Four Reference Quality Genome Assemblies of *Pyrenophora teres* f. *maculata*: A Resource for Studying the Barley Spot Form Net Blotch Interaction

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Abstract

Pyrenophora teres is the causal agent of net blotch, the most devastating foliar disease of barley. In nature, net blotch is seen in two forms, net form net blotch, caused by *P. teres* f. *teres*, and spot form net blotch, caused by *P. teres* f. *maculata*. To date, 11 *P. teres* f. *teres* genomes have been sequenced and deposited in publicly available repositories, but only one *P. teres* f. *maculata* genome has been publicly deposited. Here, we present four additional reference-quality full-genome sequences of *P. teres* f. *maculata* isolates with good geographical and phenotypic diversity, with accompanying RNA sequencing-based genome annotations. These additional *P. teres* f. *maculata* genomes will aid in the understanding of the genomic complexities of this important barley pathogen.

Genome Announcement

Net blotch is the most economically important foliar disease of barley, as it is present across all barley-growing regions and typical yield losses range from 10 to 40%, with potential for total crop loss given environmental conditions favorable to the pathogen. Net blotch is caused by the necrotrophic fungus *Pyrenophora teres* that exists in two forms, *P. teres* f. *teres*, causal agent of net form net blotch and *P. teres* f. *maculata*, causal agent of spot form net blotch (Smedegård-Petersen 1971). The two forms can be distinguished by the lesions formed on infected barley leaf tissue, with *P. teres* f. *teres* forming necrotic striated lesions with a distinct net-like pattern and *P. teres* f. *maculata* forming oval necrotic lesions with a chlorotic halo (Shipton et al. 1973; Smedegård-Petersen 1971). Previous work has argued that the two forms of *P. teres* could be classified as separate species, based on their genetic isolation and the rarity of hybridization of the two forms in nature, despite their presence in the same field and often infecting the same leaf (Clare et al. 2020; Ellwood et al. 2012; Jalli 2011; Rau et al. 2007; Syme et al. 2018). Though the two forms of *P. teres* are often present in the same field, one form is often dominant within a geographical region, due to the susceptibility of the predominant cultivars planted, for which the two forms vary in virulence (Clare et al. 2020).

Recent genomic resources for *P. teres* f. *teres* have been released and provided critical information about the genome of this important plant pathogen. The first *P. teres* f. *teres* genome sequenced was the Canadian isolate 0-1, which was sequenced using Illumina sequencing (Ellwood et al. 2010), and later, the 0-1 genome was resequenced using Pacific

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Biosciences long-read sequencing to obtain a near chromosome level reference genome (Wyatt et al. 2018). Due to the decreased costs in sequencing technologies, it is now feasible to have multiple representative reference-quality genomes for a single species (Goodwin et al. 2016; Thomma et al. 2016). Four additional *P. teres* f. *teres* isolates were sequenced and assembled to near chromosome resolution and were used in a comparative analysis with the reference isolate 0-1 (Wyatt et al. 2020). The comparative analysis of the five genomes revealed important genomic architecture relating to known virulence loci in *P. teres* f. *teres* and established common trends within the *P. teres* f. *teres* genomes (Wyatt et al. 2020). The first and only *P. teres* f. *maculata* genome sequence released was the Australian isolate SG1. SG1 was sequenced using long-read technology and was optically mapped to produce near chromosome level scaffolds (Syme et al. 2018). A comparative analysis was performed between the *P. teres* f. *maculata* SG1 genome and the *P. teres* f. *teres* genomes W1-1 and 0-1, revealing differences between the genomes of the two forms primarily relating to expansions of repeat elements in *P. teres* f. *teres* that were absent from *P. teres* f. *maculata* (Syme et al. 2018).

