Genome Resource for Two *Stemphylium vesicarium* Isolates Causing Stemphylium Leaf Blight of Onion in New York

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Abstract

Stemphylium leaf blight caused by *Stemphylium vesicarium* was recently identified as an emerging disease and dominant in the foliar disease complex affecting onion in New York. Here, we report the genomes of two isolates of *S. vesicarium*, On16-63 and On16-391. The availability of the genomes will accelerate genomic studies of *S. vesicarium*, including population biology, sexual reproduction, and fungicide resistance. Additionally, comparative genomics with the other published genome of *S. vesicarium* causing brown spot of pear will help understand pathogen biology and underpin the development of management strategies for this disease.

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Keywords

Allium cepa, foliar disease

Genome Announcement

Stemphylium vesicarium causes disease of a broad range of economically important plants including vegetables, herbaceous crops, and fruit trees (Falloon et al. 1987; Lamprecht et al. 1984; Rossi et al. 2005; Suheri and Price 2000). Stemphylium leaf blight (SLB) caused by *S. vesicarium* was first reported in the United States in Texas in 1976 (Miller et al. 1978) and was usually considered a minor component of the foliar disease complex affecting onion worldwide but has recently emerged as dominant in New York (Hay et al. 2019) and Ontario, Canada (Foster et al. 2019; Paibomesai et al. 2012). This has been attributed to the development of resistance against commonly used fungicides (Hay et al. 2019). Despite the importance of SLB on onion, genomic resources and population biology studies on *S. vesicarium* are limited. The first genome of *S. vesicarium* associated with brown spot of pear was only recently released (Gazzetti et al. 2019). To accelerate genomic studies, develop tools to understand the population biology and reproduction, and identify genes responsible for fungicide resistance, the genomes of *S. vesicarium* isolates On16-63 and On16-391, associated with SLB epidemics of onion in New York (Hay et al. 2019), were sequenced and assembled.

S. vesicarium On16-63 and On16-391 were isolated from SLB on onion from New York in 2016. The identity of the isolates was confirmed through multilocus sequencing and phylogenetic analysis (Hay et al. 2019). DNA was extracted from single-spore cultures using a modified CTAB extraction protocol (Leslie and Summerell 2006). DNA quantity was assessed with a Nanodrop ND 1000 spectrophotometer and quality with a Qubit fluorometer. Genomic

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Table 1. Genome features of *Stemphylium vesicarium* isolates On16-63 and On16-391 from *Stemphylium* leaf blight of onion compared with the published genome of *Stemphylium vesicarium* 173-1a13F1M3 associated with brown spot of pear (Gazzetti et al. 2019)

Features	On16-63	On16-391	173-1a13F1M3
Number of contigs	1,734 (>500 bp)	2,395 (>500 bp)	1,127 (>0 bp)
N50 (bp)	118,769	70,493	167,978
L50 (bp)	92	150	63
Largest contig (bp)	644,906	572,557	974,405
Assembly length (all contigs)	40,827,444	39,846,678	38,664,579
Genome coverage	97x	110x	60x
GC content (%)	50.68	51.04	50.72
BUSCO completeness (%)	98.6	98.7	96
Predicted genes	13,064	12,810	12,309
tRNAs	380	366	N/A

DNA (5 µg for each isolate, On16-63 and On16-391) was submitted to the Cornell University Institute of Biotechnology Genomics Facility, Ithaca, New York, for PCR-free library preparation. Libraries were prepared with a TrueSeq Nano DNA library preparation kit, and 2x 300-bp paired-end sequencing was performed using an Illumina MiSeq. Both isolates were sequenced within a single lane. The quality of the reads was determined with FastQC v 0.11.8 (Andrews 2010). Poor-quality reads with an average quality score less than 30 were trimmed and discarded with Trimmomatic version 0.36 (Bolger et al. 2014). High-quality reads were assembled with AbySS version 1.9.0 (Simpson et al. 2009). QUAST-LG (Mikheenko et al. 2018) was used to predict the size of the genomes and sequencing coverage. The completeness and quality of the assembled genomes was analyzed with Benchmarking Universal single-copy orthologs (BUSCO v 3.1.0) (Simão et al. 2015). Putative genes in the isolates were predicted with AUGUSTUS version 3.3.2 (Stanke and Morgenstern 2005), using gene sets of *Fusarium graminearum*. tRNAscan-SE version 1.3.1 (Lowe and Chan 2016) was used to predict the number of transfer RNAs (tRNAs) in the genomes. To identify the loci associated with the mating types and resistance against commonly used fungicides, BLAST databases for the *S. vesicarium* isolates On16-63 and On16-391 were created, using Geneious version 11.0.5 (Kearse et al. 2012). Nucleotide sequences of *cytochrome b*, *hexokinase*, *succinate dehydrogenase* (SDHB, C, D) and *cyp51* from *Stemphylium* or closely related *Alternaria* species were retrieved from GenBank and searched as queries against the local BLAST database created for isolates On16-63 and On16-391. Similarly, sequences of the mating type (MAT) genes previously deposited in GenBank (AY335178.1) (Inderbitzin et al. 2005) were downloaded and searched as queries against the database created for the isolates.

A single-lane Illumina MiSeq 2x 300 sequencing of the libraries produced 2x 8.47 million reads and 2x 9.81 million reads for *S. vesicarium* isolates On16-63 and On16-391, respectively. The genomes were assembled into 1,734 (On16-63) and 2,393 (On16-391) contigs greater than 500 base pairs with a coverage of approximately 97x and 103x (Table 1). The estimated genome sizes were 40.83 Mb and 39.85 Mb, with a GC content of 50.68% (On16-63) and 51.04% (On16-391). The predicted sizes of the genomes were similar to a *S. vesicarium* isolate from pear (Gazzetti et al. 2019). For On16-63, the N50 value was 118,769 and the largest contig was 644,906 bp. For On16-391, the N50 value was 70,493 and the largest contig was 572,557 bp. BUSCO analysis predicted the genome assemblies to be 98.7 and 98.6% complete for isolates On16-63 and On16-391, respectively. AUGUSTUS predicted 13,064 genes for the assembled genome of isolate On16-63 and 12,810 genes for isolate On16-391. Based on the results of tRNAscan-SE, 380 and 366 tRNAs were predicted in the assembled genomes of isolates On16-63 and On16-391, respectively. Furthermore, BLAST analyses of genes for fungicide resistance against the genomes successfully identified different loci associated with some commonly used fungicides. Both *S. vesicarium* isolates contained fused MAT1-1 and MAT1-2 genes in reverse orientation, a feature previously demonstrated in some *Stemphylium* spp. (Inderbitzin et al. 2005). Single copies of MAT1-1 and MAT1-2 were identified in both genomes.

To the best of our knowledge, this is the first report of the genome of *S. vesicarium* causing SLB of onion. These genomes will be a useful resource to enable pathogen and population biology studies and to develop molecular-based tools for fungicide resistance monitoring.

The *S. vesicarium* isolates were deposited in the International Collection of Microorganisms from Plants (ICMP) (Auckland, New Zealand) (On16-63 = ICMP 23434; On16-391 = ICMP 23435). The whole genome shotgun projects have been deposited at DDBJ/ENA/GenBank under accessions VHPT00000000 and VHPU00000000. Draft assemblies described herein are versions VHPT01000000 and VHPU01000000 for isolates On16-63 and On16-391, respectively. Raw sequencing reads are available in GenBank as SRA accession PRJNA549936.

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Author-Recommended Internet Resource

Geneious version 11.0.5: <https://www.geneious.com>

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