Improved Genome Sequence and Gene Annotation Resource for the Potato Late Blight Pathogen *Phytophthora infestans*

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Abstract

Phytophthora infestans is a devastating pathogen causing potato late blight (*Solanum tuberosum*). Here we report the sequencing, assembly and genome annotation for two *Phytophthora infestans* isolates sampled in Republic of Korea. Genome sequencing was carried out using long read (Oxford Nanopore) and short read (Illumina Nextseq) sequencing technologies that significantly improved the contiguity and quality of *P. infestans* genome assembly. Our resources would help researchers better understand the molecular mechanisms by which *P. infestans* causes late blight disease in the future.

Funding

This work was carried out with the support of Rural Development Administration, Republic of Korea “Cooperative Research Program for Agriculture Science & Technology Development (project number PJ01418603)”.

Keywords

genomics, potato late blight, *Phytophthora infestans*, *Solanum tuberosum*

Genome Announcement

Phytophthora infestans is one of the major threats to the potato and tomato industry all over the world. This oomycete pathogen causes late blight in potato and tomato and it has been a serious concern throughout the history of humankind, with the most damaging outbreak resulting in the Irish potato famine (Bourke et al. 1993; Fry 2016). Due to its scientific and economic importance, *P. infestans* has been extensively studied. Recently, the research focus has been to better understand the coevolution between *P. infestans* and its host plants at the molecular level (Kamoun et al. 2015; Leesutthiphonchai et al. 2018).

Similar to other plant pathogens, *P. infestans* is expected to deliver many effector proteins into the host plant cells and intercellular space. The main function of these effector proteins is to suppress plant immunity responses and promote pathogen proliferation in the host tissue (Hogenhout et al. 2009; Kamoun 2006). Most *P. infestans* effectors carry N-terminal signal peptide sequences for secretion (Haas et al. 2009; Kamoun 2006). In addition, many *P. infestans* effector

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

The author(s) declare no conflict of interest.

Accepted for publication 18 April 2020.

proteins carry a RXLR or crinkler (CRN, for crinkling and necrosis; LXLFLAK) motif required for translocation into host cell (Zhang et al. 2016). Interestingly, some of these effectors can be recognized by specific intracellular NLR (nucleotide-binding domain leucine-rich repeat-containing) receptors and activate plant immunity (Birch et al. 2008; Morgan and Kamoun 2007).

In order to gain insights into *P. infestans* epidemiology and its interaction with host plants, several genome studies have been conducted. It was found that the *P. infestans* genome is the largest of all sequenced species of the *Phytophthora* genus and a significant part of it (approximately 74%) consists of repetitive sequence (Haas et al. 2009). Such high repeat content is not only the evidence for genome expansion under a strong evolutionary pressure but also a significant obstacle for reliable genome assembly (Gijzen 2009; Thomma et al. 2016). The current *P. infestans* reference genome has been generated by Sanger-based random shotgun sequencing (Haas et al. 2009). In addition, the short read-based draft genome assemblies of multiple *Phytophthora* species have been reported (Liu et al. 2016; Longmuir et al. 2017; Reyes-Tena et al. 2019; Studholme et al. 2019; Vetukuri et al. 2018). Recently, several long-read sequencing-aided high contiguity genome assemblies of *Phytophthora ramorum* (Malar C et al. 2019a and b), *P. capsici* (Cui et al. 2019), *P. cactorum* (Yang et al. 2018), and *P. sojae* (Fang et al. 2020) became available. Utilizing the long-read sequencing technologies was shown to dramatically improve the assembly contiguity, especially around the highly repetitive DNA sequences. Therefore, we used the combination of long- and short-read sequencing technologies in order to achieve high contiguity and accuracy of genome assembly for one of the most important plant pathogens, *Phytophthora infestans* (Kamoun et al. 2015).

In the Republic of Korea, four major clonal lineages, KR_1_A1, KR_2_A2, SIB-1, and US-11, have been identified by genotyping with 12 simple sequence repeat (SSR) markers. KR_1_A1 and KR_2_A2 are Korea-specific genotypes. KR_1_A1, which is predominant in the Korean peninsula, is sensitive to metalaxyl and is the A1 mating type. KR_2_A2 is highly resistant to metalaxyl and is the A2 mating type found in Miryang, where winter cultivation of potato is common (Choi et al. 2020).

Two *Phytophthora infestans* isolates KR_1_A1 and KR_2_A2 were isolated from diseased potato plants in Daegwallyeong and Miryang cities, respectively, in the Republic of Korea. To prepare mycelia, *P. infestans* isolates were grown on V8 medium (17 g of agar, 50 mg of β -sistosterol, 900 ml of distilled water, 100 ml of V8 juice, 1.5 g of calcium carbonate) for 7 days. To increase mycelia production, each isolate was inoculated to 10 ml of liquid pea medium (120 g of frozen peas, 1 liter of distilled water) and was incubated for 14 days in a shaking incubator at 18°C and 160 rpm. Subsequently, mycelia were collected and were freeze-dried for DNA isolation. To prepare genomic DNA extraction for Oxford Nanopore Technology (ONT) sequencing, GeneAll Exgene Plant SV mini kit (GeneAll) was used for genomic DNA extraction according to manufacturer instructions. Genomic DNA libraries for ONT sequencing were constructed using the ONT 1D ligation sequencing kit, according to the manufacturer instructions. Sequencing was carried out using a single 1D flowcell for each isolate. To prepare genomic DNA extraction for Illumina sequencing, the fungi/yeast genomic DNA isolation kit (Norgen Corp.) was used, according to manufacturer instructions. Then, genomic DNA libraries with an average insert size of 350 bp were constructed according to the standard Illumina TruSeq Nano library protocol.

