High-Quality Genome Assembly of *Peronospora destructor*, the Causal Agent of Onion Downy Mildew

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Abstract

Peronospora destructor is an obligate biotrophic oomycete that causes downy mildew on onion (*Allium cepa*). Onion is an important crop worldwide, but its production is affected by this pathogen. We sequenced the genome of *P. destructor* using the PacBio sequencing platform, and de novo assembly resulted in 74 contigs with a total contig size of 29.3 Mb and 48.48% GC content. Here, we report the first high-quality genome sequence of *P. destructor* and its comparison with the genome assemblies of other oomycetes. The genome is a very useful resource to serve as a reference for analysis of *P. destructor* isolates and for comparative genomic studies of the biotrophic oomycetes.

Onion (*Allium cepa* L.) is widely cultivated and consumed worldwide because of its pungent flavor and nutritional benefits. Bulb onions were grown in Egypt more than 5,000 years ago (Brewster 1994). Onion is economically important and, according to the Food and Agriculture Organization of the United Nations, it is one of the top three horticultural crops worldwide, along with tomato and potato. Onion production is affected by several abiotic and biotic factors (Fenwick et al. 1985). Downy mildew is one of the most devastating diseases of onion. It is caused by *Peronospora destructor*, a fungus-like obligate biotrophic organism belonging to the oomycetes. During favorable cool and humid weather, its spores infect onion leaves with subsequent necrosis and serious damage to growing onions, markedly reducing yield.

The genomes of several downy mildews have been sequenced, including *Peronospora tabacina* (tobacco), *Peronospora effusa* (spinach), *Hyaloperonospora arabidopsidis* (Arabidopsis), *Pseudoperonospora cubensis* (cucurbits), *Plasmopara viticola* (grapevine), *Plasmopara halstedii* (sunflower), and *Sclerospora graminicola* (pearl millet) (Baxter et al. 2010; Derevnina et al. 2015; Dussert et al. 2016; Feng et al. 2018; Nayaka et al. 2017; Sharma et al. 2015; Tian et al. 2011). Most of them were sequenced using HiSeq short-read sequencing and are, consequently, highly fragmented. Because these pathogens are obligate biotrophic and nonculturable, it is difficult to obtain high-quality, contamination-free genomic DNA. Contiguous genome sequences are a benefit for understanding genomic approaches such as studies on

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Keywords

downy mildew, genome, onion, pathogen, *Peronospora destructor*

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*The *e-Xtra* logo stands for “electronic extra” and indicates that a supplementary figure and supplementary tables are published online.

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Table 1. Assembly statistics of the *Peronospora destructor*

Feature	<i>P. destructor</i>
Total contig size (bp)	29,334,786
GC content (%)	48.48
Contig N ₅₀ (bp)	727,749
Largest length (bp)	2,289,071
No. of contigs	74
No. of contigs matching to Oomycete	74
No. of telomeric repeat contigs	25
BUSCO genome (S/D/F/M)% ^a	97.9 (97.0:0.4:0.4:1.7)
GenBank accession no.	WBRY000000000

^a S = single copy, D = duplicated, F = fragmented, M = missing BUSCOs.

annotations of effector genes, telomeric and centromeric regions, repeat elements, coregulated gene clusters, and structural rearrangements (Thomma et al. 2016).

In this study, we sequenced the genome of *P. destructor*, the causal agent of downy mildew of onion, based on long-read sequencing using PacBio single-molecule real-time (SMRT) sequencing technology to obtain a high-quality assembled genome. We collected sporangia by scraping onion leaves sporulated with downy mildew in an onion field in Muan, South Korea. We confirmed that the collected pathogen was *P. destructor* using internal transcribed spacer sequencing analyses. We prepared high-molecular weight genomic DNA with a phenol extraction method (Cho et al. 1994). The DNA was sequenced on a PacBio Sequel system at DNA Link (South Korea). Running one Sequel cell with a 20-kb insert size library-incorporated v3.0 chemistry produced 686,600 subreads containing 7.8 Gb of filtered subread bases (coverage 256×) and a subread N₅₀ of 17,048 bp. When we analyzed filtered subreads using GenomeScope, the genome size was expected to be 30.2 Mb with $k = 17$ (Supplementary Fig. S1) (Vurture et al. 2017) and heterozygosity was displayed 0.83% with one peak, which was similar to other oomycetes (less than 1%) (Fletcher et al. 2019). Using HGAP.4, the data were assembled to 74 contigs with a total size of 29,334,786 bp (97% of the expected size), a GC content of 48.48%, contig N₅₀ of 727,749 bp, and longest contig length of 2,289,071 bp (Supplementary Table S1). When we mapped with 686,600 subreads to the assembled genome, allowing duplicates, a total of 1,379,984 reads were aligned and 1,274,620 reads were completely mapped (accounted for 92.4% as mapping rate). To check the contamination of DNA fragments of other microbes, each contig was subject to a National Center for Biotechnology Information BLAST search. All 74 contigs matched the oomycete genome sequences, showing no similarity with mitochondrial sequences, and were determined to be the *P. destructor* genome. Completeness was confirmed by BUSCO (v3) (Simão et al. 2015). Of the 234 core alveolata and stramenopile benchmarking universal single-copy orthologs (BUSCOs), 229 (97.9%) were complete (228 singles and one duplicated), while one was fragmented and four were missing (Table 1). This result was significantly in accordance with the other oomycetes. Among 74 contigs, 25 contigs presented telomeric sequence (TTAGGG) repeats at the end of the contig (Supplementary Table S2). Where, in the case of ribosomal DNA sequence, one contig had ribosomal RNA (rRNA) genes encompassing 6,082-bp homology with the rRNA genes of *Peronospora* or *Phytophthora* species. Thus, we postulate that *P. destructor* comprised at least 13 chromosomes.

In summary, the assembled genome of *P. destructor* is nearly complete in accordance with the significant level of conserved BUSCOs and expectable chromosome numbers. To the best of our knowledge, this is the first genome sequence of onion downy mildew and is an improved, concise, and contiguous genome sequence of biotrophic oomycetes. It will serve as a genome resource for plant-pathogenic oomycetes and promote the study of molecular pathogenic mechanisms.

This whole genome shotgun project has been deposited at the DDBJ/ENA/GenBank database under accession number WBRY000000000. The version described in this paper is version WBRY000000000.

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