Complete Chromosome-Scale Genome Sequence Resource for *Sporisorium panici-leucophaei*, the Causal Agent of Sourgrass Smut Disease

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Abstract

Here, we present the first complete chromosome-level genome assembly of the smut fungus strain *Sporisorium panici-leucophaei* SPL10A, the causal agent of the sourgrass (*Digitaria insularis*) smut disease. Combining Illumina paired-end and Nanopore long reads, we generated a final assembly composed of 23 chromosomes (22 nuclear and one mitochondrial) with 18,915,934 bp. Gene prediction accomplished using extrinsic evidence from the sugarcane smut fungus *Sporisorium scitamineum* originated a total of 6,402 protein-encoding genes. The secretome (388 proteins) and the effectorome repertoires (68 candidates) were also predicted, given their crucial roles in plant-pathogen interactions. The complete telomere-to-telomere chromosome sequences of this poorly studied fungus will provide a valuable resource for future comparative genomic studies among smuts to unravel their underlying pathogenicity mechanisms.

Genome Announcement

Fungi from the Ustilaginaceae family are the primary causal agents of smut diseases in members of the Poaceae family. The best-studied smut fungi (genera *Ustilago* and *Sporisorium*) infect species of agronomic importance, such as cereals, forages, and sugarcane (Horst 2013). Infected crops result in variable yield losses to farmers, ranging from negligible to severe proportions, depending on the cultivar and environmental conditions (Quijano et al. 2016; Sundar et al. 2012). Smuts, a nonmonophyletic group composed of more than 600 species, are biotrophic fungi dependent on the host intimate physiological interaction to complete their life cycle (Bakkeren et al. 2008). Some species are nonpathogenic and have potential biotechnological value (Geiser et al. 2014). In pathogenic interactions, the secretion of effector molecules by smut fungi is a critical element to overcome host defense and

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Keywords

genome assembly, nanopore sequencing, plant-pathogen interaction, smut fungi, *Sporisorium scitamineum*

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Fig. 1. Sourgrass smut disease symptom and the complete *Sporisorium panici-leucophaei* version 1 genome assembly. The small image shows the whip-shaped structure developed in an infected sourgrass plant from which we collected spores for fungal isolation. From the outside in, the genome image represents the following tracks: chromosomes in kilobase pairs of each chromosome; predictions of effectors in red tiles; a histogram with Illumina sequencing coverage ranging from 0 to 30x; green tiles representing repetitive elements; and colored links represent alignments of repetitive sequences (100 bp < length < 500 bp) obtained by BLASTn (e-value < 1e-5 and identity of 95%) on the first and last 500 bp of each chromosome. Genome figure generated by Circos (Krzywinski et al. 2009).

establish host colonization. Common symptoms of smut diseases include the formation of tumors, abnormal floral components, and whip-like structure development (Benevenuto et al. 2018; Lo Presti et al. 2015).

Sporisorium panici-leucophaei (Bref.) M. Piepenbr., also known as *Anthracocystis panici-leucophaei* and formerly described as *Lundquistia panici-leucophaei* (Cunnington et al. 2005; Piepenbring 1999; Stoll et al. 2005) infects several plant genera of the Poaceae family. However, sourgrass [*Digitaria insularis* (L.) Fedde], a well-known invasive weed species resistant to glyphosate, is considered the fungus primary host (Clayton and Renvoize 1986; Piątek 2006). The characteristic disease symptom is the growth of a whip-like structure from the culm apex, similar to those caused by another smut fungi infecting sugarcane, *Sporisorium scitamineum* (Taniguti et al. 2015). Studies on the pathogenicity and genomic features of this smut species are scarce. Here, we present a complete chromosome-scale genome sequence and features of the predicted secretome and effectorome of *S. panici-leucophaei*.