To date there are 11 total genome sequences for *P. teres* f. *teres* publicly available but only one *P. teres* f. *maculata* genome. The lack of available *P. teres* f. *maculata* genomes presents an obstacle to comparative analysis both within and between the two forms of *P. teres*. In order to produce additional *P. teres* f. *maculata* genomic resources, we used Pacific Biosciences long-read sequencing to sequence four *P. teres* f. *maculata* isolates from diverse geographical locations, FGOB10Ptm-1 (North Dakota, U.S.A.), P-A14 (Montana, U.S.A.), and Den2.6 (Denmark), and NZKF2 (New Zealand), to complement the already available Australian isolate SG1. Additionally, we performed RNA sequencing of in vitro culture and four in planta infection timepoints for isolate FGOB10Ptm-1, to aid in annotating these *P. teres* f. *maculata* genomes. Isolates FGOB10Ptm-1, P-A14, Den2.6, and NZKF2 were chosen based on their geographical diversity and their previous use in disease evaluations (Carlsen et al. 2017; Neupane et al. 2015; Tamang et al. 2015, 2019).

High-molecular weight DNA was extracted from isolates FGOB10Ptm-1, P-A14, Den2.6, and NZKF2, following a modified CTAB method as outlined by Wyatt et al. (2020). For isolate FGOB10Ptm-1, high-molecular weight DNA was then shipped on dry ice to the Mayo Clinic Genome Analysis Core facility in Rochester, Minnesota, for sequencing on a Pacific Biosciences RSII, using nine single-molecule real-time cells. For isolates P-A14, Den2.6, and NZKF2, high-molecular weight DNA was shipped on dry ice to the Genomics and Bioinformatics Research Unit at the United States Department of Agriculture Agricultural Research Service (USDA-ARS) station in Stoneville, Mississippi. Each isolate was sequenced using a 20-kb size-selected library on a Pacific Biosciences RSII or Sequel to >200× coverage. Genomes were assembled as described by Wyatt et al. (2020); briefly, Canu v1.8 (Koren et al. 2017) was used with FASTQ file inputs for correction, trimming, and assembly. Genome assemblies were polished using 20× coverage Ion Torrent sequencing, using the genome polishing program Pilon (Walker et al. 2014). Briefly, Ion Torrent sequencing was performed following a two restriction enzyme digest of genomic DNA with the restriction enzymes *HhaI* and *ApeKI* and Ion Torrent adapter ligation (Leboldus et al. 2015). Genome assemblies for isolates FGOB10Ptm-1, Den2.6, and NZKF2 resulted in less than complete chromosome level assemblies and were therefore aligned to the genome assembly of isolate P-A14 for scaffolding and gap estimation, using the program RagTag (Alonge et al. 2019).

RNA sequencing was performed as described by Wyatt et al. (2018) with samples taken from pure culture and in planta timepoints of 48, 72h, 96, and 120 h postinoculation. Pure culture consisted of *P. teres* f. *maculata* spores inoculated in Friesie medium and allowed to grow in the dark for 72 h. The highly susceptible Pinnacle barley was sown into a 96-conetainer rack and was grown under greenhouse conditions for approximately 2 weeks. Pinnacle barley was inoculated with spores from isolate FGOB10Ptm-1 at a concentration of 2,000 spores/ml and were placed into a lighted mist chamber for 24 h, with 100% relative humidity. After 24 h, inoculated plant material was moved to a growth chamber with a temperature of 21°C and a 12-h photoperiod. Inoculated leaf tissue was collected at the appropriate timepoint and was immediately flash-frozen in liquid nitrogen. Total mRNA from each sample was extracted using the mRNA Direct kit (Thermo-Fisher Scientific), following manufacturer protocol. RNA sequencing library preparation was done with the Illumina Truseq v.3 kit, following the manufacturer protocol. RNA sequencing was performed on an Illumina

Table 1. Genome summary statistics

Genome statistic	P-A14	NZKF2	Den2.6	FGOB10Ptm-1
Total genome size (Mb)	42.0	42.6	42.7	38.2
Contigs	12	14	13	46
Scaffolds	12	12	12	12
N50 scaffolds (Mb) ^a	3.9	3.8	4.1	3.4
L50 ^b	5	5	5	5
Genes	9,219	9,863	9,799	9,509
BUSCO score (%) ^c	96.3	98.2	98.5	98.8
Proteins with Pfam domain (%) ^d	60%	62%	61%	60%
Predicted secreted proteins ^e	1,004	695	699	1,000
Predicted effectors ^f	258	180	187	257

^a N50 is the largest length L, such that 50% of all nucleotides are contained in scaffolds of size at least L (Bradnam et al. 2013; Earl et al. 2011; Lander et al. 2001).

