Genome Sequence of *Verticillium dahliae* Race 1 Isolate VdLs.16 From Lettuce

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Abstract

Verticillium dahliae is a widespread fungal pathogen that causes Verticillium wilt on many economically important crops and ornamentals worldwide. Populations of *V. dahliae* have been divided into two distinct races based upon differential host responses in tomato and lettuce. Recently, the contemporary race 2 isolates were further divided into an additional race in tomato. Herein, we provide a high-quality reference genome for the race 1 strain VdLs.16 isolated from lettuce in California, U.S.A. This resource will contribute to ongoing research that aims to elucidate the genetic basis of *V. dahliae* pathogenicity and population genomic diversity.

Genome Announcement

Verticillium dahliae is a soilborne fungal pathogen that causes Verticillium wilt on over 200 plant species, and yield losses often approach 100% in some crops (Atallah et al. 2011; Inderbitzin and Subbarao 2014; Klosterman et al. 2009). Microsclerotia produced by *V. dahliae* serve as resting structures and remain viable in the soil for many years, even in the absence of hosts, and serve as the primary inoculum for subsequent crop infection (Inderbitzin et al. 2013; Klosterman et al. 2009; Vallad and Subbarao 2008). The microsclerotia of *V. dahliae* germinate in response to signals from host root exudates and initiate plant infection (Fitzell et al. 1980; Mol and Riessen 1995). In lettuce, the hyphal colonies are visible at the root tips and root-elongation zones within five days after inoculation, reaching cortical tissues and penetrating the xylem tissues within two weeks (Vallad and Subbarao 2008). The soilborne and xylem-limited nature of *V. dahliae*, coupled with its broad host range

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Keywords

genomic diversity, fungal pathogen, Verticillium wilt

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Table 1. Genome statistics of *Verticillium dahliae* VdLs.16

Genome assembly	Scaffold	Contig
Total number	14	17
Total length (bp)	36,092,517	36,089,483
Gap number (bp)	3,034	–
Average length (bp)	2,578,037	2,122,911
N50 length (bp)	4,722,744	3,663,785
N90 length (bp)	2,532,149	2,375,583
Maximum length (bp)	8,087,087	8,087,087
Minimum length (bp)	50,807	41,068
GC content (%)	53.8	53.8
Genome prediction		
Protein coding genes	10,799	
Length of genes (bp)	18,863,221	
Mean gene length (bp)	1,747	
Gene GC %	58.56	
Number of exons	31,232	
Coding gene with introns	1.89	
Mean exons per gene	2.89	
Mean exon length (bp)	529	
Number of introns	20,433	
Mean intron length (bp)	114	
Mean intergenic length (bp)	1,584	
Number of transposons	4,870	
Total length of transposons (bp)	3,879,742	
Percentage of transposons in genome	10.7494	
Genome annotation		
Empowered homologs in eggNOG database	9,775	
Empowered homologs in InterPro database	7,576	
Empowered homologs in KEGG database	3,929	
Secretory proteins	797	
CAZymes (carbohydrate-active enzymes)	588	
CAZymes (secreted)	266	
Small cysteine-rich proteins	302	
LysM (lysine motif) effectors	6	
NEPs (necrosis and ethylene-inducing proteins)	7	
Protein kinases	150	
Transcription factors	432	
PHI (pathogen-host interaction) homologs	2,797	

and prolonged survival in the soil, makes this pathogen challenging to manage and becomes a perennial problem once the soil is infested.

A sexual cycle for *V. dahliae* has not been reported despite the preservation of the genes for sexual reproduction in the genome (Short et al. 2014a), and asexual organisms are often considered evolutionary dead-ends (Felsenstein 1974; McDonald and Linde 2002). Despite being an obligate asexual fungus, marked differences between *V. dahliae* populations, including six vegetative compatibility groups (Bhat et al. 2003; Joaquim and Rowe 1991; Milgroom et al. 2014), at least two races (Vallad et al. 2006), with an additional race 3 recently reported in tomato (Usami et al. 2017), two mating-type idiomorphs, *MAT1-1* and *MAT1-2* (Usami et al. 2009), and two pathogenic phenotypes on woody hosts, described as defoliating and nondefoliating (Schnathorst and Mathre 1966) have been documented.