S. panici-leucophaei spores were isolated from infected sourgrass plants developing a whip-like structure (Fig. 1). Infected plants were collected in the Teaching, Research, and Extension Farm, FEPE, Universidade Estadual Paulista, Ilha Solteira Campus in the city of Selvíria, Mato Grosso do Sul, Brazil (approximate geographic coordinates: latitude 20° 22' S

Table 1. Summary of the genome sequencing, assembly and annotation statistics

Feature	<i>Sporisorium panici-leucophaei</i>
Raw data (Nanopore) (Mbp)	417
Raw data (Illumina) (Mbp)	418
Assembly software	Canu
Genome (bp)	18,915,934
Total sequence coverage	32×
Number of chromosomes	22
Largest chromosomes (bp)	2,102,384
Smallest chromosome (bp)	152,803
Mitochondrial genome (bp)	78,051
Mitochondrial contigs	1
Genome N50 (bp)	969,070
GC content (%)	53.99
BUSCO completeness (genome mode) (%)	97.7
BUSCO completeness (protein mode) (%)	97.2
Repeat elements (%)	2.71
Number of predicted protein-encoding genes	6,402
Number of predicted secreted proteins	388
Number of predicted effectors	68

and longitude 51° 22' W). Sequence analysis of the PCR-amplified internal transcribed spacer (ITS) region using universal ITS primers (Stoll et al. 2003) revealed >99% identity with sequences of *S. panici-leucophaei* and *A. panici-leucophaei* available in GenBank (accession numbers AY344986, AY998102, and AY740035).

Yeast-like haploid cells germinated from a single spore (SPL10) were tested for mating-type (SPL10A) and were deposited at the Collection of Reference Fungi on Health Surveillance (CFRVS), FIOCRUZ, Instituto Oswaldo Cruz, under accession numbers 40436 and 40437. Haploid cells were grown as described by Benevenuto et al. (2016) for further DNA extraction and sequencing (Benevenuto et al. 2018). We used a hybrid assembly strategy by combining short reads from Illumina and long reads from Oxford Nanopore platforms to sequence and assemble the *S. panici-leucophaei* genome SPL10A.

High-molecular weight DNA was extracted using the Genomic-tip 20G kit according to manufacturer instructions for yeasts (Qiagen, Inc.). DNA integrity and quantity were checked on pulse-field gel electrophoresis (Taniguti et al. 2015) and were measured using a Qubit 2.0 Fluorometer (Invitrogen, ThermoFisher Scientific). The MinION sequencing library was prepared according to the Genomic DNA by Ligation kit (Oxford Nanopore Technologies), NEBNext Ultra II End-prep reaction/FFPE DNA repair (New England Biolabs), ligation buffer, NEBNext Quick T4 DNA ligase, and adapter mix, all according to manufacturer instructions. A sample of 1 µl of the final library eluted in 15 µl of Nanopore elution buffer was quantified and 5 to 50 fmol was used for loading the flow cell with 45 pores available (MinION flow cells (R9.4.1) (Oxford Nanopore Technologies). The MinION device sequenced for 48 h, and MinKNOW (MKE_1013_v1_revAR_11Apr2016) ran in mode base-calling real-time. We obtained a total of 69,189 reads (N50 = 8,852 bp, maximum length = 89,048 bp, minimum length = 1,004 bp, average length = 6,016 bp) comprising 417 Mbp.

We then prepared a second DNA library using the Nextera DNA sample preparation workflow kit to obtain short paired-end sequences, using the MiSeq Illumina platform (2× 300 bp). Reads were analyzed using FASTQC (v.0.10.1) and Seqclean (v.1.8.10) to filter low-quality sequences (Phred quality below 20). The resulting 715,363 reads generated approximately 418 Mbp of DNA sequenced.

For the first step of genome assembling, we used Canu v1.8 (Koren et al. 2017) to assemble the long reads with the following parameters: genomeSize = 25m, correctedErrorRate = 0.144, and -nanopore-raw to specify the Nanopore reads (available on GitHub). All other parameters used were default set values, including minReadLength of 1 kb. Second, we used the Illumina short reads to improve the assembly. BWA-MEM v0.7.17 was used to align short reads on the first raw assembly, and then, Pilon v1.23 (Walker et al. 2014) with the parameter “-fix bases,gaps” was used to correct potential misassemblies. Cross_match (Gordon et al. 1998) was used to close gaps in the scaffolds, generating an assembly with 23 contigs. Finally, we used Pilon again (×2) to polish this final assembly. We covered each chromosome

sequence with an average 15-fold of Illumina reads (Fig. 1) and approximately 17-fold of Nanopore reads (Table 1), resulting in a total 32-fold coverage assembly.

