

Short Research Paper

Genome Resource of Raspberry Root Rot Pathogen *Phytophthora gonapodyides*

Rishi R. Burlakoti¹✉, Sanjib Sapkota^{1,3}, Mark Lubberts²

1. Science and Technology Branch, Agassiz Research and Development Centre, Agriculture and Agri-Food Canada, Agassiz, BC, Canada.
2. Science and Technology Branch, Summerland Research and Development Centre, Agriculture and Agri-Food Canada, Summerland, BC, Canada.
3. Abbotsford Agriculture Centre, Ministry of Agriculture and Food, Abbotsford, BC, Canada.

✉ Corresponding author: Rishi R Burlakoti, rishi.burlakoti@agr.gc.ca.

© His Majesty the King in Right of Canada, as represented by the Minister of Agriculture and Agri-Food Canada, 2024. This is an open access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>). See <http://ivyspring.com/terms> for full terms and conditions.

Received: 2024.01.01; Accepted: 2024.04.21; Published: 2024.04.23

Abstract

Phytophthora gonapodyides is a newly reported oomycetes pathogen associated with root rot of red raspberry. We generated high-quality whole genome resource for *P. gonapodyides*, which was pathogenic on red raspberry. The genome size was 88,717,598 bp with a BUSCO completeness score of 93.9%. This genome resource provides insight on pathogen biology of *Phytophthora* spp. causing root rot of raspberry. To our best knowledge, this is the first complete genome assembly of plant pathogenic *P. gonapodyides*.

Keywords: *Phytophthora gonapodyides*, oomycete, Illumina Sequencing, red raspberry

Introduction

Phytophthora root rot and wilt caused by *Phytophthora* spp. is the most serious disease of raspberry (*Rubus idaeus* L.) worldwide, including the Pacific Northwest regions of Canada and USA [1]. The pathogen initially infects root of the plant and causes serious damage to entire plants with severe wilting symptoms. *P. rubi* is considered as a major pathogen causing Phytophthora root rot and wilt of raspberry, but multiple species of *Phytophthora* including *P. gonapodyides*, *P. cryptogea*, *P. citricola*, and *P. megasperma* were reported to cause the disease from different regions [1–5]. *Phytophthora* spp. survive in infected tissues or soil in the form of mycelia or oospores (sexual spores). When adequate moisture is available on soil, both mycelia and oospores can produce sporangia (asexual spores), which can directly infect root tissue or produce motile spores, zoospores for the infection. *Phytophthora* spp. colonize in plant roots and crown and block water and nutrient movement in the vascular system and ultimately cause root rot, wilting and other symptoms on foliage (yellowing, necrosis, and scorching). High rainfall and average temperatures of 15 to 20°C is favourable for the disease development [1].

Whole genome resources of *P. rubi* strains infecting raspberry in Canada were previously reported and their population diversity was assessed [2]. *P. gonapodyides* was reported as a new species of *Phytophthora* causing root rot of raspberry in Canada [3,4]. This species was also reported from raspberry in Chile [6]. However, the complete genome resource of *P. gonapodyides* infecting raspberry has not been published yet. Here, we report the whole genome resources of *P. gonapodyides*, which is a first step towards understanding molecular mechanisms of pathogenicity and host adaptation of this pathogen. The information can also be useful for managing root rot and wilt of raspberry.

Materials and Methods

We used the isolate BC14 for the whole genome assembly in this study. The detail of pathogen isolation, culture and DNA extraction methods are described in the previous studies [2–4]. The isolate (accession number SRR20227809) was identified as *P. gonapodyides* using multiplex targeted sequencing with degenerate primers of three nuclear genes: heat shock protein90, elongation factor 1 alpha and beta

