

# A Genome Resource for the Apple Powdery Mildew Pathogen *Podosphaera leucotricha*

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## Abstract

Powdery mildew, caused by *Podosphaera leucotricha*, is an economically important disease of apple and pear trees. A single monoconidial strain (PuE-3) of this biotrophic fungus was used to extract DNA for Illumina sequencing. Data were assembled to form a draft genome of 43.8 Mb consisting of 8,921 contigs, 9,372 predicted genes, and 96.1% of complete benchmarking universal single copy orthologs (BUSCOs). This is the first reported genome sequence of *P. leucotricha* that will enable studies of the population biology, epidemiology, and fungicide resistance of this pathogen. Furthermore, this resource will be fundamental to uncover the genetic and molecular mechanisms of the apple–powdery mildew interaction, and support future pome fruit breeding efforts.

## Genome Announcement

Powdery mildew, caused by *Podosphaera leucotricha* (Ellis & Everh.) E. S. Salmon, is one of the most important and widespread diseases of cultivated apple (*Malus × domestica*) and pear (*Pyrus* spp.). Chronic effects of powdery mildew on tree vigor, especially in highly susceptible cultivars, can be detrimental to the profitability of orchards and nurseries (Butt et al. 1983; Serdani et al. 2005; Yoder 2000). Although young leaves are the most susceptible organs, powdery mildew infections also occur in blossoms and fruits, thus reducing crop yield and fruit market value because of fruit russet symptoms (Spotts 1984; Yoder 2000). Besides apple and pear, *Podosphaera leucotricha* has been reported from other host members of the Rosaceae family including peach (*Prunus persica*) (Jankovics et al. 2011), the African cherry tree (*Prunus africana*) (Mwanza et al. 2001), and ornamental photinias (*Photinia* spp.) (Garibaldi et al. 2005; Liang et al. 2012).

The biotrophic nature of *Podosphaera leucotricha* hinders research on the biology, genetic diversity, and host–pathogen interactions, which remain poorly understood (Amiri and Gañán 2019). Genetic and genomic approaches might overcome these limitations and help unravel some of the many unanswered questions. Despite the economic importance of powdery mildew on apple and pear crops, only 52 nucleotide sequences, all shorter than 1,600 bp, are publicly available in the NCBI database currently. Compared with other powdery mildew fungi, such as *Blumeria graminis* f. sp. *tritici*, *B. graminis* f. sp. *hordei*, and *Erysiphe necator*, genetic sequence resources available for *Podosphaera leucotricha* are much more limited. To overcome this limitation, we undertook a whole-genome shotgun sequencing approach to support the development of research studies on this significant plant parasite.

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## Keywords

biotrophic, mycology, pome fruit, whole-genome shotgun sequencing

**Table 1.** *Podosphaera leucotricha* draft genome assembly summary

Genome assembly feature	Value
Total length (bp)	43,868,508
Number of contigs $\geq 1,000$ bp	8,921
Size of largest contig (bp)	60,133
Contig $N_{50}$ (bp)	8,371
Contig $N_{90}$ (bp)	1,921
GC content (%)	43.69
BUSCO complete (%)	96.1
BUSCO duplicated (%)	0.4
BUSCO fragmented (%)	1.7
Number of predicted genes	9,372
Interspersed repeat content (%)	77.8
Genome accession	JAATOF000000000
Version accession	JAATOF010000000

