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Version 2

🌐 Draft genome of the switchgrass head smut pathogen *Tilletia maclaganii* V.2

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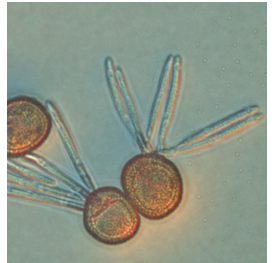
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Abstract

The head smut (*Tilletia maclaganii*) is a significant pathogen of the bioenergy crop switchgrass. *T. maclaganii* typically is more prevalent in older stands of switchgrass and can contribute to significant biomass loss. Here, we outline the methods for the sequencing, assembly, and annotation of the first reference genome for *Tilletia maclaganii*.

Image Attribution

Photo by Christine Layton



Guidelines

NA

Materials

DNA and RNA isolation:

- Macherey-Nagel Nucleobond HMW DNA kit
- New England Biolabs Monarch Total RNA Miniprep Kit (New England Biolabs, MA, USA)
- Thermo Scientific Nanodrop (Thermo Scientific, MA, USA)
- Agilent 5200 Fragment Analyzer (Agilent Technologies, Santa Clara, CA, USA)

Genome sequencing:

- PacBio Sequel IIe sequencing platform (Pacific Biosciences, CA, USA)
- Megaruptor 3 (Diagenode, Seraing, Belgium)
- SMRTbell Barcoded Adapter Plate 3.0 (Pacific Biosciences, CA, USA)
- SMRTbell Express Template Prep Kit 2.0 (Pacific Biosciences, CA, USA)
- AMPure PB Beads (Pacific Biosciences, CA, USA)
- BluePippin (Sage Science, MA, USA)
- SMRT Link 10.2, 8M v1 SMRT cells, Version 2.0 sequencing chemistry

Transcriptome sequencing:

- PerkinElmer Sciclone NGS robotic liquid handling system
- Illumina TruSeq Stranded mRNA HT sample prep kit
- KAPA Biosystems next-generation sequencing library qPCR kit (KAPA Biosystem, MA)
- Roche LightCycler 480 real-time PCR instrument (Roche, MA, USA)
- Illumina NovaSeq 6000 sequencing platform
- NovaSeq XP v1.5 reagent kits, S4 flow cell

Bioinformatic analysis:

- bbmap suite (<http://sourceforge.net/projects/bbmap/>)
- BBtools version 38.79 [kmercountexact.sh default; bbnorm.sh pigz passes=1 bits=16 target=9999999 min=174; bbduk.sh maxgc=0.4].
- Flye version 2.9-b1768 [-g 100k --asm-coverage 100 --pacbio-hifi]
- Prodigal version 2.6.3 [-p meta]
- HMMER version 3.1b2 [--domtblout]
- BBDuk [bbduk.sh k=25 mm=f kmask=N]
- BBTools [k=25 mm=f mkf=0.03 ordered ow]
- RACON version 1.4.13 [-u -t 36]
- Trinity version 2.11.0

Safety warnings

 NA

Ethics statement

NA

Before start

NA

Fungal isolation and DNA extraction

- 1 *Tilletia maclaganii* was isolated from switchgrass cultivar Shawnee in Ithaca NY, USA (42.4654130 N, -76.4590049 W) in 2021.

Approximately 100 mg of teliospores collected from infected switchgrass panicles were transferred to a 1.5 ml microcentrifuge tube. The teliospores were then surface sterilized by the addition of 1.0 ml of 0.165% (v/v) sodium hypochlorite, vortexing, and incubating for approximately 20 seconds. The teliospore suspension was then centrifuged for 10 seconds at 1000 X g, followed by removal of the supernatant with a pipettor. Sterile distilled water (1.0 ml) was then added and teliospores vortexed to rinse. The teliospore suspension was again centrifuged for 10 seconds at 1000 X g and supernatant removed with a pipettor. Two additional rinses with sterile distilled water were performed in the same manner.

