High Contiguity Whole Genome Sequence and Gene Annotation Resource for Two *Venturia nashicola* Isolates

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Abstract

Venturia nashicola is a fungal pathogen that causes Asian pear scab disease. This pathogen is of particular importance in Northeast Asian countries, where Asian pears are grown industrially. Scab disease in Asian pear is currently controlled by fungicide spraying and this situation calls for developing scab resistant cultivars. High-quality genome data are therefore required for in-depth comparative genome analysis of different isolates of *V. nashicola* and *V. pyrina*, a closely related species, which only infects European pear plants. Here, we report the high-contiguity whole genome assembly of two *V. nashicola* isolates, which is expected to enable genome comparisons for identification of the genes involved in host range determination of *V. nashicola*.

Genome Announcement

Venturia nashicola Tanaka & Yamamoto, a causal agent of scab disease in Asian pears (e.g., *Pyrus pyrifolia*, *P. ussuriensis*, *P. betulaeifoila*, and *P. bretschneideri*) (Abe et al. 2008; Ishii and Yanase 2000; Li et al. 2007; Tanaka and Yamamoto 1964), is a major threat for the pear industry in Northeast Asia. Although, *V. nashicola* is limited to countries where Asian pears are grown industrially, including Japan, Korea, Taiwan, and China (Plantwise database), it is considered as a critical quarantine threat in the European Union, United States, Israel, and Turkey (Jeger et al. 2017). As there is no commercially available resistant cultivar, scab disease is controlled by multiple fungicide applications every year (Ishii and Yanase 2000; Park et al. 2000). Unfortunately, development of *V. nashicola* resistance to the commonly used fungicides has been reported (Ishii 2011).

The genus *Venturia* is phylogenetically assigned to the order *Venturiales* within the class *Dothideomycetes* (Zhang et al. 2011). Species of this genus can infect hosts in six plant

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Keywords

fungus-plant interactions, genomics

Table 1. Statistics of the whole genome assemblies of *Venturia nashicola* isolates

Assembly parameters	<i>Venturia nashicola</i>	
	MAFF615029	PRI2
Sequencing technology	Pac-Bio	Illumina
Sequencing coverage	79×	220×
Package used for assembly	HGAP2	bbmap
Number of ^a contigs or ^b scaffolds	198 ^a	55 ^b
Total residues	41,878,255	40,199,691
Mean length	211,506.34 ^a	730,903.5 ^b
Minimum length	3,885 ^a	9,793 ^b
Maximum length	2,819,867 ^a	2,807,685 ^b
L50	1,515,729 ^a	1,787,749 ^b
L90	469,434 ^a	711,231 ^b
NN content	0	158,627
GC content (%)	46.2	46
Repeat content of genome (%)	2.5	2.5
Number of predicted genes	12,163	11,760
Percentage completeness	98.3	97

^a Statistic provided is that of a contig.^b Statistic provided is that of a scaffold.

families, including *Acaraceae*, *Betulaceae*, *Cornaceae*, *Oleaceae*, *Rosaceae*, and *Salicaceae* (Sivanesan 1977). In general, *Venturia* species are host-specific pathogens and each can only infect one plant genus or close relatives in a given host family (González-Domínguez et al. 2017). The best studied and most economically damaging species is *V. inaequalis*, which causes scab disease in apple (Bowen et al. 2011; MacHardy 1996). In contrast, fewer studies have been reported for related *Venturia* species such as *V. nashicola*, *V. pyrina*, and *V. carpophila* infecting Asian pear, European pear, and peach, respectively (Ishii and Yanase 2000; Sivanesan 1974; Sivanesan and Waller 1974). *V. nashicola* and *V. pyrina* were initially considered as one species but were later separated based on distinct morphological and pathological characteristics (Ishii and Yanase 2000). Moreover, *V. nashicola* and *V. pyrina* are unable to cause disease in European pear and Asian pear, respectively, indicating that the pear scab pathogens have highly restricted host ranges (Abe et al. 2008; Ishii and Yanase 2000; Park et al. 2000). Recently, it was shown that disease resistance (*R*) genes play a major role in the nonhost resistance of *Arabidopsis thaliana* to *Albugo candida* (white rust) (Cevik et al. 2019). However, the extent to which effector-triggered *R* gene-dependent immunity plays a role in nonhost resistance of European and Asian pear to *V. nashicola* and *V. pyrina*, respectively, is currently unknown. A draft genome of *V. pyrina* has been recently published (Deng et al. 2017). The draft genome sequence and annotation of *V. nashicola* isolate Yasato 2-1-1 was reported recently (Johnson et al. 2018). However, since this assembly seems to be highly fragmented (40,800 scaffolds), we aimed to provide improved *Venturia nashicola* genome assembly and annotations.

