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Gapless Genome Assembly of the Potato and Tomato Early Blight Pathogen *Alternaria solani*

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Abstract

The *Alternaria* genus consists of saprophytic fungi as well as plant-pathogenic species that have significant economic impact. To date, the genomes of multiple *Alternaria* species have been sequenced. These studies have yielded valuable data for molecular studies on *Alternaria* fungi. However, most of the current *Alternaria* genome assemblies are highly fragmented, thereby hampering the identification of genes that are involved in causing disease. Here, we report a gapless genome assembly of *A. solani*, the causal agent of early blight in tomato and potato. The genome assembly is a significant step toward a better understanding of pathogenicity of *A. solani*.

Genome Announcement

Alternaria solani is an important pathogen that causes early blight on potato and tomato. It belongs to the *Alternaria* genus, which is also comprised of species that are pathogenic on other plants as well as species that are saprophytic (Rotem 1994; Thomma 2003). The *Alternaria* genus has recently been restructured based on conserved DNA sequences (Lawrence et al. 2013; Ozkilinc et al. 2017; Woudenberg et al. 2013, 2014). It was recognized that *A. protenta* and *A. grandis*, which are closely related to *A. solani*, can also cause early blight on potato (Ayad et al. 2017; Bessadat et al. 2016; Duarte et al. 2014; Woudenberg et al. 2014).

Genomes of various *Alternaria* species, including *A. solani*, have been sequenced in the past (Bihon et al. 2016; Dang et al. 2015; Hu et al. 2012; Nguyen et al. 2016; Woudenberg et al. 2015). Most of these genome assemblies are based on short-read sequencing technologies and, as a result, are highly fragmented. Still, these studies have been helpful to discover new genes and to clarify the taxonomy of *Alternaria* species and they facilitate comparative genomics.

The use of long reads derived from Pacific Biosciences (PacBio) single-molecule real-time (SMRT) sequencing technology is a powerful approach to produce high-quality assemblies of fungal genomes (Faino et al. 2015; Seidl et al. 2015; Van Kan et al. 2017), as was also recently demonstrated for *A. alternata* (Nguyen et al. 2016). A contiguous genome

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assembly is important when studying plant-pathogenic fungi, because genes encoding effector proteins that are involved in the development of disease are often present in fast-evolving, repeat-rich parts of the genome that are difficult to assemble (Lo Presti et al. 2015; Thomma et al. 2016). Moreover, knowledge about the organization of genes and chromosomes helps the identification of gene clusters that are involved in the production of secondary metabolites and to characterize potential conditionally dispensable chromosomes, which can both be important factors when studying pathogenicity (Thomma et al. 2016).

Here, we sequence the genome of *A. solani* isolate altNL03003 that was originally sampled from a potato field in Rutten (The Netherlands). We confirmed the identity of the *A. solani* isolate through the analysis of the sequences of multiple conserved genes (Woudenberg et al. 2014) and deposited a culture that was grown from a single spore in the CBS-KNAW culture collection (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, accession number CBS 143772). Pathogenicity on potato and a good capacity for sporulation was already previously reported for the isolate (Iftikhar et al. 2017).

About 20 μg of genomic DNA was isolated from mycelium that was grown in a liquid culture, was sheared, and was used to prepare a PacBio sequencing library with an insert size of approximately 20 kb. The library was loaded onto four SMRT cells and was sequenced on a PacBio RSII system (Keygene, Wageningen, The Netherlands), yielding over 7 Gb of sequencing data. Initial assembly using HGAP (Chin et al. 2013) yielded a total of 23 contigs with sizes from 4.7 kb to 6.9 Mb. The combined length of all contigs is 33.1 Mb, yet the 12 largest contigs (ranging from 245 kb to 6.9 Mb) comprise 99% of the total length. These results are in line with previous studies, in which genome sizes of 32.6 to 32.9 Mb were reported for *A. solani* (Dang et al. 2015; Woudenberg et al. 2015).

To assess the quality of the genome assembly, we determined the presence of telomeric repeats (TTAGGG) in the contigs (Faino et al. 2015). Contigs that contain telomeric repeats on both ends are likely fully assembled chromosomes (Seidl et al. 2015). We identified 20 telomeric repeats, which were all found on the ends of the assembled contigs, suggesting that *A. solani* has 10 chromosomes. The 12 largest contigs, which make up almost the entire assembly, all contain telomeric repeats on at least one end and seven of them have telomeric repeats on both ends. The contigs that contain these telomeric repeats all have a comparable coverage of about 150×, whereas the remaining contigs (representing only about 1% of the total assembly length) all lacked telomeric repeats, had much lower coverage, or consisted entirely of mitochondrial or ribosomal DNA, singly or in combination.

Closer inspection of the six contigs with single telomeric repeats revealed that two of these contigs could be combined, as they had an overlapping sequence of 14 kb. Two other contigs both overlapped with a third contig that consisted entirely of ribosomal DNA and that lacked telomeres, so these could also be combined with each other. The remaining two contigs with single telomeres did not overlap but could be joined by searching for PacBio reads spanning these contigs, using PBJelly (English et al. 2012). The final assembly of *A. solani* that was thus produced consists of 10 chromosomes with telomeres on both ends and a combined size of 32.8 Mb (Table 1).

Table 1. Overview of the assembly of the 10 chromosomes from *Alternaria solani* (altNL03003/CBS 143772)[a]

Chromosome	Length (bp)	Coverage	GC content (%)
NL03003_chromosome1	6,940,169	158×	51
NL03003_chromosome2	5,062,150	177×	52
NL03003_chromosome3	3,306,264	159×	50
NL03003_chromosome4	2,866,555	157×	52
NL03003_chromosome5	2,771,896	156×	51
NL03003_chromosome6	2,590,027	158×	51
NL03003_chromosome7	2,546,252	155×	51
NL03003_chromosome8	2,508,373	158×	51
NL03003_chromosome9	2,309,181	156×	52
NL03003_chromosome10	1,878,275	157×	51

[a] The higher sequencing coverage of chromosome 2 is explained by the presence of the ribosomal RNA gene cluster.

The genome sequence of *A. solani* that we report here presents a major improvement over previous genome assemblies of *A. solani*, which consist of over 100 separate contigs. It is also the first finished genome of an *Alternaria* species and, thus, provides a solid basis for performing comparative genomics as well as for studying the molecular basis of pathogenicity of *A. solani*.

The genome project, which includes the genome assembly as well as raw PacBio reads, has been deposited as National Center for Biotechnology Information BioProject PRJNA391093.

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