Following surface sterilization, the teliospores were incubated in 1.0 ml sterile distilled water for 24 hours at 24 C to stimulate production of basidiospores from teliospore surfaces. After 24 hours, basidiospores were dislodged from teliospores by vortexing the 1.5 ml tube for approximately one minute. The basidiospore/teliospore suspension was then left to settle for 5 minutes at 24 C, with heavier teliospores separating from the lighter basidiospores by gravity. After 5 minutes, a clearer upper layer and brown lower layer in the suspension became visible within the suspension, with greater numbers of basidiospores populating the clearer upper layer and teliospores populating the lower layer. 100 µl aliquots of the upper suspension layer were transferred and spread onto 100 mm diameter water agar (1% w/v) plates. The plates were immediately inspected under a stereomicroscope, and ungerminated single basidiospores identified and transferred with a sterile, cool scalpel to Malt Extract Peptone Agar (MPA) medium [1] plates amended with 20 µg/ml ciprofloxacin and 10 µg/ml gentamicin [2].

Mycelium from a single basidiospore (isolate Tm001-NY21) was grown in Malt Extract Peptone Broth (MPB) medium for 14 days at 24°C and shaking at 100 rpm [1]. DNA was extracted with a Macherey-Nagel Nucleobond HMW DNA kit, with the manufacturer's protocol, with the following modifications: the volumes of lysis buffer H1 and Proteinase K added to the disrupted mycelium in step 2 were increased from 5 ml and 200 µl to 10 ml and 400 µl respectively, and the volume of binding buffer H2 added to the sample in step 6 of the protocol was also doubled from 10 µl to 20 µl. The remainder of the protocol was performed as directed by the manufacturer. Final DNA quality was assessed with a Thermo Scientific Nanodrop and Agilent Femto Pulse System fragment analyzer. A sequence check of the internal transcribed spacer (ITS) region of the genomic DNA was performed using universal fungal ITS PCR primers ITS1 and ITS4 [3]

followed by Sanger sequencing and NCBI BLAST analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Fungal isolation and RNA extraction

- 2 For RNA isolation from fungal tissue, mycelium of *Tilletia maclaganii* Tm001-NY21 was grown in 100 ml MPB medium for 14 days with shaking (50 rpm) at 24°C. Three hours prior to harvest, the fungus was challenged with pentachloronitrobenzene (PCNB) fungicide at a final concentration of 1 ppm. After incubation with PCNB, the mycelium was harvested and filtered, flash-frozen in liquid nitrogen, and disrupted with mortar and pestle. Total RNA was then extracted using a New England Biolabs Monarch Total RNA Miniprep Kit (New England Biolabs, MA, USA) according to the manufacturer's instructions.

Total RNA extraction from switchgrass infected with *Tilletia maclaganii* was performed by first harvesting infected plant tissue (stems and panicles), flash freezing in liquid nitrogen, then disrupting the plant tissue with mortar and pestle. Approximately 100 mg of disrupted tissue was then processed for total RNA extraction in a manner identical to that described for *T. maclaganii* Tm001 NY21 mycelium above.

Final RNAs quality was assessed with a Thermo Scientific Nanodrop (Thermo Scientific, MA, USA) and Agilent 5200 Fragment Analyzer (Agilent Technologies, Santa Clara, CA, USA).

DNA sequencing

- 3 DNA was sequenced using the PacBio Sequel IIe sequencing platform (Pacific Biosciences, CA, USA). An input of 1.5 µg of genomic DNA was sheared around 10 kb using the Megaruptor 3 (Diagenode, Seraing, Belgium). The sheared DNA underwent exonuclease treatment to remove single-stranded ends. Subsequently, it was subjected to a DNA damage repair enzyme mix and an end-repair/A-tailing mix. The DNA was then ligated with the SMRTbell Barcoded Adapter Plate 3.0 (Pacific Biosciences, CA, USA), using barcoded overhang adapters from the SMRTbell Express Template Prep Kit 2.0 (Pacific Biosciences, CA, USA). Lastly, the DNA was purified using AMPure PB Beads (Pacific Biosciences, CA, USA). Libraries were pooled based on the required amount of reads per sample, ensuring each sample received adequate coverage for analysis. Pooled libraries are then size-selected using the 0.75% agarose gel cassettes with Marker S1 and High Pass protocol on the BluePippin (Sage Science, MA, USA). PacBio Sequencing primer was then annealed to the SMRTbell template library and sequencing polymerase was bound to them using Sequel II Binding kit 2.0. The prepared SMRTbell template libraries were then sequenced on a PacBio Sequel IIe sequencer using SMRT Link 10.2, 8M v1 SMRT cells, and Version 2.0 sequencing chemistry with 1×1800 movie run times.

