A Genome Resource of *Setosphaeria turcica*, Causal Agent of Northern Leaf Blight of Maize

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Abstract

The heterothallic ascomycete *Setosphaeria turcica* (anamorph *Exserohilum turcicum*) causes northern corn leaf blight, which results in devastating yield losses and a reduction in feed value. Although genome sequences of two model strains of the pathogen are available (<https://mycocosm.jgi.doe.gov/mycocosm/home>), previous drafts were assembled using short read technologies, making evolutionary and genetic linkage inferences difficult. Here, race 23N of *S. turcica* strain Et28A was sequenced again using Illumina HiSeq and PacBio Sequel technologies, and assembled to approximately 43,480,261 bp on 30 scaffolds. In all, 13,183 protein-coding genes were predicted, 13,142 of them were well annotated. This *S. turcica* genome resource is important for understanding the genetics behind pathogen evolution and infection mechanisms.

Genome Announcement

Setosphaeria turcica (anamorph *Exserohilum turcicum*, formerly known as *Helminthosporium turcicum*) is a fungal pathogen that causes northern corn leaf blight (NCLB) and is increasingly problematic worldwide (Mueller et al. 2016; Wu and Turgeon 2013). NCLB has been recorded in Europe, the Americas, Australasia, Asia, and Africa. Infection that occurs prior to flowering can cause grain yield losses exceeding 50% (Martin 2011; Mueller et al. 2016; Raymundo and Hooker 1981). Performing high-resolution genome sequencing and dissecting the molecular mechanisms underlying infection by *S. turcica* are critical to gaining a full understanding of the disease but also for successful breeding for resistance.

Genome sequencing of *S. turcica* Et28A (race 23N) was performed using a combination of single molecule real-time (SMRT) DNA sequencing and next generation sequencing technology. Genomic DNA extraction was performed using the Omega E.Z.N.A Fungal DNA Maxi Kit (Omega Bio-Tek, Inc.). For SMRT sequencing, the extracted DNA was prepared using a SMRTbell library with the PacBio SMRTbell Template Prep Kit (Pacific Biosciences). After sequencing and quality control, 2,381,468 PacBio subreads and 20,661,698,287 total bases were used for further assembly. In addition, a DNA library with an insert average length of 300 bp was constructed using a TruSeq PE Cluster Kit. This library was sequenced with the Illumina HiSeq platform (150 paired-end reads), and 42,452,942 raw and 37,248,211 clean, short-sequence paired-end reads were generated for further assembly. Before assembling, K-mer analysis on the read sequence data with the condition of 15-mers indicated that the genome size was ~44.7 Mbp.

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Keywords

evolution, fungal pathogens, genome sequence, genomics, infection, microbe-genome sequencing, *Setosphaeria turcica*

Table 1. Genome assembly statistics of *Setosphaeria turcica* Et28A

Statistics	<i>S. turcica</i> Et28A
Number of scaffolds	30
Total length (bp)	43,480,261
N50 length (bp)	2,274,269
N90 length (bp)	878,248
GC content (%)	50.68
BUSCO completeness	97.6%
Gene number	13,183
Annotated gene number	13,124

Genome assembly was performed using the Celera Assembler (version 8.3) with default parameters after subreads correction (Berlin et al. 2015). The whole genome assembly was 42.2 Mbp, on 30 scaffolds (Table 1). The N50 and N90 sizes of the scaffolds were 2,274,269 and 878,248 bp, respectively (Table 1). Four gene prediction programs (Augustus, Genewise, SNAP, and GeneMark) were used to predict protein-coding genes from the genome assembly with default parameters, and 13,381 gene models were predicted (Birney et al. 2004; Johnson et al. 2008; Stanke et al. 2008; Ter-Hovhannisyan et al. 2008). Benchmarking universal single-copy orthologs (BUSCO v4) analysis was used to assess the completeness of the predicted genes (Seppey et al. 2019). By comparison with the fungi_obd9 database, the genome was estimated to be 97.6% complete, with 283 complete BUSCOs from a total of 290 BUSCO groups (with one duplication, one fragmented, and five missing).

Functional annotation was accomplished by analysis of protein sequences. The highest quality alignment was chosen as the gene annotation. Functional annotation was completed by blasting genes against different databases, such as nonredundant (Nr), gene ontology (GO), Kyoto encyclopedia of genes and genomes (KEGG), cluster of orthologous groups of proteins (COG), pathogen host interactions (PHI), fungal cytochrome P450 database, carbohydrate-active enZymes database (CAZy), etc. (Galperin et al. 2015; Kanehisa and Goto 2000; Park et al. 2008; Pruitt et al. 2005; The Gene Ontology Consortium 2019; Urban et al. 2019). This resulted in 13,124 genes that were well annotated with at least one annotation.

The results provide the foundation for a comprehensive understanding of *S. turcica* at a high resolution genome level. For example, a previous analysis of genome sequence of *S. turcica* strain 28A (43.1 Mbp, 407 scaffolds) (Mideros et al. 2018) suggested there may be a significant duplication of a portion of the sequence, that likely complicated assignment of key effector encoding genes (Mideros et al. 2018). These issues should be resolved by in depth analyses of the present long read assembly and remapping of markers used by Mideros et al. (2018) to construct the genetic map with 21 linkage groups.

The genome sequences have been deposited in GenBank under accession numbers CP054627 to CP054656 (PRJNA638013). Raw reads from PacBio platform have been deposited in NCBI SRA database with the accession number PRJNA635532.

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