The First Genomic Resources for *Phymatotrichopsis omnivora*, a Soilborne Pezizomycete Pathogen with a Broad Host Range

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Abstract

Phymatotrichopsis omnivora is a destructive plant pathogen causing root rot disease of alfalfa, cotton, pecan, grape, and many other important dicotyledonous species. A member of the family Rhiziniaceae, in the class Pezizomycetes, *P. omnivora* is a soilborne ascomycete fungus that is difficult to maintain in culture, currently genetically intractable, and for which there are no publicly available genomic resources. We have generated draft genome sequences of four *P. omnivora* isolates obtained from cotton and alfalfa, growing in Texas and Oklahoma. These genome sequences will provide new insights into the biology of the fungus, including the factors responsible for its broad host range and pathogenicity.

Genome Announcement

The ascomycete fungus *Phymatotrichopsis omnivora* belongs to the family Rhiziniaceae (Marek et al. 2009) and represents one of the few known plant pathogens from the order Pezizales, which is notably comprised of saprobes (e.g., *Ascochytium immersus*) and symbionts (e.g., *Tuber melanosporum*). *P. omnivora* is a destructive plant pathogen causing root rot disease (commonly referred as cotton root rot or Phymatotrichum root rot) of many plants including alfalfa, cotton, pecan, grape, and vegetable crops. This fungus has an extraordinarily broad host range and has been shown to exhibit high plasticity, infecting as a necrotrophic or hemibiotrophic pathogen (Kankanala et al. 2020; Taubenhuis and Ezekiel 1936). Of the 2,116 plant species tested, 81% were susceptible to the pathogen, but notably, all monocotyledonous plants were not affected (Taubenhuis and Ezekiel 1936). *P. omnivora* has no known sexual stage, and the pathogen is endemic to southwestern United States and Mexico, where the disease is visibly active during summer (Percy 1983; Young et al. 2015). Despite its economic importance, there are no public genomic resources available for *P. omnivora*. Several attempts to transform this fungus have been made, but to date, this fungus remains genetically intractable (Uppalapati et al. 2010). Furthermore, this fungus is difficult to maintain in the lab as active cultures readily lose pathogenicity. To better understand this recalcitrant pathogen both from a biological and a management perspective, genomes of four isolates of *P. omnivora* were sequenced and assembled. To capture knowledge of pathogen diversity, we selected *P. omnivora* isolates that originated from two different host crops and three locations, and represented two different mating types (Table 1). One of the selected isolates, NFPO1 was used to study *P. omnivora*–host and –nonhost interactions with *Medicago truncatula* (susceptible), *Arabidopsis thaliana* (partially resistant), and *Brachypodium distachyon* (resistant/nonhost) (Kankanala et al. 2020).

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*The e-Xtra logo stands for “electronic extra” and indicates there are supplementary materials published online.

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Keywords

alfalfa, ascomycete, cotton root rot, Phymatotrichum root rot

Table 1. Genome assembly features of *Phymatotrichopsis omnivora* isolates

Isolate	NFPo1 ^a	NFPo18 ^a	NFPo20 ^a	NFPo30
Host	Alfalfa	Alfalfa	Alfalfa	Cotton
Isolation year	2014	2014	2014	2007
Location (county, state)	Carter, Oklahoma	Love, Oklahoma	Love, Oklahoma	Reeves, Texas
Putative mating type ^b	MAT1-2	MAT1-2	MAT1-1	MAT1-1
Genome assembly size (Mb)	109.0	107.1	108.7	107.8
Number of contigs	22,063	25,419	25,171	26,805
Genome N50 (kb)	9,663	7,705	8,205	7,114
Genome largest contig (kb)	93,987	123,073	93,965	139,063
Repeat regions	38%	39%	39%	40%
GC content (%)	43%	44%	43%	44%
Number of predicted genes	14,827	14,728	15,033	14,747
BUSCO completeness (fungal lineage) (%)	94	94	93	93
Small secreted proteins	380	413	428	403
CAZymes	332	325	326	324

^a Isolate NFPo1 is described in Kankanala et al. (2020), NFPo18 and NFPo20 originated from the study site described in Mattupalli et al. (2015, 2018, 2019).

^b Mating-type genes were identified using TBLASTN with the mating-type genes from *Tuber melanosporum* (MAT1-1-1 accession number HM370280) and *Tuber indicum* (MAT1-2-1 accession number KF318364) as query sequences. The accession numbers of the *P. omnivora* mating-type sequences are MW357816 to MW357819.

