Chromosome-Scale Genome Assembly of *Fusarium oxysporum* Strain Fo47, a Fungal Endophyte and Biocontrol Agent

Bo Wang,¹ Houlin Yu,^{2,3} Yanyan Jia,¹ Quanbin Dong,⁴ Christian Steinberg,⁵ Claude Alabouvette,⁵ Veronique Edel-Hermann,⁵ H. Corby Kistler,⁶ Kai Ye,^{1,4,†} Li-Jun Ma,^{2,3,†} and Li Guo^{1,4,†}

¹ MOE Key Laboratory for Intelligent Networks & Network Security, Faculty of Electronic and Information Engineering, Xi'an Jiaotong University, Xi'an 710049, China

² Graduate Program of Plant Biology, University of Massachusetts Amherst, Amherst, MA 01003, U.S.A.

³ Department of Biochemistry and Molecular Biology, University of Massachusetts Amherst, Amherst, MA 01003, U.S.A.

⁴ School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049 China

⁵ Agroécologie, AgroSup Dijon, INRA, University of Bourgogne Franche-Comté, F-21000 Dijon, France

⁶ USDA ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, MN 55108, U.S.A.

Abstract

Here, we report a chromosome-level genome assembly of *Fusarium oxysporum* Fo47 (12 pseudomolecules; contig N₅₀: 4.52 Mb), generated using a combination of PacBio long-read, Illumina paired end, and high-throughput chromosome conformation capture sequencing data. Although *F. oxysporum* causes vascular wilt to over 100 plant species, the strain Fo47 is classified as an endophyte and is widely used as a biocontrol agent for plant disease control. The Fo47 genome carries a single accessory chromosome of 4.23 Mb, compared with the reference genome of *F. oxysporum* f. sp. *lycopersici* Fo4287. The high-quality assembly and annotation of the Fo47 genome will be a valuable resource for studying the mechanisms underlying the endophytic interactions between *F. oxysporum* and plants as well as for deciphering the genome evolution of the *F. oxysporum* species complex.

Genome Announcement

The filamentous ascomycete fungus *Fusarium oxysporum* represents a species complex of diverse members that cause soil-borne vascular wilt diseases to over 100 plant species in a host-specific fashion (Edel-Hermann and Lecomte 2019; Kistler 1997; Ma et al. 2013). Strains pathogenic to specific host species are classified as *formae speciales* (f. sp.), and, together with related nonpathogenic strains, they are collectively known as the *F. oxysporum* species complex (Edel-Hermann and Lecomte 2019; Kistler 1997; Michielse and Rep 2009). Although notorious as plant pathogens, *F. oxysporum* strains are commonly plant endophytes often beneficial to host plants (Alabouvette et al. 2009; de Lamo and Takken 2020). Fo47, a strain isolated from plant disease-suppressive soils (Alabouvette 1986), grows endophytically and is widely used as a biological control agent for preventing vascular wilt of tomato, caused by *F. oxysporum* f. sp. *lycopersici* (Alabouvette et al. 2009), and other plant diseases as well (He et al. 2002; Paparu et al. 2007; Veloso and Díaz 2012). In general, beneficial microbes

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Keywords

biocontrol, fungal endophyte, *Fusarium oxysporum*, genome assembly, plant-fungal interactions

[†]Corresponding authors: K. Ye; kaiye@xjtu.edu.cn, L.-J. Ma; lijun@biochem.umass.edu, and L. Guo; guo_li@xjtu.edu.cn

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contribute to the fitness of host plants, potentially through diverse mechanisms including induced systemic resistance, promoting nutrient assimilation, and conferring antagonisms against microbial pathogens (Alabouvette et al. 2009; Aimé et al. 2013; de Lamo and Takken 2020; Pieterse et al. 2014; Van der Ent et al. 2009). The biocontrol effect of Fo47 is widely exploited in agriculture, but the molecular mechanisms behind it remain poorly understood. Decoding the genome of Fo47 will provide a valuable resource, not only facilitating dissection of such mechanisms but also contributing to comparative genomic studies targeted toward understanding the genome evolution of the *F. oxysporum* species complex in terms of endophytism, pathogenesis, and host specificity. A previous genome assembly was deposited at the National Center for Biotechnology Information (NCBI) under accession number GCA_000271705.2.

High-molecular weight genomic DNA was extracted from 4-day-old Fo47 mycelia using the CTAB (cetyltrimethylammonium bromide) method. A total of 6.85 Gb of paired-end short sequence reads (2×150 bp) and 18.09 Gb of single-molecule long-read sequence reads were generated, using the Illumina NovaSeq 6000 and PacBio SMRT RS II platforms, respectively. K-mer (17 mer) frequency analysis of 6.85 Gb of Illumina reads (138× coverage) using the Jellyfish-GenomeScope pipeline with default parameters (Marçais and Kingsford 2011; Vurture et al. 2017) revealed an estimated genome size of 49.54 Mb for Fo47. The 18.09-Gb (341× coverage) PacBio SMRT data (1.24 million reads) has a read length N_{50} of 18,156 bp after filtering. We then assembled a Fo47 genome from the PacBio reads using the assembler NextDenovo v2.0, with parameters 'read_cutoff = 1k' and 'seed_cutoff = 34,834'. The PacBio assembly was further polished by Illumina PE reads using Pilon v. 1.22 with parameters '-changes-vcf-frags' (Walker et al. 2014) to correct 2,040 (0.004%) erroneous positions. To make the chromosome-scale assembly, the contigs were scaffolded using chromatin contact maps produced by high-throughput chromosome conformation capture (Hi-C) reads. Basically, a single Hi-C library prepared from cross-linked chromatin of fungal cells, using a standard Hi-C protocol (Belton and Dekker 2015), were sequenced by NovaSeq 6000 to yield 5.76 Gb (115× coverage) Illumina PE reads. The Hi-C data were used to anchor all contigs on 12 pseudomolecules (chromosomes), using Juicer v. 1.5 with default parameters (Durand et al. 2016b) and Juicebox v. 1.11.08 (Durand et al. 2016a), followed by manual checks. The number of chromosomes was determined by centromeric interaction regions detected in Hi-C contacted map figures (Marbouty et al. 2014).

