity.

Data Availability

This Whole Genome Shotgun project has been deposited in the DNA Data Bank of Japan/European Nucleotide Archive (DDBJ/ENA)/GenBank under accession number JAE-LUR000000000 (BioProject number PRJNA686475, BioSample number SAMN17118324) for *Forpi*Tf1262 and accession number JAELUQ000000000 (BioProject number PRJNA686475, BioSample number SAMN17118325) for *Forpe*Tf1208. Raw Illumina sequencing reads for RNA sequencing have been deposited in DDBJ/ENA/GenBank under accession number PRJNA686475. These genome-sequenced strains have been deposited at the Japan Collection of Microorganisms (JCM), RIKEN BioResource Research Center, Tsukuba, Japan under accession numbers JCM39197 for *Forpi*Tf1262 and JCM39198 for *Forpe*Tf1208.

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Author-Recommended Internet Resources

1,000 Fungal genomes project:

<http://1000.fungalgenomes.org/home/wp-content/uploads/2013/02/genomicDNAProtocol-AK0511.pdf>

RepeatMasker v.4.0.7: <http://www.repeatmasker.org/>

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