Genome Sequence Data of *MAT1-1* and *MAT1-2* Idiomorphs from *Verticillium dahliae*

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Abstract

Though *Verticillium dahliae* is an asexually reproducing fungus, it is considered heterothallic owing to the presence of only one of the two mating-type idiomorphs (*MAT1-1* or *MAT1-2*) in individual isolates. But sexual reproduction has never been observed either in nature or in the laboratory. All of the genomic information in the literature thus far has therefore come from studies on isolates carrying only the *MAT1-2* idiomorph. Herein, we sequenced and compared high-quality reference genomes of *MAT1-1* strain S011 and *MAT1-2* strain S023 obtained from the same sunflower field. The two genomic sequences displayed high synteny, and encoded similar number genes, a similarity especially notable among pathogenicity-related genes. Homolog analysis between these two genomes revealed that 80% of encoded genes are highly conserved (95% identity and coverage), but only 20% of the single copy genes were identical. These novel genome resources will support the analysis of the structure and function of the two idiomorphs and provide valuable tools to elucidate the evolution and potential mechanisms of sexual reproduction in *V. dahliae*.

Sexual reproduction in heterothallic ascomycetes is controlled by alternative forms of the mating-type locus *MAT1-1* and *MAT1-2* (Coppin et al. 1997; Dyer et al. 2016; Pöggeler 2001; Turgeon and Yoder 2000). *MAT1-1* encodes an alpha-box transcription factor and *MAT1-2*, a high mobility group (HMG) transcription factor domain. These transcription factors target pheromone precursor and receptor genes that mediate cell attraction and fusion during sexual reproduction but also affect other cellular processes (Becker et al. 2015; Keszthelyi et al. 2007). The alpha1 gene present at *MAT1-1* is designated '*MAT1-1-1*', and the HMG box gene at *MAT1-2* is referred to as *MAT1-2-1*. In addition, other genes are present at *MAT* depending on the species, and these include *MAT1-1-2*, *MAT1-1-3*, and *MAT1-1-4* at the *MAT1-1* idiomorph and similarly, *MAT1-2-2*, *MAT1-2-3*, and *MAT1-2-4* at the *MAT1-2* idiomorph (Keszthelyi et al. 2007; Klis et al. 2010; Turgeon and Yoder 2000).

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V. dahliae is considered an asexual pathogen. The most intuitive evidence for this is the lack of sexual structures in either natural or laboratory conditions (Short et al. 2014). Several population genetic studies also support clonal propagation in *V. dahliae* (Atallah et al. 2011; Baroudy et al. 2019; Milgroom et al. 2014, 2016; Rafiei et al. 2018; Short et al. 2015). However, *V. dahliae* has preserved two different mating type loci (*MAT1-1* and *MAT1-2*) and is heterothallic (Usami et al. 2008, 2009). Interestingly, the mating type frequencies in *V. dahliae* in nature is heavily skewed toward the *MAT1-2* idiomorph (Milgroom et al. 2014; Short et al. 2015; Usami et al. 2008). Comparison of genome structures revealed extensive chromosomal rearrangements among the different *V. dahliae* genomes (Chen et al. 2018; de Jonge et al. 2013), which are expected to interfere in the correct pairing of homologous chromosomes during meiosis (Kistler and Miao 1992). Therefore, *V. dahliae* is mainly considered to be an asexual species that propagates clonally (Seidl and Thomma 2014).

Several recent studies, however, have speculated outcrossing in *V. dahliae*, a feature that was likely in ancestral species or an extant ability that conferred cryptic sexual reproduction (Atallah et al. 2010, 2012; Inderbitzin et al. 2013; Milgroom et al. 2014; Short et al. 2014; Usami et al. 2009). As noted above, *V. dahliae* isolates contain either the *MAT1-1* or *MAT1-2* idiomorph, and the sex-related genes are conserved, active, and are under purifying selection (Short et al. 2014). Moreover, several *V. dahliae* recombinant haplotypes are likely to have arisen by recombination between closely related lineages (Milgroom et al. 2014). In addition, although the parasexuality was speculated as the likely origin of the diploid *V. longisporum* (Karapapa et al. 1997), it has been discounted for lack of evidence. However, recent studies have clearly demonstrated the origin of this species via hybridization between *V. dahliae* and an unknown species (Depotter et al. 2016; Inderbitzin et al. 2011a, b). The stability of *V. longisporum* as a hybrid suggests that interspecific hyphal fusion followed by nuclear fusion as more plausible than parasexual processes for its origin (Inderbitzin et al. 2011b).

