A High-Quality Genome Resource of *Botrytis fragariae*, a New and Rapidly Spreading Fungal Pathogen Causing Strawberry Gray Mold in the United States

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Abstract

Although *Botrytis fragariae* was only recently identified as a new *Botrytis* species that causes strawberry gray mold, its prevalence extends to many states of the eastern United States. Compared with *B. cinerea*, which is known to be the causal agent of gray mold on many crop plants including strawberry, *B. fragariae* appears to have specifically adapted to strawberry and exhibits distinct fungicide sensitivity. This is the first presentation of a high-quality genome assembly of *B. fragariae* with gene annotation based on sequence homology and deep transcriptome data. The genome sequence information from *B. fragariae* is expected to help reveal genomic features underlying its host specialization and evolution of distinct fungicide resistance and other novel pathogenicity mechanisms.

Genome Announcement

Gray mold is arguably the most important fungal disease in many strawberry growing regions worldwide (Petrusch et al. 2019). As the second largest strawberry producer in the world (FAOSTAT 2018), the United States produced 1.3 billion kilograms of strawberries in 2018, valued at \$2.7 billion (USDA-NASS 2018). The main causal agent of gray mold, *Botrytis cinerea*, was ranked as the second most important fungal pathogen by the plant pathology community (Dean et al. 2012) and was previously thought to be the sole causal agent of strawberry gray mold in the United States (Sutton 1998). However, a new species, *Botrytis fragariae*, has recently been identified to also cause gray mold on strawberry in the United States and Germany (Rupp et al. 2017). A recent study has shown that 35.1% of the 188 *Botrytis* isolates collected in five eastern U.S. states were *B. fragariae* (Dowling et al. 2018), indicating that *B. fragariae* is widely spread in strawberry fields in this region.

Despite the wide distribution of *B. fragariae*, only a few studies have been conducted to characterize its biological features at the phenotypic level. Compared with *B. cinerea*, *B. fragariae* was slightly more aggressive on strawberry flowers albeit causing smaller lesions on leaves and fruits (Rupp et al. 2017). Interestingly, *B. fragariae* was unable to infect Gerbera petals, apple fruit, or tomato leaves, all of which can be readily infected by *B. cinerea*, suggesting that *B. fragariae* has a narrower host range and may have specifically adapted to strawberries (Rupp et al. 2017). There also are marked differences regarding fungicide sensitivity between the two *Botrytis* species. Single-site fungicides, including phenylpyrroles (PPs) and succinate dehydrogenase inhibitors (SDHIs), are key chemical classes for strawberry gray mold control. Our recent work showed that some U.S. *B. fragariae* isolates have higher levels of resistance to

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Table 1. A comparison of the *Botrytis fragariae* genome assembly and gene annotation with those of three other *Botrytis* species

Species	<i>B. fragariae</i>	<i>B. paeoniae</i> ^a	<i>B. tulipae</i> ^a	<i>B. cinerea</i> ^b
Scaffolds	28	1,833	653	18
Assembly size (Mb)	41.92	46.36	45.45	42.65
Largest scaffolds (Mb)	3.9	0.9	0.74	4.1
N50 (Mb)	2.43	0.13	0.17	na
BUSCO completeness	98.50%	99.00%	98.80%	99.80%
Predicted genes	12,506	12,138	12,656	13,703
smCOGs ^c	527	506	526	594
CAZyme ^d	452	435	453	508
Secreteome ^e	1,184	1,192	1,205	1,381
Effectorome ^f	154	145	153	173
Species specific effector	67	42	46	57

^a The assembly information is from Valero-Jimenez et al. (2019).

^b The genome sequence is from NCBI (*Botrytis cinerea* B05.10, assembly ASM14353V4).

^c Secondary metabolite clusters of orthologous groups.

^d CAZyme: annotated on dbCAN2 website (<http://bcb.unl.edu/dbCAN2/index.php>).

^e Secretome: proteins have signal peptide, but without a transmembrane domain.

^f Effectorome: predicted by software EffectorP.

fludioxonil compared with other resistant *Botrytis* isolates from Europe and the United States (Hu et al. 2019). These isolates overexpressed *atrB*, a gene that encodes a multidrug efflux pump belonging to the ABC transporter family (Vermeulen et al. 2001). Overexpression of *atrB* is normally linked to various activating mutations in the transcription factor *mrr1* in resistant *B. cinerea* or German *B. fragariae* isolates (Kretschmer et al. 2009; Rupp et al. 2017). However, no mutations in *mrr1* were found in the U.S. *B. fragariae* isolates with resistance to fludioxonil, warranting an investigation to reveal a possible novel mechanism (Hu et al. 2019). Furthermore, no resistance to boscalid (SDHI) has been found in *B. fragariae*, whereas an average of 20% of *B. cinerea* isolates were found to be resistant to boscalid (Hu et al. 2016). Perhaps resistance-conferring mutations in SDH subunit(s) carry a significant fitness cost in *B. fragariae* that make such isolates unable to compete with the endemic population. A high-quality genome sequence will be useful for understanding the genomic and molecular bases of the unique attributes of *B. fragariae*. Thus, we selected U.S. *B. fragariae* isolate BVB16 for genome sequencing and assembly, with the expectation that it will serve as the reference genome of *B. fragariae* for exploring the molecular mechanisms underlying its distinct host adaptation, fungicide resistance and other biological attributes.