The final assembly of the strain Fo47 genome is 50.36 Mb placed in 12 chromosomes, proximate to its estimated genome size. Each chromosome is represented in a single contig, with a contig N_{50} of 4.52 Mb, demonstrating the high continuity of this assembly (Table 1). Of 12 chromosomes, nine had more than three copies of the telomeric repeat unit TTAGGG on both ends, whereas the other three contained those copies on only one end, indicating a high-quality Fo47 genome assembly of chromosome level. Compared with a previous Fo47 genome assembly (GenBank accession GCA_000271705.2) at NCBI (Table 1), the most important improvement concerns chromosome 7, as 73 contigs (3.96 Mb) of the initial assembly can be aligned to the single 4.23-Mb Chr7 (telomeric repeats on both ends) of the new assembly, using Mummer v. 3.1 with default parameters (Kurtz et al. 2004).

For genome annotation, GeneMark-ES with parameters '-ES-fungus' (Borodovsky and Lomsadze 2011) was first used for de novo gene-structure prediction. A *Fusarium*-specific gene model was then used to train Augustus v. 3.1 (Stanke and Morgenstern 2005). MAKER2 pipeline (Cantarel et al. 2008) was used to find protein-coding genes integrating gene models predicted from GeneMark-ES and Augustus and protein sequences of the *F. oxysporum* reference genome of a tomato-pathogenic isolate, Fol4287 (GenBank assembly accession GCA_000149955.2) (Ma et al. 2010), with the RepeatMasker v. 4.0.7 (Tarailo-Graovac and Chen 2009) option on to find and mask repetitive elements. In total, the Fo47 genome has 17,127 protein-coding genes and 3.42% repeat content (Table 1). The genome assembly completeness was assessed by BUSCO (benchmarking universal single copy ortholog) v. 1.22 with parameters '-m OGS' (Simão et al. 2015), using proteome sequences showing BUSCO score of 99% based on the 'fungi_odb9' library (Table 1), suggesting a high degree of completeness for this Fo47 genome assembly.

Compared with the reference genome Fol4287 (Ma et al. 2010), Fo47 has a smaller genome size and fewer genes and repeat elements (Table 1). A whole-genome sequence alignment between Fo47 and Fol4287 using Mummer v. 3.1 with default parameters (Kurtz et al. 2004) reveals that the size of sequence mapped to the core genome of Fol4287 (11

Table 1. Summary of the genome assembly and annotation statistics of *Fusarium oxysporum* Fo47 compared with a previous assembly of Fo47 (GenBank accession GCA_000271705.2) and *F. oxysporum* f. sp. *lycopersici* Fol4287 (GenBank accession GCA_000149955.2)

Strains	Fo47 (this study)	Fo47 (GCA_000271705.2) ^a	Fol4287 ^b
Number of contigs	12	124	88
Number of chromosomes	12 ^c	NA	15
Number of lineage-specific chromosomes	1	NA	4
Genome size (Mb)	50.36	49.67	61.47
GC content (%)	47.71	47.68	48.48
Contig N ₅₀ (bp)	4,523,040	3,844,136	4,589,937
Number of protein-coding genes ^d	17,127	16,985	17,628
Repeat content (%) ^d	3.42	2.77	5.43
Predicted proteome completeness (%) ^e	99	99	98

^a NA = unavailable.

^b Reported by Ma et al. (2010).

^c Number of putative centromeres was determined using Hi-C contact map.

^d Protein-coding genes and repeat content were identified by the same pipeline (this study) to ensure a fair comparison.

^e Completeness = (total BUSCOs – missing BUSCOs)/total BUSCOs × 100.

chromosomes) (Ma et al. 2010) was 46.10 Mb, suggesting a set of 11 core chromosomes in Fo47. A single Fo47 chromosome, Chr7 (4.23 Mb), did not align to any Fol4287 chromosome, indicative of a lineage-specific chromosome in Fo47. Chr7 is highly enriched for transposable elements (TEs), with 51% of total TE sequences located on Chr7 and 49% located on the rest of genome. Overrepresentation of repetitive sequences in this chromosome explains the fragmentation in the initial assembly. Chr7 has a smaller average gene density (290 per megabase) compared with core chromosomes (347 per megabase). The 1,229 genes on Chr7 are significantly enriched (P value = $1.5e-4$) for regulators of G-protein signaling functions, suggesting their likely role in external sensing and organism response to external nutrient sources and other environmental signals (Bayram and Braus 2012; Kim et al. 2017). This genome assembly of Fo47 will be a useful resource for comparative genomics of *F. oxysporum* and future functional genomic studies of Fo47 and host endophytic interactions. This genome project is indexed at GenBank under BioProject accession number PRJNA624237. The chromosomal sequence and gene annotation of Fo47 can be found at GenBank with accession numbers CP052038 to CP052049. PacBio reads, Illumina reads, and Hi-C Illumina data are available in the NCBI Sequenced Read Archive under the accession numbers SRR11526760, SRR11523115, and SRR11523120, respectively.

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Author-Recommended Internet Resource

NextDenovo: <https://github.com/Nextomics/NextDenovo>

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