At present, more than 20 *V. dahliae* genome sequences (~35 Mb each) have been publicly released (<https://www.ncbi.nlm.nih.gov/genome/genomes/832>) and all of them carry the *MAT1-2* idiomorph, except the whole genome sequences of 85S strain (GenBank assembly accession: GCA_004798885.1, 39 of scaffolds) and Vd39 strain (GCA_011032905.1, 1578 of scaffolds) isolated from sunflower in France and Germany, respectively, that carry the *MAT1-1* idiomorph (Depotter et al. 2019). Therefore, the relative dearth of genome information for the *MAT1-1* idiomorph has limited our understanding and research into the sexual mode of reproduction in *V. dahliae*.

The distribution of the two *MAT* idiomorphs in relative equilibrium was observed in the sunflower and potato cropping systems in Inner Mongolia, China (Verticilli-Omics, <https://db.cngb.org/Verticilli-Omics/>), which suggested that *MAT1-1* idiomorph has adapted and expanded under some environments in China, resulting in enhanced risk of sexual reproduction in *V. dahliae*. To determine the genomic basis of potential sexual mating and to facilitate the study of this phenomenon, we sequenced the genomes of *V. dahliae* strains S011 and S023 isolated from a sunflower field in Inner Mongolia, carrying the *MAT1-1-1* and *MAT1-2-1* sequences, respectively.

The high-quality genomic DNA of these two strains was extracted for genome sequencing using a Quick-DNA Fungal/Bacterial Midiprep Kit (Zymo Research, Orange, CA, U.S.A.). For whole-genome sequencing, 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.), and then sequenced using PacBio Sequel and Illumina HiSeq 2000 platforms, respectively. The adapter sequence, low-quality reads (<Q30), and *N* bases of PacBio and Illumina data were filtered. The assembly procedure was employed to assemble the genomes as described previously (Chen et al. 2020), including de novo assembly by SMARTdenovo (version 170825), extended by the SSPACE (version STANDARD-3.0) program using sequencing reads (Boetzer and Pirovano 2014), minding gap by PBjelly2 program (English et al. 2012, version 15.8.24), error correction by the variantCaller program (version 2.2.2) from the SMRTlink v4 package (Pacific Biosystems), and polished the assembled sequences by GATK (McCormick et al. 2015, version 3.4.0), SOAPsnp (version 1.05), and SOAPindel (version 1.08) programs.

The final genome assemblies of S011 and S023 consist of 12 and 13 scaffolds, for a total genome size of 37,324,492 bp (N50 length of 4,479,799 bp) and 35,377,336 bp (N50 length of 3,798,651 bp), respectively. The length of the top eight assembled sequences of S011 and S023 were 34,287,596 and 31,582,067 bp, amounting to 91.86 and 89.27% of the genome sizes,

Table 1. Genome statistics of *Verticillium dahliae* strains S011 and S023^a

Statistics	Scaffold	
	S011 (MAT1-1)	S023 (MAT1-2)
Genome assembly		
Total number	12	13
Total length (bp)	37,324,442	35,377,336
Gap number (bp)	25	25
Average length (bp)	3,732,449	2,721,334
N50 length (bp)	4,479,799	3,798,651
N90 length (bp)	3,001,655	1,616,047
Maximum length (bp)	5,985,695	7,438,647
Minimum length (bp)	61,794	95,361
GC content (%)	53.33	53.65
Coverage between genomes (%)	90.07	93.48
Genome content		
Protein coding genes	10,773	10,517
BUSCO	97.9%	97.9%
Length of genes (bp)	19,057,009	18,460,127
Mean gene length (bp)	1,769	1,755
Gene GC %	58.51	58.60
Exon number	31,037	30,524
Coding gene with introns	1.88	1.90
Mean exons per gene	2.88	2.90
Mean exons length (bp)	540	530
Intron number	20,264	20,007
Mean intron length (bp)	114	114
Mean intergenic length (bp)	1,689	1,589
Number of transposons	4,693	4,415
Total length of transposons (bp)	5,024,491	3,589,160
Percentage of transposons in genome (%)	13.46	10.15
Genome annotation		
Secretome	798	793
CAZymes	587	586
CAZymes (secreted)	301	302
Protein kinases	150	153
Transcription factors	429	426
SCRPs	303	299

^a CAZymes, carbohydrate-active enzymes; SCRPs, small cysteine-rich proteins; and BUSCO: benchmarking universal single-copy orthologs.

respectively (Table 1). Transposable elements were identified by de novo prediction, RepeatMasker (open 3.2.8, detailed parameters: -no_is, -norna, -engine, -s, -parallel=1, used Rebase version 15.08) and RepeatProteinMask (-noLowSimple, -P value = 1e-4, version open-4.0) (<http://www.repeatmasker.org>). The analysis revealed that 13.46 and 10.15% of the S011 and S023 genomes, respectively, were composed of transposons.

