Genome Sequence Resource for *Stemphylium vesicarium*, Causing Brown Spot Disease of Pear

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Abstract

Stemphylium vesicarium is the causal agent of several plant diseases as well brown spot of pear (BSP), which is one of the most economically important fungal diseases in European pear-production areas. In addition to the relevance of the economic impact, conidia spread widely from plant material infected by the pathogen can trigger respiratory allergy. Here, we report the first genome of a *S. vesicarium* strain, 173-1a13F11M3, isolated from pear and sensitive to the mostly used fungicide classes currently authorized in Europe against BSP. The availability of this draft genome could represent a first important step in understanding the physiology and the infection mechanism of the pathogen. Furthermore, this contribution could be fundamental in order to design more effective and sustainable strategies to control the disease.

Genome Announcement

Stemphylium vesicarium (Wallr.) E. G. Simmons belongs to the class Dothideomycetes, order Pleosporales, and its teleomorph is *Pleorospira allii* (Rabenh.) Ces. & De Not (Simmons 1969). This fungus can cause disease of noncrop plants, herbaceous crops, and fruit trees (Falloon et al. 1987; Lamprecht et al. 1984; Rossi et al. 2005; Suheri and Price 2000). Brown spot of pear (BSP), caused by *S. vesicarium*, is the most economically relevant fungal disease for many varieties of pear in Italy and in other European countries, because it may cause loss of production up to 100% (Llorente et al. 2012). During the infection process, the germinating conidia produce two host-specific toxins, SV-I and II (Singh et al. 1999), able to induce ultrastructural changes in the plasma membrane of susceptible leaf cells (Singh et al. 2000). Throughout the season, both leaves and fruit can be damaged and, thus, many fungicide applications are required from petal fall to fruit ripening to protect pear orchards (Alberoni et al. 2010b). The fungus has been shown to be capable of developing field resistance toward key products such as dicarboximides, fludioxonil (Alberoni et al. 2005; Gazzetti et al. 2017) and strobilurins (Alberoni et al. 2010a). Furthermore, it is known that conidia of *Stemphylium* spp. can induce respiratory allergy (Sáenz-de-Santamaría et al. 2006; Weber 2015). Therefore, understanding the *S. vesicarium* genome sequence is a fundamental step toward increasing our knowledge about this important organism, both for plant protection and human health.

S. vesicarium strain 173-1a13F11M3 was grown from a single conidium, isolated from a culture originally sampled on 2 August 2013 from leaves of pear affected by BSP, belonging to the highly BSP susceptible cultivar Abate Fétel collected in an untreated orchard located in

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Table 1. Core metrics of the *Stemphylium vesicarium* 173-1a13FI1M3 genome draft and average nucleotide identity (ANI) comparison between *S. vesicarium* 173-1a13FI1M3 and other plant pathogens

Genome features		ANI comparison			
Core metrics, pathogens	Numbers	Strain	Accession ^a	NUCmer ^b	Blast ^c
Metrics					
Number of contigs (≥0 bp)	1,127	...	...	...	...
Number of contigs (≥25,000 bp)	278	...	...	...	...
N50 (bp)	167,978	...	...	...	...
L50 (bp)	63	...	...	...	...
Largest contig (bp)	974,405	...	...	...	...
Assembly length (bp)	38,664,579	...	...	...	...
Genome coverage	60x	...	...	...	...
CG content (%)	50.72	...	...	...	...
BUSCO completeness (%)	96	...	...	...	...
Number of predicted genes	12,309	...	...	...	...
Number of coding sequences ^d	12,230	...	...	...	...
Pathogens					
<i>Stemphylium vesicarium</i>	...	173-1a13FI1M3	QXCR000000000	1.000	1.000
<i>Tuber melanosporum</i>	...	Mel28	ASM15164v1	0.0001	0.003
<i>Botrytis cinerea</i>	...	B05.10	ASM14353v4	0.0006	0.017
<i>Pyrenophora teres</i>	...	W1-1	OCTH01000000	0.0834	0.330
<i>Alternaria alternata</i>	...	SRC1lrK2f	LXPP01000000	0.1195	0.608
<i>Stemphylium lycopersici</i>	...	CIDEFI 216	LGLR01000000	0.7374	0.774

^a Accession number.

^b ANI-NUCmer values.

^c ANI-Blast values.

^d Number of predicted complete coding sequences.

Altedo (Emilia-Romagna region, Italy). This strain is deposited at the CBS-KNAW culture collection (accession number CBS 145331; Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands). The deposited culture is sensitive to dicarboximides, fludioxonil, strobilurins, and succinate dehydrogenase inhibitor (SDHI) fungicides, as verified by biological assays conducted as described by Alberoni et al. (2005, 2010a,b) and Ciriani et al. (2016). The sensitivity of strain 173-1a13FI1M3 to these fungicides was confirmed by Sanger sequencing. Indeed, wild-type alleles were obtained by sequencing of *SvHK1* and the *S. vesicarium* orthologous of *cyt b* and of the SdhB, -C, and -D subunit encoding genes, involved in the resistance toward dicarboximides and fludioxonil (Alberoni et al. 2010b), strobilurins (Alberoni et al. 2010a), and SDHI fungicides (unpublished data), respectively.