To prepare the DNA samples for genome sequencing, *Venturia nashicola* isolates MAFF615029 and PRI2, sampled from scab-infected pear in Japan and the Republic of Korea, respectively, were grown on sterile Miracloth filter paper placed on potato dextrose agar media at 20°C for 30 days before mycelia were harvested and ground with liquid nitrogen for genomic DNA extraction, using the CTAB method (Doyle 1987) followed by purification on NucleoSpin columns (Macherey-Nagel GmbH & Co. KG). For *V. nashicola* isolate MAFF615029, 5 µg of genomic DNA was used for construction of a single-molecule real-time (SMRT) bell library (Pacific Biosciences). Small fragments less than 20 kb of SMRT bell template were removed, using BluePippin size selection system for large-insert library. Subsequently, the SMRT bell library of MAFF615029 isolate was sequenced using the PacBio RS II sequencing platform. We aimed to use the PacBio sequenced MAFF615029 genome sequence as a reference for assembly of the PRI2 genome that was sequenced using the Illumina Hi-seq platform.

For *V. nashicola* MAFF615029, genome assembly of the PacBio reads was performed using the HGAP2 assembly pipeline with post-assembly Quiver polishing at DNA-link (Seoul, Korea), which resulted in a high-contiguity genome assembly consisting of 198 scaffolds totaling 41,878,255 base pairs (Table 1). Prior to assembly of the *V. nashicola* PRI2 genome, Illumina short reads were trimmed with Trimmomatic v0.38 (Bolger et al. 2014) and were assessed for quality, using FastQC (Andrews 2014). Illumina short reads were initially de novo assembled with the

MaSuRCA assembler (Zimin et al. 2013), generating 1,849 contigs totaling 33,716,288 base pairs. This was significantly shorter than the PacBio–based assembly of the *V. nashicola* isolate, which yielded more than 40 Mb. This was expected, as short reads do not allow proper repeat region assembly and, overall, produce shorter de novo contigs. Therefore, a reference guided assembly of the *V. nashicola* PRI2 Illumina short reads was performed using bbMap, with *V. nashicola* isolate MAFF615029 PacBio assembly as a reference (Bushnell 2014). Further scaffolding was performed with Ragout (Kolmogorov et al. 2014) and polishing was done with Pilon (Walker et al. 2014), reusing the Illumina data. *V. nashicola* PRI2 genome assembly resulted in 55 scaffolds totaling 40,227,028 base pairs which is comparable to the MAFF615029 isolate (Table 1). The fact that the PRI2 assembly yielded fewer scaffolds than the MAFF615029 assembly is due to less stringent contig linking at the scaffolding step, allowing gaps to be filled with N bases.

Gene models were predicted using the modified version of the FunGAP gene annotation pipeline (Min et al. 2017). All the FunGAP steps relying on transcriptome data were changed to work without it or were removed from the pipeline. FunGAP takes advantage of several gene prediction tools, such as BRAKER (Hoff et al. 2016), MAKER (Cantarel et al. 2008) and AUGUSTUS (Stanke et al. 2006). Originally BRAKER requires transcriptome data; thus, we altered its subcommand to self-train based on the translated transcripts from *V. inaequalis* ICMP13258 gene prediction aided by its own transcriptome data (Deng et al. 2017). We also performed AUGUSTUS training on the same gene set (Deng et al. 2017). There were 12,163 genes predicted in *V. nashicola* MAFF615029 isolate and 11,760 genes predicted in *V. nashicola* PRI2 isolate. These numbers are comparable to the gene content reported for other *Venturia* genomes of similar size (Deng et al. 2017).

Completeness of genome assemblies and gene predictions were assessed using BUSCO (Simão et al. 2015). Assessment of genome completeness with the *Ascomycota* BUSCO dataset (1,325 BUSCO groups total) showed 98.3% completeness with 1,292 complete, 19 fragmented, and four missing BUSCO orthologs in MAFF615029 and 97% completeness with 1,275 complete, 17 fragmented, and 23 BUSCO orthologs in PRI2.

In contrast, assessment with the BUSCO Fungi dataset (290 BUSCO groups) showed 98.6% completeness with 286 complete, two fragmented, and two missing BUSCO orthologs in MAFF615029 and 97.9% completeness with 284 complete, one fragmented, and five missing BUSCO orthologs in PRI2 (Table 1).

Assemblies of fungal genomes are challenging due to their size in the tens of megabases and the presence of genomic repeats. Short read–only based assemblies suffer from high rates of fragmentation and unresolved genomic repeats (Thomma et al. 2016). The high contiguity genome sequences of two *V. nashicola* isolates presented in this resource will provide a platform for genomic comparisons, phylogenetic analyses, and host determinant mining in order to understand the basis of high host specificity in *Venturia* spp. It can be further used, in conjunction with optical mapping, to achieve chromosome level assemblies of *V. nashicola* completed reference genome in future studies (Faino et al. 2015).

Full genome assembly data and genome annotations are deposited in the NCBI database under BioProject accession number PRJNA527174.

Author-Recommended Internet Resource

Plantwise database: <https://www.plantwise.org>

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