^b L50 is the number N of sequences that comprise the N50 statistic.

^c BUSCO scores are calculated with BUSCO v4, using the Ascomycota dataset (Simão et al. 2015).

^d Proteins with Pfam domains were identified using the Funannotate accessory scripts (Palmer and Stajich 2017).

^e Secreted proteins were predicted with SignalP 5.0 (Almagro Armenteros et al. 2019).

^f Effector proteins were predicted with EffectorP 2.0 (Sperschneider et al. 2018).

NextSeq at the USDA-ARS Small Grains Genotyping Center (Fargo, North Dakota) to produce 150-bp single-end reads.

Gene models for each genome were generated in a similar manner to that of Wyatt et al. (2018), using the Maker2 genome annotation software (Holt and Yandell 2011). Briefly, amino acid sequences from the closely related species *Pyrenophora tritici-repentis*, *P. teres* f. *teres*, and the *P. teres* f. *maculata* isolate SG1 were input into the Maker2 software in conjunction with both de novo assembled and alignment-based transcripts from RNA sequencing data. Transcripts were de novo assembled using the program Trinity (Haas et al. 2013) and were aligned to the genomes of each isolate using HISAT2 (Kim et al. 2019), for subsequent transcript assembly with Stringtie (Pertea et al. 2016). Gene models were predicted from these inputs with a maximum intron length set to 3,000 bp and were used as a training set for the *ab initio* genome annotation software SNAP (Johnson et al. 2008). This process was iteratively done twice more to produce final gene models. Gene models were then subject to Funannotate functions to provide functional annotations (Palmer and Stajich 2017). Secreted proteins were predicted with SignalP 5.0 (Almagro Armenteros et al. 2019) and effectors were predicted with EffectorP 2.0 (Sperschneider et al. 2018). Genome completeness was estimated using the program BUSCO with the Ascomycota data set (Simão et al. 2015).

The genome assembly of isolate P-A14 produced 12 total contigs representing the 12 chromosomes present in *P. teres* f. *maculata*, while the genome assemblies of Den2.6 and NZKF2 produced 13 and 14 total contigs, respectively, that were then aligned to the P-A14 genome and scaffolded into 12 chromosomes each (Table 1). Isolate FGOB10Ptm-1 assembly resulted in 46 contigs and was scaffolded into chromosome-based synteny with the P-A14 genome and was subsequently compared with the previously published FGOB10Ptm-1 × SG1 genetic map (Table 1). Genome size of the four *P. teres* f. *maculata* genomes ranged from 38.2 to 42.7Mb (Table 1). Genome completeness was estimated for each genome, using the single copy orthologs from the BUSCO Ascomycota dataset with the four *P. teres* f. *maculata* genomes scoring between 96.3 to 98.8% complete (Table 1).

Genome annotation of the four *P. teres* f. *maculata* genomes produced RNA sequencing guided gene models. Total gene models ranged from 9,219 to 9,863 with between 60 and 62% containing predicted Pfam domains (Table 1). Secreted proteins are particularly interesting for plant pathogens, as they are often involved in the pathogen-host interaction and 695 to 1,004 secreted proteins were identified in the four *P. teres* f. *maculata* genomes, with 180 to 258 predicted by EffectorP 2.0 to be effectors (Table 1).

These newly generated genomes and annotations of *P. teres* f. *maculata* isolates will provide necessary genomic resources for the investigation of spot form net blotch of barley. The genomic resources generated will aid in effector identification and characterization in *P. teres* f. *maculata* and will also increase the speed of resistance and susceptibility gene identification in barley. Additionally, these new genomic resources will be useful for comparative studies both within *P. teres* f. *maculata* and between *P. teres* f. *maculata* and *P. teres*

f. *teres*. Genomes and genome annotation data for isolate FGOB10Ptm-1 (SAMN08007490) has been submitted to NCBI under the BioProject PRJNA417860 along with isolates P-A14 (SAMN15340022), Den2.6 (SAMN15340081), and NZKF2 (SAMN15340079).

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