RNA sequencing

- 4 RNA was sequenced using the Illumina NovaSeq 6000 sequencing platform. Plate-based RNA sample preparation was performed with the PerkinElmer Sciclone NGS robotic liquid handling system, utilizing Illumina's TruSeq Stranded mRNA HT sample prep kit. Poly-A selection of mRNA was carried out following the protocol outlined by Illumina in their user guide (https://support.illumina.com/sequencing/sequencing_kits/truseq-stranded-mrna.html). The PCR conditions were as follows: 1 µg of total RNA starting material per sample and 8 cycles of PCR were used for library amplification. The prepared library was then quantified using KAPA Biosystems next-generation sequencing library qPCR kit (KAPA Biosystem, MA) and run on a Roche LightCycler 480 real-time PCR instrument (Roche, MA, USA).
Once quantified, the library was multiplexed with other libraries, and the pooled libraries sequenced on an Illumina NovaSeq 6000 sequencing platform using NovaSeq XP v1.5 reagent kits, S4 flow cell, following a 2×150 indexed run recipe.

Bioinformatic analysis

- 5 Circular consensus sequencing (CCS) reads were initially filtered using the BBMap suite (<http://sourceforge.net/projects/bbmap/>) to remove low-quality reads, sequencing errors, and other artifacts. The mitochondrial genome was assembled separately from the nuclear genome using CCS reads. Organelle-derived reads were isolated based on coverage and GC content, excluding nuclear sequences using a maximum coverage threshold of 1.5 times the k-mer coverage peak and a GC content cutoff of 0.40 using BBTools version 38.79 [kmercountexact.sh default; bbnorm.sh pigz passes=1 bits=16 target=9999999 min=174; bbduk.sh maxgc=0.4]. An initial mitochondrial assembly was generated with Flye [4] version 2.9-b1768 [-g 100k --asm-coverage 100 --pacbio-hifi], followed by gene prediction using Prodigal [5] version 2.6.3 [-p meta]. Predicted genes were searched against a curated mitochondrial HMM database using HMMER [6] version 3.1b2 [--domtblout]. Contigs with putative mitochondrial genes were masked for ribosomal loci using BBTools [bbduk.sh k=25 mmf=kmask=N] and a curated eukaryotic ribosomal database. These masked contigs were used to recruit additional CCS reads using BBTools [k=25 mmf= mkf=0.03 ordered ow], and a second Flye assembly was performed with the same parameters. An additional iteration of read recruitment and assembly followed. Contigs shorter than 1 kpb were removed from the assembly and polish was performed with two rounds of Racon [7] version 1.4.13 [racon -u -t 36] to produce the final mitochondrial assembly.
- 6 The mitochondrial assembly was used to remove organelle-derived reads from the CCS . The filtered nuclear reads were assembled using Flye version 2.9-b1768 [-t 32 --pacbio-hifi], followed by two additional rounds of Racon [8] polishing.
- 7 Genome and mitochondrial assemblies were annotated using the JGI Annotation Pipeline [8-9] with supporting Illumina transcriptome RNA-Seq data.

- 8 RNA-Seq reads were filtered using BBduk from the BBmap suite. Raw reads were evaluated for artifact sequences by kmer matching [kmer=25], allowing for 1 mismatch. Detected artifacts were trimmed from the 3' end of the reads. RNA spike-in reads, PhiX reads, and reads containing any N's were removed. Quality trimming was performed using the phred trimming method with a threshold of Q6. After trimming, reads shorter than the length threshold were discarded (minimum length 25 bases or 1/3 of the original read length, whichever was longer). Finally, the trimmed and filtered reads were assembled into consensus sequences using Trinity [10] version 2.11.0.

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