The genomic DNA of *S. vesicarium* strain 173-1a13FI1M3 was isolated from fresh mycelium, following a cetyltrimethylammonium bromide-based method (Alberoni et al. 2010a), with modifications: nucleic acids were separated by chloroform/isoamyl alcohol (24:1) and precipitated in isopropanol (1V) and 3 M NaAc, pH 5.2 (0.1V). The pellet was dissolved in 10 mM Tris-HCl, pH 8, and yield, purity, and integrity of DNA were accurately determined by gel electrophoresis and by both spectrophotometric and fluorometric quantification. Shotgun libraries were prepared following the manufacturer's recommendation for the TrueSeq DNA Sample Preparation Kit, and Illumina MiSeq v3 was used to perform the 2 × 300-bp paired-end sequencing, producing 2 × 23,806,218 paired-end sequences (2 × 6.45 Gb). Library preparation and sequencing was done by BMR Genomics (Padua, Italy). The obtained raw sequences were cleaned up and filtered in order to discard reads with average quality of phred score lower than 30 by Trimmomatic (Bolger et al. 2014). The genome was de novo assembled in 1,127 contigs by SPAdes 3.6 (Bankevich et al. 2012). QUAST was used to assess assembly quality (Gurevich et al. 2013). Total assembly length was 38.66 Mb (genome coverage 60x), similar to the genome assembly size of *S. lycopersici* (Franco et al. 2015), with a GC content of 50.72% and a N50 value of 167,978. The longest contig consisted of 974,405 bp.

Average nucleotide identity (ANI) is proposed as the appropriate in silico substitute for DNA-DNA hybridization and, thus, is useful for delineating species boundaries (Richter and Rosselló-Móra 2009). ANI was calculated between the *S. vesicarium* 173^{1a}_{13FI1M3} genome and other filamentous plant pathogens, some of which belonging to the order Pleosporales. In order to achieve a wider vision of overall genomes relatedness, the ANI calculation was made by both MUMmer2 NUCmer (Delcher et al. 2002) and NCBI BLAST (Goris et al. 2007), based

on two different algorithm structures. *Tuber melanosporum* was used as the outgroup. Obtained ANI values are reported in Table 1 together with the core metrics of the *S. vesicarium* 173^{1a}_{13FI1M3} genome.

For de novo gene prediction, PASA (Haas et al. 2003) was used as the ab initio gene finding for AUGUSTUS 3.2.1 (Stanke and Morgenstern 2005) and GENEMARK (Borodovsky and Ekisheva 2006). Results showed 12,309 genes, of which 12,230 were complete coding sequences that covered 44.6% of the genome sequence. Mean gene length was 1,585 bp and the mean number of exons per gene was 3. The prediction was analyzed by BUSCO v12 (Simão et al. 2015) in order to evaluate the completeness with a set of common fungal single-copy orthologs, estimating the genome to be 96% complete. A primary functional annotation was made using the hierarchical orthology framework eggNOG 4.5 (Huerta-Cepas et al. 2016) providing the annotations of orthologous groups, as well gene ontology (GO) terms, KEGG pathways, and SMART/Pfam domains for each group. Combined prediction of transmembrane topology and signal peptide was carried out by Phobius (Käll et al. 2004). Genome sequence was analyzed by AntiSMASH 3.0 fungal version (Weber et al. 2015), for the automatic genomic identification and analysis of biosynthetic gene clusters.

Overall, the annotation analysis predicted 40 genes described by the GO term defining involvement in pathogenesis (GO 0009405). Searching the PHI-base for possible orthologs of pathogenicity genes from other plant pathogens, at default setting, identified three matches involved in appressorial development and function: the orthologs of *MoERR1* (Goh et al. 2017), *MGG_04128* (Jeon et al. 2007), and the tetraspanine *CIPLS1* (Veneault-Fourrey et al. 2005). In total, 521 genes were predicted as coding for carbohydrate-active enzymes; 77 of them are defined as components located in an extracellular region (GO 0005576) and putatively code for glycoside hydrolase (GH), auxiliary activity, polysaccharide lyase, and carbohydrate esterase enzymes families, all classes that are considered cell-wall-degrading enzymes and may have a role in plant infection (Hou et al. 2016; Walton 1994). Among members of the GH11 family, an orthologous gene for *xyn11A* was predicted, an effector of the necrotrophic fungus *Botrytis cinerea* (Noda et al. 2010). The annotation process classified 553 predicted genes as involved in secondary metabolism. These genes could be investigated for a possible role in several biosynthetic pathways, with particular interest directed to those leading to the production of secondary metabolites related to fungal pathogenesis and virulence such as the SV-I and II HSTs that have been little studied to date. In total, 444 predicted genes have functions associated with signal transduction mechanisms and, in particular, 4 are predicted to encode for histidine kinases and could be candidate targets for fungicides inhibiting signaling in osmoregulation pathway (Alberoni et al. 2010b). Moreover, the annotation was able to detect the orthologs of *alt a 1 major* and *alt a 7 minor* allergens (Gabriel et al. 2016), probably involved in respiratory allergies triggered by *Stemphylium* conidia (Agarwal et al. 1982; Sáenz-de-Santamaría et al. 2006).

The draft genome presented here is the first available genome sequence of *S. vesicarium*, and only the second of the *Stemphylium* genus. Knowledge of this genome sequence opens a new scenario in the investigation of fungal lifestyle, epidemiology, and molecular plant-pathogen interaction. The sequence of *S. vesicarium* strain 173-1a13FI1M3 could be used in comparative studies and, as reference genome, to discover mutations in fungicide target genes of resistant strains. Therefore, this resource could improve the possibility of an integrated control of BSP and of other plant infections. Moreover, the new data available could make it possible to investigate *S. vesicarium* as a potential allergenic fungus in humans. The genome sequence has been deposited in the GenBank under accession number QXCR000000000 (BioProject: PRJNA470620, BioSample: SAMN09098503).

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