Four hyphal-tipped pure cultures of *P. omnivora* isolated from alfalfa and cotton in Texas and Oklahoma (NFPo1, 18, 20, and 30; Table 1) were grown from mycelial plugs in liquid modified ATCC medium 1078 (Marek et al. 2009) in the dark without shaking at 28°C for 8 to 10 days. Genomic DNA was extracted using the Quick-DNA Fungal/Bacterial DNA MiniPrep kit (Zymo Research Corporation, Irvine, CA). Sequencing libraries were prepared using Nextera XT DNA library kit and each *P. omnivora* isolate was sequenced on its own flow cell using the v3 paired end 600 cycle kit (600 bp paired end reads) on the Illumina MiSeq platform (San Diego, CA) at the University of Kentucky HealthCare Genomics Center (Lexington, KY). De novo genome assembly was performed using SPAdes (Bankevich et al. 2012), and repetitive DNA sequences were identified with RepeatScout version 2.1.4 (Price et al. 2005). The genomes were masked using RepeatMasker version 4.0.9 (Smit et al. 2015) before gene prediction was performed using AUGUSTUS v3.3.2 with the incorporation of RNA-seq (150 bp paired end reads) data from NFPo1 in vitro growth on modified ATCC medium 1078 and half-strength Murashige-Skoog medium supplemented with vitamins (PhytoTechnology Laboratories, Shawnee Mission, KS) for 7 days at 28°C. Genome completeness was evaluated using BUSCO (Simão et al. 2015), and a phylogeny of selected *Pezizomycetes* species (Fig. 1) was generated using 100 BUSCO genes (Supplementary Table S1). Amino acid sequences of each gene were aligned separately using MAFFT (Nakamura et al. 2018), and then a super alignment was created by concatenating all individual alignments. The phylogeny was estimated from this alignment using RAxML version 8.2.12 (Stamatakis 2014) with the GAMMAWAG. The phylogenetic relationships observed in the multigene phylogeny (Fig. 1) are consistent with trees based on large subunit and small subunit rDNA sequences and the deduced amino acid sequences of RPB2 (Marek et al. 2009). SignalP version 4.1 (Petersen et al. 2011) and dbCAN2 (Zhang et al. 2018) were used to identify predicted secreted proteins or carbohydrate-active enzymes (CAZymes), respectively.

The draft genome assemblies of the isolates were larger than most fungal genomes and rich in repeats. Assembly length ranged from 107.1 to 109.0 Mb with repeats accounting for 38 to 40% of the sequences (Table 1). Given the repetitiveness of the genomes and because they were assembled without long reads, it is likely that the genomes are larger than these estimates. The number of predicted genes was ~15,000. Despite the fragmented genomes, a high proportion of the gene-coding regions were captured in these assemblies given that BUSCO scores ranged between 93 and 94%.

These genomes will be a useful resource for those studying *P. omnivora* and cotton root rot disease. It will now be possible to generate genetic tools to study population structure and develop sensitive in-field detection methods for growers and stakeholders whose land might be a reservoir for this pathogen. Although a sexual stage has never been reported for this fungus, the sequenced isolates possessed different mating types, with NFPo20 and NFPo30 containing the *MAT1-1* idiomorph, whereas NFPo1 and NFPo18 contained *MAT1-2*. This may indicate the potential for *P. omnivora* to be heterothallic and able to sexually recombine in nature.

Fig. 1. Phylogeny of *Phymatotrichopsis omnivora* isolates sequenced in this study with selected representatives of Pezizomycetes and Orbiliomycetes (outgroup). The phylogeny was estimated on a super alignment of 100 concatenated BUSCO gene alignments (genes listed in Supplementary Table S1). Species included in the phylogeny have been annotated by lifestyle as either, plant pathogen (red), animal pathogen (black), saprobe (blue), or symbiont (green).

Beyond the use to the immediate *P. omnivora* research community, these genomes will enable useful genomic comparisons within the class Pezizomycetes. This class is of interest due to its inclusion of the choice edible taxa, ectomycorrhizal truffles (Tuberaceae and Terfeziaceae) and saprotrophic morels (Morchellaceae), and the few plant-pathogenic species in the Rhiziniaceae clade, where *P. omnivora* groups with *Rhizina undulata* and *Caloscypha fulgens* (Fig. 1). Currently, many genomic resources are available for this class (<https://mycocosm.jgi.doe.gov/pezizomycetes/pezizomycetes.info.html>) and have been used to investigate the biology of the ectomycorrhizal truffle lifestyle (Marqués-Gálvez et al. 2021; Martin et al. 2010; Murat et al. 2018). Likewise, the addition of these genomes from *P. omnivora* will be useful for future comparative genomics projects investigating how the root-pathogenic lifestyle evolved in the Pezizomycetes.

The genome sequences have been deposited into GenBank under BioProject PRJNA641071 with BioSamples SAMN15340118 to SAMN15340121, and sequence read archive accessions SRR13120354 to SRR13120357. The RNAseq data are found under BioProject PRJNA642231 with BioSamples SAMN15390343 to SAMN1539035. The accession numbers of the mating-type gene sequences are MW357816 to MW357819.

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