Races 1 and 2 of *V. dahliae* were originally identified in tomato based on the responses of host differentials (Grogan et al. 1979; Schaible et al. 1951). In tomato, *V. dahliae* race 1 strains possess the *Ave1* gene, which encodes a virulence factor that acts on plants lacking the plant immune receptor encoding the *Ve1* gene (de Jonge et al. 2012), and the strains lacking *Ave1* are assigned to race 2. Similarly, in lettuce, two races of *V. dahliae* have been identified based on the responses of the differential host genotypes, i.e., isolates avirulent on the cultivar La Brillante and virulent on Salinas, are characterized as race 1, whereas isolates virulent on both genotypes are characterized as race 2 (Vallad et al. 2006), and, just as in tomato, the race 1 isolate in lettuce also carries the *Ave1* gene (Short et al. 2014b). Resistance against race 1 in cultivar La Brillante is conferred by the single dominant locus *Verticillium resistance 1* (*Vr1*) (Hayes et al. 2007, 2011; Inderbitzin et al. 2019). In lettuce, race 1 is most prevalent (>93%) among the pathogen populations evaluated in coastal California (Gurung et al. 2014).

Dozens of *V. dahliae* genome sequences (approximately 35 Mb) have been publicly released (National Center for Biotechnology Information *V. dahliae* database) and most of these belong to race 2, owing to the lack of *Ave1*, including the VdLs.17 strain from lettuce (Klosterman et al. 2011) and the Vd991 strain from cotton (Chen et al. 2018). Of the race 1 strains, the high-quality genome of JR2 from tomato has been published (de Jonge et al. 2012). This reference genome was used to characterize chromosomal rearrangements in *V. dahliae*, a potentially important mechanism driving genetic changes and host adaptations in some asexual pathogens (de Jonge et al. 2013). To obtain additional reference genomes of race 1 strains from different hosts, the VdLs.16 isolate from lettuce was cultivated in complete medium (including 6 g of yeast extract, 6 g of casein acid hydrolysate, and 10 g of sucrose in 1 liter of distilled water), and high-quality genomic DNA was extracted for genome sequencing using a Quick-DNA fungal/bacterial midiprep kit (Zymo Research, Orange, CA, U.S.A.) according to manufacturer protocol, with a few modifications.

For whole-genome sequencing of the VdLs.16 strain, two libraries with insertion sizes of 20 kb and 400 bp were constructed using SMRTbell template prep kits (Pacific Biosciences, Menlo Park, CA, U.S.A.) and Illumina TruSeq Nano DNA library prep kits (Illumina, San Diego, CA, U.S.A.), respectively. The 20 kb and 400 bp libraries were sequenced by the PacBio Sequel platform and Illumina HiSeq 2000 platform, respectively. To ensure high-quality reads for use in assembling the genome, the adapter sequence, low-quality reads, and N bases of PacBio data and Illumina data were filtered. The genome sequence was de novo assembled using the PacBio subreads with the SMARTdenovo program, and the designated parameters (-c 1 -t 8 -k 16) were extended by the SSPACE program using subreads (parameters: -o 50 -l 3) and Illumina clean reads (parameters: -o 20 -m 32 -k 5 -n 15) (Boetzer and Pirovano 2014). The PBJelly2 program (English et al. 2012) was used for minding the gap of assembled sequences with the default parameters. Error corrections were carried out using subread by the variantCaller program from the SMRTlink v4 package (Pacific Biosystems). Subsequently, the GATK program (McCormick et al. 2015) was used for further error corrections with the designated parameters (-cluster 2 -window 5 -stand_call_conf 50 -stand_emit_conf 10.0 -dcov 200 MQ0 ≥4); finally, the assembled sequences were corrected by the SOAPsnp (parameters: -u -t -z @ -Q i -q) and SOAPindel program (parameters: -c 3 -h 1 -u 2 -m 2) (Li et al. 2013), using Illumina clean reads.

