Genome Resources for the Ex-Type of *Phytophthora citricola*, and Well-Authenticated Isolates of *P. hibernalis*, *P. nicotianae*, and *P. syringae*

Subodh K. Srivastava,^{1,2,†} Leandra M. Knight,^{1,2} Mark Nakhla,¹ and Z. Gloria Abad^{1,†}

¹ United States Department of Agriculture–Animal and Plant Health Inspection Service Plant Protection and Quarantine, S&T, Pathogen Confirmatory Diagnostics Laboratory, Beltsville, MD 20705

² Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695

Abstract

Phytophthora is one of the most important genera of plant pathogens, with many members causing high economic losses worldwide. To build robust molecular identification systems, it is very important to have information from well-authenticated specimens and, in preference, the ex-type specimens. The reference genomes of well-authenticated specimens form a critical foundation for genetics, biological research, and diagnostic applications. In this study, we describe four draft *Phytophthora* genome resources for the ex-type of *Phytophthora citricola* BL34 (P0716 WPC) (118 contigs for 50 Mb), and well-authenticated specimens of *P. syringae* BL57G (P10330 WPC) (591 contigs for 75 Mb), *P. hibernalis* BL41G (P3822 WPC) (404 contigs for 84 Mb), and *P. nicotianae* BL162 (P6303 WPC) (3,984 contigs for 108 Mb) generated with MinION long-read high-throughput sequencing technology (Oxford Nanopore Technologies). Using the quality reads, we assembled high-coverage genomes of *P. citricola* with 291× coverage and 16,662 annotated genes; *P. nicotianae* with 205× coverage and 29,271 annotated genes; *P. syringae* with 76× coverage and 23,331 annotated genes, and *P. hibernalis* with 42× coverage and 21,762 annotated genes. With the availability of genome sequences and their annotations, we predict that these draft genomes will be accommodating for various basic and applied research, including diagnostics to protect global agriculture.

Resource Announcement

Phytophthora is one of the most important genera of plant pathogens, with many members causing high economic and environmental impact diseases in nurseries, forests, and natural ecosystems. To build robust molecular identification systems for effective management of plant-pathogenic species of *Phytophthora*, it is very important to have information from well-authenticated specimens and, preferably, the ex-type specimens (Martin et al. 2012). The ex-type materials are living cultures (and duplicates of the type) deposited in international culture collections as a requirement for the official description of new species. The reference genomes from well-authenticated specimens form a critical foundation for genetics, biological research, and diagnostic applications. Until 2019, there were 83 genome assemblies for 22 species of *Phytophthora* at the NCBI; however, only two genomes were from ex-types. These genomes were for *Phytophthora lateralis* (Feau et al. 2016) and *P. agathidicida* (Studholme et al. 2015). In this article, we describe draft genome resources for four additional *Phytophthora* spp. acquired from the World *Phytophthora* Collection (WPC) at the University of California, Riverside that were generated using MinION long-read high-throughput sequencing technology

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Keywords

bioinformatics, oomycetes, pathogen detection

[†]Corresponding authors: Z. G. Abad; Gloria.Abad@usda.gov, and S. K. Srivastava; Subodh.k.srivastava@usda.gov

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Table 1. *Phytophthora* ex-type and well-authenticated species generated genome assembly statistics in this study using Nanopore sequencer and Canu assembler^a

Number	Attributes	<i>P. citricola</i>	<i>P. syringae</i>	<i>P. hibernalis</i>	<i>P. nicotianae</i>
1	Ex-type or selected isolate	ET BL34 (P0716 WPC)	SE BL57G (P10330 WPC)	SE BL41G (P3822 WPC)	SE BL162 (P6303 WPC)
2	Quality reads (>1,000 nucleotides)	2,403,276	1,301,009	328,327	2,870,407
3	Times coverage	292x	76x	42x	205x
4	Read mean length	4,994	3,787	7,135	4,621
5	Total assembled contigs	118	591	404	3,984
6	L ₅₀	14	51	46	590
7	N ₅₀	1,328,562	374,162	512,528	44,192
8	Largest contigs	2,766,784	1,955,381	2,209,908	484,771
9	Assembled genome size	50 Mb	75 Mb	84 Mb	106 Mb
10	Contigs > 100 kb	58	180	186	167
11	GC%	51.88	52.41	51.75	50.39
12	Assemble completeness (CEGMA) (%)	98	94	94	98
13	Assemble completeness (BUSCO) (%)	72	76	75	93
14	Predicted genes	16,662	23,331	21,762	29,271
15	NCBI accession number	VMRO000000000	JAAKBD000000000	JAAKBF000000000	JAAKBE000000000

^a Abbreviations: ET = ex-type material; SE = selective specimen; WPC = World Phytophthora Collection, Riverside, CA, U.S.A.; CEGMA = Core Eukaryotic Genes Mapping Approach (core eukaryotic gene set); and BUSCO = Benchmarking Universal Single-Copy Orthologs (single copy gene set).