We sequenced the *B. fragariae* genome using long read Pacific Biosciences technology (Rhoads and Au 2015). High molecular weight genomic DNA (~50 kb) was extracted using the previously published CTAB-PEG method protocols (Huang et al. 2018; Rhoads and Au 2015). Library preparation and PacBio sequencing were performed at the University of Delaware PacBio Sequencing Facility (<https://dna.dbi.udel.edu/>). In total, 252M reads were generated using three smart cells, which reached a sequence depth of ~90× coverage of the *B. fragariae* genome. Genome assembly was conducted using MECAT software (Xiao et al. 2017). The assembled genome size is 41.92 Mb, which is comparable to the genome size of other sequenced *Botrytis* species (43 to 55 Mb) (Valero-Jimenez et al. 2019). Although the genome assembly for *B. fragariae* has not reached to the level of the gapless, completely assembled 18 chromosomes for the *B. cinerea* genome (Van Kan et al. 2017), the sequenced reads were assembled into only 28 supercontigs with a large N50 length of 2.43 Mb and 98.5% BUSCO completeness (Table 1), which is more contiguous and complete than the genome assemblies recently reported for other *Botrytis* sister species, including *B. paeoniae* and *B. tulipae* (Valero-Jimenez et al. 2019). This indicates that we have obtained a high-quality whole-genome assembly for *B. fragariae*, which ensures the following accurate gene prediction and annotation.

We performed gene prediction using MAKER (2.31.8) (Holt and Yandell 2011) with three iterative runs in order to combine evidence from: (i) three ab initio prediction using SNAP (Korf 2004), AUGUSTUS (Stanke et al. 2004), and GeneMark (Ter-Hovhannisyan et al. 2008); (ii) homology-based comparison against available protein sequences of other *Botrytis* species from NCBI (<https://www.ncbi.nlm.nih.gov/datasets/genomes/?txid=33196>) and protein database from Uniprot; and (iii) expression data analysis (i.e., EST assembled from *B. fragariae* mycelial RNA-seq reads generated in this study using Trinity and EST data of *B. cinerea* downloaded from NCBI). In the first round we performed complete de novo gene annotation for *B. fragariae*

using AUGUSTUS with training models from *B. cinerea* and the est2genome and protein2genome were set to 1 based on the annotation protocol. Next, we used the gene models from the first MAKER run to train SNAP while incorporating GeneMark ab initio gene prediction in the second run. Then, we used the gene models from the second MAKER run to train SNAP again to generate the final gene set. Lastly, we performed manual curation with the final gene set using Web Apollo (Lee et al. 2013). In total, we predicted 12,506 genes from the *B. fragariae* genome, which is estimated to cover 98.8% of all genes in the *B. fragariae* genome based on the proteome-completeness assessment using BUSCO v3 (Simao et al. 2015) against the Ascomycota odb9 database (which contains 1,315 conserved Ascomycota orthologs; <https://busco-archive.ezlab.org/v3/>).

With such a high-quality gene prediction, we further performed functional annotation for the deduced *B. fragariae* proteins using EGGNOG-MAPPER (<http://eggno-mapper.embl.de/>) (Huerta-Cepas et al. 2017), which offers insights into the biological characteristics of *B. fragariae*. We found that 8,183 *B. fragariae* genes (accounting for 65.43% of the total annotated genes) can be mapped to clusters of ortholog groups (COGs), of which 527 belong to secondary metabolite COGs, suggesting that the majority of *B. fragariae* genes are highly conserved genes. Moreover, we identified 1,184 genes encoding putative secreted proteins using SignalP3.0 (Bendtsen et al. 2004) (which predicts the presence of a signal peptide) and TMHMM 2.0 (Krogh et al. 2001) (which predicts the presence of transmembrane domains) (Table 1). Finally, by using EffectorP, which can predict fungal effector proteins from secretomes (Sperschneider et al. 2018), we predicted 154 putative effector-encoding genes in *B. fragariae* (Table 1).

Compared with *B. cinerea* that has a broad host range (over 1,000 plant species), *B. fragariae* seems restricted to strawberry (Rupp et al. 2017), just like *B. paeoniae* and *B. tulipae* that only or primarily infect peonies (Munoz et al. 2016) and tulips (Coley-Smith and Javed 1972), respectively. The wide host range of *B. cinerea* is presumably due to its evolution of virulence factors that can overcome immunity of many different types of plants (Choquer et al. 2007). As a corollary to this reasoning, strawberry-restricted *B. fragariae* might have fewer effector genes. To evaluate these speculations, we performed comparative genome analyses between *B. fragariae* and other *Botrytis* species. We identified genes encoding candidate carbohydrate-active enzymes (CAZymes) (Zhang et al. 2018), secreted proteins especially those digestive enzymes required for breaking the plant cell wall, or effectors that are secreted and delivered to host tissues/cells to suppress host immunity from those 12,506 genes of *B. fragariae*, and compared them to those predicted in the genomes of the three other *Botrytis* species using the same algorithm and criteria. As shown in Table 1, compared with the three host-specific *Botrytis* species, *B. cinerea* has a relatively larger predicted CAZyme, secretome, and effectorome. Interestingly, despite having a smaller predicted effectorome relative to that of *B. cinerea*, *B. fragariae* has a significantly higher proportion of species specific effectors (67/43.5%) compared with other *Botrytis* species (29.0 to 32.9%) (Table 1).

The whole genome sequencing project has been deposited to NCBI under accession number JABFCT000000000, and the BioProject and BioSample accession number are PRJNA627915 and SAMN14689424, respectively. The raw Pacbio data and RNA-seq data were deposited to SRA under accession numbers SRR11844330 and SRR11843737.

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