The totality of de novo assembled draft genome was evaluated using BUSCO (benchmarking universal single-copy orthologs, fungi_odb10, version 3.1.0). Protein-coding genes in the S011 and S023 genomes were predicted using a combination of de novo-based GeneMark-ES (version Suite 4.10) (Borodovsky and McIninch 1993), AUGUSTUS (version 2.6) (Stanke et al. 2008) and SNAP (version 11/29/2013) (Korf 2004) programs, as well as by the homology-based approaches previously described (Chen et al. 2018). The functional annotations of all genes were interrogated with known databases using BLASTP to identify homologs, including the databases of *nr*, eggNOGs (Powell et al. 2012), and InterProScan (Jones et al. 2014). The subcellular localization predictions were identified following procedures described previously (Klosterman et al. 2011). Putative secreted carbohydrate-active enzymes (CAZymes) were identified using the HMM-based routine of the carbohydrate-active-enzymes database (Huang et al. 2018).

Overall, the S011 and S023 genomes encoded 10,773 and 10,517 putative protein-encoding genes, respectively. Among these, 798 and 793 genes encoded secreted proteins in S011 and S023, respectively, and among these 303 and 299 belonged to the small cysteine-rich proteins (SCRPs, <400 amino acids, ≥4 cysteine residues) (Table 1), respectively. A total of 587 and 586 genes that encode CAZymes were identified in S011 and S023, respectively. A total of 153 and 152 protein kinases were predicted by HMM searches locally with Kinomer version 1.0

A

B

Fig. 1. Comparative genomics between strains S011 and S023 of *Verticillium dahliae*. **A**, Sketch map of the *MAT1-1* and *MAT1-2* loci in the sequenced genomes of *V. dahliae* strains S011 and S023. **B**, Homolog (1:1, equal copies between *MAT1-1* and *MAT1-2* genomes) comparisons between the S011 and S023 genomes. The homologs were clustered at identity and coverage levels: 50, 70, 90, and 95 to 100%.

(Martin et al. 2009), while 429 and 426 transcription factors were predicted using the InterPro database in S011 and S023 (Table 1), respectively. This information revealed that the genome composition, especially in the pathogenicity-related gene numbers, was highly similar in both genomes.

The mating-type loci of S011 and S023 were identified by the BLASTN using the *MAT1-1-1* and *MAT1-2-1* sequences (Usami et al. 2009). The *MAT1-1* and *MAT1-2* loci displayed high synteny in gene orthologs, and the *MAT1-1* locus also contained the putative *VdMAT1-1-3* that encodes the HMG homolog (Fig. 1A). However, the encoded genes were differentiated between the

genomes of *MAT1-1* and *MAT1-2* idiomorphs. First, the size of these two genomes is different, and with 90.07 and 93.48% coverage when the short sequence reads from S023 or S011 were mapped to the S011 or S023 genome (Table 1), respectively. Homolog comparisons (parameters: both identities and coverage were set as up to 50, 70, 90, and 95 to 98%) between the two genomes revealed that the percent of homologs in two genomes were >75%, indicating that the genes are conserved between the *MAT1-1* and *MAT1-2* genomes. However, only around 20% homologs were identical (at identity and coverage length of 100%) between S011 and S023 genomes (Fig. 1B), indicating that minor sequence divergence is widespread among the genes in the *MAT1-1* and *MAT1-2* genomes.

In this study, high-quality genomic assemblies of *V. dahliae* *MAT1-1* (S011) and *MAT1-2* (S023) were obtained. Strains S011 and S023 were both derived from the same sunflower field. These resources will support our future genomic characterization of *MAT1-1* *V. dahliae* strains, and the study of the mechanisms of sexual and asexual reproduction in *V. dahliae*. The status of reproductive mode in *V. dahliae* will expand our knowledge of the biology of *Verticillium* spp. and facilitate studies that seek to understand the evolutionary characteristics of fungal pathogens over complex and changeable environments.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accessions JADFUI000000000 (S011) and JADFUI000000000 (S023). The version described in this paper is version JADFUI000000000 and JADFUI000000000. The BioProject accession is PRJNA666276.

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