The final genome assembly of VdLs.16 produced in this work consists of 17 contigs (N50 length of 3,663,785 bp, N90 length of 2,375,583 bp), further assembled into 14 scaffolds (N50 length of 4,722,744 bp, N90 length of 2,532,149 bp), to obtain the genome size of 36,092,517 bp (Table 1). The length of the top eight assembled sequences is 33,653,383 bp, which is 93.2% of the genome size, indicating that the sequencing and assembly of the VdLs.16 genome were of high quality and close to the total predicted eight chromosomes for *V. dahliae* (Chen et al. 2018; de Jonge et al. 2012; Klosterman et al. 2011). Transposable elements were identified by RepeatMasker (open 3.2.8, detailed parameters: -no_is, -norna, -engine, -s, -parallel = 1, using Repbase version 15.08) and RepeatProteinMask (-noLowSimple, -P value = 1e-4). The output files were summarized using a custom Perl script. The analysis revealed that 10.7% of VdLs.16 genome was composed of repetitive DNA, and nearly 30% of the transposable elements (1.15 Mb) were of the LTR/Gypsy type.

For protein-coding gene prediction, de novo-based and homology-based prediction methods were used. For the de novo prediction, the gene prediction of repeat-masked genomes was implemented by GeneMark-ES (Borodovsky and McIninch 1993) together with *V. dahliae* (VdLs.17 and VdJR2)-trained Augustus (version 2.6) (Stanke et al. 2008) and SNAP (Korf 2004). For the homology-based prediction, the protein-coding genes of VdLs.16 were collected and mapped onto the Vd991 genome using TBLASTN. The homologous sequences that aligned to the matching proteins were defined as gene models of isolate Vd991, using the GeneWise program (Birney and Durbin 2000). Finally, all gene evidences were combined using GLEAN (Mackey et al. 2005), enabling the prediction of 10,799 genes in the VdLs.16 genome (Table 1). For functional annotations of predicted genes, all the genes were interrogated to known databases, using BLASTP to identify homologs, including the databases of eggNOGs (Powell et al. 2012) and InterPro (incorporated InterPro, Gene Ontology, and KEGG [Kyoto Encyclopedia of Genes and Genomes] pathway annotation) (Jones et al. 2014). Putative extracellular proteins containing a signal peptide, lacking transmembrane domains, and distributing to extracellular subcellular localization were identified as secreted proteins; secreted proteins were predicted using SignalP v5.0 (Almagro Armenteros et al. 2019), transmembrane domains were predicted using TMHMM v2.0 (Krogh et al. 2001) and Phobius (Käll et al. 2007), and subcellular

localizations were predicted using WoLF PSORT (Horton et al. 2007). The results, shown in Table 1, indicated that a total of 797 genes encoded secreted proteins in VdLs.16. Among these, 302 belonged to the small cysteine-rich proteins. Putative secreted carbohydrate-active enzymes (CAZymes) were identified using the hidden Markov model (HMM)-based routine of the CAZymes database, revealing a total of 588 genes that encode CAZymes in VdLs.16. The homologs of known pathogen-host interaction (PHI) genes were predicted using the PHI database v4.7 (Urban et al. 2017). The results revealed that 2,797 genes from VdLs.16 were predicted to be PHI genes. A total of 150 protein kinases were predicted by running HMM searches locally with Kinomer version 1.0 (Martin et al. 2009), while 432 transcription factors were predicted using the InterPro database.

Since the reference genomes of strains VdLs.17 (Klosterman et al. 2011) and Vd991 (Chen et al. 2018) are both race 2 and only the JR2 genome was available as a representative of race 1 (de Jonge et al. 2013), additional race 1 genomes are helpful for research on elucidating the underlying mechanisms of differentiation in *V. dahliae* populations. We therefore provide the corresponding annotation information of the race 1 strain VdLs.16 and expect it to serve as an additional reference genome for researchers.

This Whole Genome Shotgun project has been deposited at the China National GenBank database under accession CNP0001022 and DDBJ/ENA/GenBank under accession JABBIU000000000. The versions described in this paper are versions CNP0001022 and JABBIU010000000, respectively.

Author-Recommended Internet Resources

National Center for Biotechnology Information *V. dahliae* database: <https://www.ncbi.nlm.nih.gov/genome/genomes/832>
RepeatMasker program: <http://www.repeatmasker.org>

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