Genome Sequence of *Venturia oleaginea*, the Causal Agent of Olive Leaf Scab

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Abstract

Olive leaf scab, also known as peacock spot disease, caused by *Venturia oleaginea* (syn. *Spilocaea oleaginea* and *Fusicladium oleagineum*) is the most widespread and economically important fungal disease attacking olive in production countries. Here, we report the first highly contiguous whole-genome sequence (46.08 Mb) of one isolate, YUN35, of *V. oleaginea*. The described genome sequence and annotation resource will be useful to study the fungal biology, pathogen-host interaction, characterization of genes of interest, and population genetic diversity.

Keywords

fungal pathogen, genome, olive leaf scab disease, *Venturia oleaginea*

Genome Announcement

Olive leaf scab, also known as olive leaf spot or peacock spot disease, is a fungal scab disease specific to olive trees. *Venturia oleaginea* (Castagne) Rossman & Crous (synonyms *Spilocaea oleaginea* (Castagne) S. Hughes and *Fusicladium oleagineum* (Castagne) Ritschel & U. Braun), the causal agent is a pathogenic fungus belonging to the phylum Ascomycota, Pleosporales, Venturiaceae. The fungus was reported for the first time in France (Castagne 1845) and is widespread in the Mediterranean olive-producing countries as well as other countries with the same weather conditions. The pathogen, which has a biotrophic mode of nutrition, can cause early defoliation of olive trees, leading to poor growth and yield reduction (López-Doncel et al. 2000). Yield losses may reach up to 20% (Obanor et al. 2010). Trees with a dense canopy increase the humidity in the grove, which increases the prevalence of leaf scab in wet weather conditions (Sanei and Razavi 2011).

Knowledge of this pathogen at the molecular level is poor, due to the technical challenges of working with a biotrophic organism. *V. oleaginea* is difficult to isolate, taking up to 6 months to culture sufficient quantities of mycelium for DNA isolation, and it rarely produces conidia on artificial media (Chen et al. 1982; González-Lamothe et al. 2002; Obanor et al. 2010). No sexual stage of *V. oleaginea* is currently known, although indirect evidence of recombination comes from the observation that all isolates collected in the major olive-growing regions of Uruguay have unique genotypes (Bernaschina et al. 2020; Graniti 1993; Viruega et al. 2013).

Until now, seven members of the genus *Venturia* have been sequenced, including *V. effusa*, *V. inaequalis*, *V. asperata*, *V. pirina*, *V. Nashicola*, *V. aucupariae*, and *V. carpophila*, the causal pathogens of pecan scab, apple scab, pear scab, sorbus scab, peach scab (Bock

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Table 1. Statistics of the whole-genome assemblies of *Venturia oleaginea* isolate YUN35

Assembly parameters	YUN35
Sequencing platform	PacBio sequel
Assembly method	HGAP 4
Genome size (Mb)	46.08
Sequencing coverage	143x
Number of contigs	61
Average contig length (Mb)	0.76
Contig N ₅₀ (Mb)	1.80
Maximum contig length (Mb)	2.54
Contigs with telomere repeats (TTAGGG) <i>n</i> /(CCCTAA) <i>n</i> /both	16/10/2
% GC content	48.62
% Repeats	23.02
BUSCO's genome complete in ascomycota (<i>n</i> = 1,315)	Genome: 94.5%, gene: 95.7%
BUSCO's genome complete in fungi (<i>n</i> = 290)	Genome: 96.9%, gene: 96.2%
Genes	11,540
Candidate secreted proteins	581
Annotated genes	9,516

et al. 2016; Chen et al. 2017; Johnson et al. 2019; Le Cam et al. 2019; Passey et al. 2018; Prokhorchik et al. 2019; Winter et al. 2020). Genome sequencing of *V. oleaginea* will provide greater options for more effective identification of the fungus, the study of genes of interest, such as those involved in host recognition, fungicide resistance, and mating type genes, and the development of specific molecular markers for population genetic diversity analysis.