Telomeric repeats were manually analyzed, searching for the canonical repeated sequence TTAGG (Benevenuto et al. 2016; Taniguti et al. 2015). Using RepeatModeler v1.0.11 and RepeatMasker v4.1.0 (Saha et al. 2008) (both available from the Institute for Systems Biology) containing the RepBaseRepeatMaskerEdition-20181026, we identified repetitive regions in the genome. Finally, BUSCO v4.1.2 (Simão et al. 2015) was applied to evaluate the genome assembly and gene prediction completeness, using the Basidiomycota database (basidiomycota_odb10.2019-11-20) as a reference.

The final complete chromosome-scale genome sequence of *S. panici-leucophaei* (version 1) comprised 18,915,934 nucleotides distributed in 22 chromosomes sequenced telomere-to-telomere (Fig. 1) and one mitochondrial DNA sequence of 78,051 bp (Table 1). The largest chromosome consisted of 2,102,384 bp (chromosome 1), whereas chromosome 22 had less than 155,000 bp (Table 1). The genome harbored around 3% repetitive sequences with no apparent biased distribution in the chromosomes (Fig. 1).

BUSCO v4.1.2 identified 97.7% completeness of the genome assembly and 97.2% of the protein prediction considering the 1,764 orthogroups in the reference database for Basidiomycota. Gene predictions accomplished with Augustus v3.3.3 (Stanke and Morgenstern 2005) (parameter-species = *ustilago_maydis*) and proteins of *S. scitamineum* (Taniguti et al. 2015) as extrinsic evidence, as described in section “alternate protocol 7” of Hoff and Stanke. (2019), generated 6,402 protein-encoding genes.

We defined the predicted secretome of *S. panici-leucophaei* proteome following the pipeline: i) scanning proteins that presented a signal peptide with SignalP v5.0b (Almagro Armenteros et al. 2019), ii) selecting those that did not present a transmembrane domain with TMHMM V2.0c (Krogh et al. 2001), and iii) no endoplasmic reticulum retention sequence (PS00014) with PS-Scan (de Castro et al. 2006). Min (2010) showed that this strategy improved the accuracy of predictions. From a total of 6,402 predicted proteins, 388 proteins defined the fungus predicted secretome (Table 1). EffectorP 2.0 (Sperschneider et al. 2016) was used to predict the candidate effectors repertoire (effectorome) from the secretome, where approximately 17.5% (68 proteins) of all secreted proteins were considered candidate effectors (Fig. 1). To explore sequence identity to *S. scitamineum* genome, we used NUCmer from MUMmer v3.1 (Kurtz et al. 2004), with parameters --maxmatch -l 20, for global alignments. The comparison of 4.1 Mb contiguous sequences between the two genomes resulted in approximately 87% identical nucleotides.

In the present study, we generated a complete genome assembly of *S. panici-leucophaei* and used it to predict the encoded proteins and the effectorome repertoire.

To the best of our knowledge, this is the first *S. panici-leucophaei* genome sequence available. We showed that a complete set of chromosomes (telomere-to-telomere) of a smut fungus could be achieved by combining long MinION nanopore and short MiSeq Illumina reads at low depth of coverage. We expect that the *S. panici-leucophaei* genome will provide a valuable resource for future comparative studies toward understanding pathogenicity in smuts.

Data availability. The complete chromosome-scale genome sequence of strain *Sporisorium panici-leucophaei* SPL10A has been deposited in GenBank under accession numbers CP060299 to CP060321 (BioProject: PRJNA648134; BioSample: SAMN15629615, Long Read Archive: SRR12314149/SRR12314150).

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Author-Recommended Internet Resource

GitHub leucophaei assembly: <https://github.com/lmtani/leucophaei-assembly>

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