Phytophthora infestans KR_1_A1 isolate genomic DNA was sequenced using ONT Gridlon and Illumina Nextseq 2000 technologies, providing 63× long-read and 35× short-read genome coverage, respectively. Illumina short reads were quality-filtered and adapters were trimmed using fastp v0.19.7 (Chen et al. 2018). Long ONT reads were corrected using Canu v1.8 (Koren et al. 2017) correct command with the following settings, which resulted in 48× corrected read genome coverage: corOutCoverage = 500 corMinCoverage = 2 minReadLength = 2000 genomeSize = 250m. The corrected reads were assembled using SMARTdenovo v1.0, with -k 17, -c 1 preferences, to generate the long-read preassembly. The preassembly was iteratively polished using the Illumina short reads. For this, read mapping was performed using bwa-mem v0.7.17 (Li and Durbin 2009) and polishing was carried out using Pilon v1.23 (Walker et al. 2014). Each polishing iteration was followed by the genome completeness assessment, using BUSCO v3 (Simão et al. 2015) with protists set, until the BUSCO score did not improve with the following polishing iteration. The final assembly BUSCO score was 99.1% with 213 complete BUSCO ortholog groups presented of a total 215 in the Protista set (Table 1).

Table 1. Genome assembly and gene prediction statistics for *Phytophthora infestans* isolates

Parameters	T30-4	KR_1_A1	KR_2_A2
Genome assembly size (bp)	228,543,505	200,673,608	230,945,478
Number of contigs	18,288	1,510	3,344
Contig N50 (bp)	44,484	291,816	154,690
Longest contig (bp)	582,831	1,705,040	1,781,277
Spanned gaps (bp)	38,410,029	0	1,700
Repeats (%)	74	72.2	71.99
Total genes predicted	17,797	20,172	23,771
Number of RXLR effectors ^a	306	433	310
Number of CRN effectors ^a	54	40	50
Completeness (BUSCO score)	96.3%	99.1%	98.6%

^a RXLR and CRN effectors for all isolates were predicted in this study using EffectR and SignalP5.

KR_2_A2 genome sequencing yielded 49× long-read and 30× short-read genome coverages (Supplementary Table S1). However, after Canu read correction, the long-read N₅₀ length for KR_2_A2 was 5,579 bp, in contrast to 8,226 bp for KR_1_A1. We could not achieve satisfying results with Canu and SMARTdenovo assembly in terms of genome completeness and contiguity. Therefore, we used MaSuRCA v3.3.1 (Zimin et al. 2013), which polishes long reads with Illumina data prior to assembly, to generate the KR_2_A2 genome. The final assembly BUSCO score for KR_2_A2 was 98.6%, with 212 complete BUSCO ortholog groups presented of a total 215 in the Protista set (Table 1).

We further predicted gene models for *P. infestans* KR_1_A1 and KR_2_A2 isolates using the FunGAP gene annotation pipeline (Min et al. 2017). We utilized the transcriptome data available for *P. infestans* T30-4 (Short Read Archive accession SRS3443412) to aid our gene model prediction, since its read alignment rates were 92.3% and 92.33% for KR_1_A1 and KR_2_A2, respectively. We could predict a total of 20,172 genes, for KR_1_A1, and 23,771 genes, for KR_2_A2, which is significantly more than the 17,797 genes predicted in the previous *P. infestans* genome release (Haas et al. 2009). This difference is likely due to the newly discovered sequences that were previously reported as gaps and the use of different gene annotation tools. Next, we set out to identify the RXLR and CRN effector genes in the genomes of Korean *P. infestans* isolates. We first generated the six-frame translation of the T30-4 reference isolate and discarded any open reading frame (ORF) less than 100 bp in length. The resulting ORFs were used as input data for the effectR (Tabima and Grünwald 2019) tool to predict RXLR and CRN effectors. Subsequently, the RXLR and CRN candidate effectors were further analyzed for the presence of eukaryotic signal peptide, using SignalP5 (Almagro Armenteros et al. 2019). We have adjusted the SignalP5 sensitivity threshold to 0.7 as, in the same condition, functionally validated *P. infestans* effectors including PexRD36, PexRD1, Avrblb1, Avr4, and Avr3a were identified (Tabima and Grünwald 2019). As a result, we identified a total of 306 RXLR and 54 CRN effectors in the reference T30-4 genome. These numbers are smaller than what were reported previously (Tabima and Grünwald 2019), likely due to the stringent SignalP5 threshold used in this study. In the same conditions, we identified 433 RXLR and 40 CRN effector genes in KR_1_A1 and 310 RXLR and 50 CRN effector genes in KR_2_A2 genomes. Interestingly, we could identify significantly more effector candidates from KR_1_A1 than KR_2_A2 genome. We believe that the discrepancy was caused by a difference in the contiguity of genome assemblies of the two isolates.

Full genome assembly data and genome annotations were deposited to the National Center for Biotechnology Information database under BioProject accession number PRJNA592863.

Author-Recommended Internet Resource

GitHub SMARTdenovo v1.0: <https://github.com/ruanjue/smartdenovo>

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