V. oleaginea monospore isolate YUN 35 was isolated from an infected olive leaf (cv. Pecual) collected from the Yunnan Academy of Forestry garden, Kunming, Yunnan, China, and was cultured with olive leaf extract agar media as described by Obanor et al. (2010). The colony morphology of isolate YUN35 is circular, convex, grayish green, texture floccose, reverse black. These are similar to the morphological traits described by Benkada et al. (2015). DNA was extracted using the CTAB method, and strain identification was confirmed based on the DNA sequence of the internal transcribed spacer (ITS) amplified with primers ITS4 and ITS5 (White et al. 1990), the partial small subunit nuclear ribosomal DNA (SSU) region amplified with primers NS1 and NS4 (White et al. 1990), and the partial larger subunit nuclear ribosomal DNA (LSU) region amplified with LROR and LR5 (Vilgalys and Hester 1990).

The isolate YUN35 was sent for sequencing, at Beijing Novogene Bioinformatics Technology Co. Ltd., using the PacBio Sequel platform. Single molecule real-time (SMRT) cell data were generated, the low-quality reads with SMRT link (v6.0.) were filtered, and finally, 6.58 Gb of long-read data (143x genome coverage, N₅₀ 11 kb, and average length 7 kb) (Table 1) was obtained. We then conducted de novo genome assembly using HGAP4 (expected genome length = 45 Mb), a pipeline in SMRT Link. The preassembly was polished, using the Arrow algorithm, by mapping raw long reads back to the preassembled genome. A genome sequence of 46.08 Mb was assembled into 61 contigs with N₅₀ of 1.80 Mb, maximum contig length of 2.54 Mb, and average contig length of 0.76 Mb (Table 1). Among these contigs, 16 and 10 contigs contain telomere repeats (TTAGGG)*n* and (CCCTAA)*n*, respectively, at the distal end. Interestingly, two contigs of 1.90 and 1.83 Mb have telomere repeats at both ends, which indicates that these two contigs represent entire chromosomes (Table 1). Based on telomere representation, we conclude that *V. oleaginea* has at least 16 chromosomes, which is similar to the number of 20 chromosomes identified in a recent chromosome level assembly for *V. effusa* isolate FRT5LL7 (Winter et al. 2020). The completeness of the YUN35 assembly was assessed using BUSCO v3.0 (benchmarking universal single-copy orthologs) (Waterhouse et al. 2018). Evaluation with the ascomycota data set (*n* = 1,315) showed 94.5% completeness with 1,243 complete, 40 fragmented, and 32 missing BUSCO orthologs. Whereas evaluation with the fungal dataset (*n* = 290) showed 96.2% completeness with 281 complete, three fragmented, and six missing BUSCO orthologs. The repeat sequences were identified by Repeat Masker v4.0.9, using a de novo repeats library generated by RepeatModeler v1.08. Approximately 23.02% of the genome was associated with repetitive sequences. The soft repeat masked assembly was used for the gene predictions using AUGUSTUS v3.3.3 (Stanke et al. 2008), integrating the RNA-seq data

of the isolate YUN35 mycelia cultured in potato dextrose broth in the dark, with shaking, at 18°C. A total of 11,540 genes were found combining RNA-seq and the de novo strategy. The BUSCO completeness assessment at the gene level showed that 95.7% completeness with the ascomycota data set ($n = 1,315$) and 96.2% completeness with the fungi dataset ($n = 290$). Gene annotation analysis showed that there were 581 candidate secreted proteins (Table 1) identified following our previous pipeline (Bao et al. 2017), and a total of 9,516 genes (82%) can be annotated by the Pfam 32.0, Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, and EggNOG databases.

Data availability. The ITS, SSU, and LSU sequences of YUN 35 have been deposited in GenBank under accession numbers MT379576, MT379578, and MT379577, respectively. The genome Sequence has been deposited into NCBI under the BioProject accession number PRJNA595014 and the DDBJ/ENA/GenBank accession number is JABMLP000000000.

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Author-Recommended Internet Resources

Arrow algorithm: <https://github.com/PacificBiosciences/GenomicConsensus>
EggNOG database: <http://eggnog5.embl.de>
Gene Ontology resource: <http://geneontology.org>
Kyoto Encyclopedia of Genes and Genomes database: <https://www.kegg.jp>
Pfam 32.0: <http://pfam.xfam.org>

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