(Oxford Nanopore Technologies [ONT]). These species include the ex-type of *P. citricola* isolate BL 34 (P0716 WPC) as well as the well-authenticated *P. hibernalis* isolate 41G (P3822 WPC), *P. nicotianae* isolate BL162 (P6303 WPC), and *P. syringae* isolate BL57G (P10330 WPC). We selected well-authenticated specimens for *P. hibernalis*, *P. nicotianae*, and *P. syringae* for species that have lost the ex-types at all international culture collections with information available at the "IDphy" International Online Resource for *Phytophthora* <http://idtools.org/id/phytophthora/about.php> (Abad et al. 2019). High-quality DNA (approximately 100 to 200 ng) was obtained from pure mycelial cultures using the Qiagen DNeasy Mericon Food Kit protocol for DNA extraction, modified to be used for *Phytophthora* spp. from the Abdel-Latif and Osman (2017) protocol for corn. The quality of the genomic DNA was determined using Qubit, Nanodrop, and Tape station (Agilent Technologies) and the genomic DNA libraries were prepared from 100 to 200 ng using the ONT ligation sequencing kit SQK-LSK109 (Abdel-Latif and Osman 2017; Srivastava et al. 2020). The raw reads were generated on a flowcell R9.4.1 in a MinION device for 48 h using ONT's sequencing protocol, resulting in 2.4 million reads for *P. citricola*, 1.3 million reads for *P. syringae*, 300,000 reads for *P. hibernalis*, and 2.8 million reads for *P. nicotianae*, and quality filtered reads (after filtered low quality and < 7 Q-score) were processed for sequence data conversion from Fast5 to FastQ with the guppy basecaller (Wick et al. 2019). We used filtered quality data having median read quality of 10.3, 11.6, 12.3, and 12.4 in these four assembled genomes (i.e., *P. citricola*, *P. hibernalis*, *P. nicotiana*, and *P. syringae* respectively). These quality reads were assembled and received high coverage for genomes using Canu assembly (Koren et al. 2017) and the contigs were polished with ONT reads to recorrect any error bases using Pilon (Walker et al. 2014). Finally, we evaluated the assembled contigs with quality assessment tools for genome assemblies known as QUAST (Gurevich et al. 2013) and CEGMA model sets (Nishimura et al. 2017; Simão et al. 2015). We trained the prediction model using a previously sequenced species (*P. capsici*) as a model organism for AUGUSTUS software (Cui et al. 2019; Stanke and Waack 2003; Stanke et al. 2008; Srivastava et al. 2020) and predicted proteins (Table 1). The analyses of available genomes of *P. nicotianae* genomes at the NCBI were used for comparative analysis with the *P. nicotianae* isolate BL162 (P6303 WPC) from *Gramatophyllum* sp. from Indonesia, mating type (A2) within species. The Canu assembly reported 834 repeats of a total length of 11,869,197 bp adding to this genome with the ex-type. This assembled genome showed variation with other previously submitted genomes (races) of the size of *P. nicotianae* race 0 and race 1 (Blair et al. 2008; Liu et al. 2016). Therefore, we did comparative genome analysis between sequenced species genic regions and predicted genes using the AUGUSTUS pipeline described previously (Srivastava et al. 2020, 2021), and extended the analysis with other sequenced *P. nicotianae* subspecies (BL162 with 29,260 genes, race 0 with 23,619 genes, race 01 with 20,170 genes, and GM01 with 16,776 genes). The comparative gene analysis

with sequenced species (BL162) revealed 5,641 additional genes in *P. nicotianae* BL162, and 318 were unique genes compared with the ex-type. We extended analysis using the NCBI non-redundant database, resulting in 135 unique genes. We analyzed PFAM domain annotations among these genomewide and found significant protein domain numbers for some important species; that is, RXLR phytopathogen effector protein, protein kinase domain, and pectate lyase in *P. citricola* = 111, 320, and 46 domains, respectively; in *P. hibernalis* = 157, 304, and 48 domains; in *P. nicotianae* = 240, 370, and 104 domains, respectively; and in *P. syringae*; 71, 336, and 47 domains, respectively.

In conclusion, we developed draft genome assemblies for four additional *Phytophthora* spp. from type and well-authenticated isolates for various research and diagnostic applications and have deposited these at the NCBI GenBank databases.

The whole-genome sequences of this project have been deposited into NCBI GenBank project PRJNA555328 for *P. citricola* BL34 (P0716 WPC) accession number VMRO000000000 with Sequence Read Archive (SRA) submission SRR9942578; the other three under project PRJNA605765 include *P. syringae* BL57G (P10330 WPC) accession number JAAKBD000000000 with SRA submission SRR11103462, *P. nicotianae* BL162 (P6303) accession number JAAKBE000000000 with SRA submission SRR11103463, and *P. hibernalis* BL41G (P3822 WPC) accession number JAAKBF000000000 with SRA submission SRR11103464.

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