The monoconidial strain PuE-3 of *Podosphaera leucotricha* was isolated from infected leaves of apple ('Golden Delicious'  $\times$  'White Angel') plantlets maintained in a growth chamber at Washington State University, Pullman, WA. *Podosphaera leucotricha* PuE-3 was grown and maintained on detached leaves from in vitro-propagated plantlets of apple genotype 02O3R5-75 (Wan and Fazio 2011). Conidia were collected from 14-day-old colonies using sterile acupuncture needles, followed by genomic DNA isolation using the Omniprep for fungi kit (G-Biosciences, St. Louis, MO), according to the manufacturer's protocol. A partial sequence of the internal transcribed spacer (ITS) region of ribosomal DNA was amplified using the primer pair ITS 5/ITS 4 (White et al. 1990). Results from a NCBI-BLASTn search revealed that the sequence obtained was 99% identical to the voucher specimen BPI 878262 of *Podosphaera leucotricha* (GenBank accession EU148597), thus confirming the identity of the PuE-3 strain. Genomic DNA was processed using KAPA Hyper Prep Kit (Kapa Biosystems, Wilmington, MA) to construct a paired-end library, which was then sequenced ( $2 \times 150$  bp) using the NextSeq 550 system (Illumina, San Diego, CA) at RTL Genomics (Lubbock, TX), producing a total of 50,255,101 read pairs. Genome data were filtered using the ATLAS framework (White et al. 2017a) with a custom database containing potential contaminants including human, apple, phiX, *Pseudomonas* spp., and other members of the phyllosphere microbiome. Local and global alignments were performed using Bowtie 2 (Langmead and Salzberg 2012), with the very-sensitive option, and then parsed using samtools and bbdudk (part of bbmap). Finally, the filtered data were trimmed using bbdudk and assembled in MEGAHIT (Li et al. 2015). k-mer lengths were preassessed for step length for MEGAHIT assembly using MerCat (White et al. 2017b).

Assembly of the sequenced reads yielded a draft genome of 43,868,508 bp in 8,921 contigs ( $\geq 1,000$  bp) with an  $N_{50}$  value of 8,371 and GC content of 43.7% (Table 1). Gene prediction and annotation was performed using the Genome Sequence Annotation Server (GenSAS 6.0) (Humann et al. 2019), following an eukaryotic annotation process. Repeat sequences were identified and masked using a combination of de novo and homology-based approaches. A de novo prediction of repetitive elements was performed using RepeatModeler v1.0.11, and the resulting library was inputted into RepeatMasker v4.0.5 (<http://www.repeatmasker.org>). Overall, 77.8% of the *Podosphaera leucotricha* PuE-3 genome was made of interspersed repeat elements and 0.1% of simple repeats. Protein-coding genes were predicted using a combination of *ab initio* gene prediction using GenMark-ES (Ter-Hovhannisyan et al. 2008), followed by a gene model refinement with the tool EvidenceModeler (Haas et al. 2008). A total of 9,372 putative protein-coding genes were identified, of which 7,223 contained PFAM domains annotated by InterProScan v5.29-68.0 (Jones et al. 2014). Genome completeness analysis was estimated based on evolutionarily-informed expectations of gene content using the Universal Single-Copy Ortholog tool BUSCO v3 (Waterhouse et al. 2018; Simão et al. 2015). Benchmarking universal single copy ortholog (BUSCO) results reported 1,264 complete BUSCOs (C: 96.1%) of a total of 1,315 core genes of the Ascomycota\_odb9 lineage data set.

This draft genome sequence of *Podosphaera leucotricha* PuE-3 represents the first genome resource available for any powdery mildew fungus infecting economically important members of the family Rosaceae. This resource provides new data that will accelerate the development of molecular tools needed to conduct population biology and epidemiology studies of *Podosphaera leucotricha*. The newly developed genomic sequences can also be used as a

reference for future genomic and transcriptomic studies in *Podosphaera leucotricha*. Specifically, these data will be useful for uncovering *Podosphaera leucotricha* effector genes involved in the host–pathogen interactions, assist future pome fruit breeding efforts, and provide insights into the evolution of powdery mildew fungi.

This whole genome shotgun project has been deposited at DDBJ/ENA/GenBank under accession JAATOF000000000 (BioProject number PRJNA562291). In this manuscript, we describe the version JAATOF010000000. Raw reads from the Illumina sequencing are available in the NCBI Sequence Read Archive under the accession SRR11471384. A voucher specimen of *Podosphaera leucotricha* PuE-3 was deposited in the Washington State University, Charles Gardner Shaw Mycological Herbarium (WSP), under accession number WSP73997, and the partial sequence of the ITS region, used for identity confirmation, was deposited in GenBank under accession MT180425. Assembly code is available on GitHub ([https://github.com/raw937/pleucotricha\\_mildew](https://github.com/raw937/pleucotricha_mildew)), and other data files mentioned herein are stored and publicly accessible in the Open Science Framework database (<https://osf.io/reh8b>).

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