tubulin as described in [3]. Genomic DNA of the pathogen was extracted using the MagMAX kit (ThermoFisher Scientific Inc.) following the manufacturer's instructions. For whole genome sequencing, 2 × 150 bp paired-end configuration libraries were constructed using KAPA Hyperprep PCR-free library kit according to the manufacturer's instructions. Sequencing was performed on an Illumina HiSeqX next-generation sequencing device at Admera Health LLC (Plainfield, NJ) according to the manufacturer's directions. We used methods from our previous study [2], to assemble the genome of *P. gonapodyides* isolate BC14. In brief, Spades v3.15 [7], was used for the genome assembly and was annotated was performed using Funannotate v1.8.13 (<https://github.com/nextgenusfs/funannotate>) with Interproscan [8] and SignalP v6 [9] additionally used for functional annotation. Genome completeness was evaluated with BUSCO v5.4.4 using the Eukaryota Odb10 gene set [10].

Results and Discussion

Illumina sequencing generated a total of 23.7 Gbp of raw sequence data, with a mean coverage of 178x of the *P. gonapodyides* isolate BC14. The detail of genome assembly is provided in Table 1. The assembled genome was 88,717,598 bp in size. The *P. gonapodyides* genome assembled into 29,771 scaffolds and a N50 of 4,047 bp, with a BUSCO completeness score of 93.9%. The total number of predicted genes and proteins were 33,892 and 34,011, respectively. The genome assembly and BUSCO values of *P. gonapodyides* from our study are comparable to previous genome assemblies of *Phytophthora* spp. obtained from short-read and long-read sequences. For example, the genome assembly size and BUSCO values of *P. rubi* recovered from raspberry was 65,960,000 bp with 93.3% completeness [2] and *P. cinnamomi* isolated from avocado was 109,700,000 bp with 97.5% [11]. We also compared genome assembly data of *P. gonapodyides* from our study with McGowan *et al.* [12] (Table 1). McGowan *et al.* [12] reported the assembled genome size of 61,088,431 bp and BUSCO value (87.2%) of *P. gonapodyides*, which was isolated by baiting with *Rhododendron* leaves from stream water in Ireland. However, the pathogenicity of the *P. gonapodyides* reported in their study is unknown [12]. *P. gonapodyides* isolate from our study was isolated from raspberry plants showing root rot and foliage symptoms and we confirmed the Koch' postulate by inoculating the isolate to the raspberry variety 'Chemainus' [4].

The genome resource of *P. gonapodyides* will be essential for understanding the pathogen biology,

developing diagnostic tools for pathogen detection, and exploring potential targets for effective disease management. This genomic data will also be a valuable resource for future comparative genomic and evolutionary studies of plant pathogenic species of *Phytophthora* and other oomycetes.

Table 1. Genome assembly statistics of *Phytophthora gonapodyides* isolate BC14 from our study compared with study from McGowan *et al.* [12].

Genome Assembly	<i>P. gonapodyides</i> Isolate BC14 from our study	<i>P. gonapodyides</i> from McGowan <i>et al.</i> [12]
Assembled genome Size (bp)	88,717,598	61,088,431
Number of Scaffolds	29,771	16,449
N50 (bp)	4,047	5455
GC Content	54.59%	55.7%
BUSCO Completeness	93.9%	87.2%
Numbers of predicted genes	33,892	23,348

Acknowledgments

Funding was provided by Canadian Agricultural Partnership (CAP)-AgriScience Program, the BC Raspberry Industry Development Council and the BC Lower Mainland Horticultural Improvement Association (Project ID: CAP-AgriScience Project No: ASP-007). We also thank Mr. Patrick Chapman for assisting in data analyses.

Data Availability

The genome sequence of the *P. gonapodyides* BC14 isolate has been deposited in NCBI GenBank database. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/NCBI GenBank under the accession JAZAPM000000000. The version described in this paper is version JAZAPM010000000. The SUBID, BioProject, and BioSample, and SRA accession numbers are SUB14081636, PRJNA856328, SAMN29767710, and SRR26681417, respectively.

Competing Interests

The authors have declared that no competing interest exists.

References

- Sapkota S, Burlakoti RR, Punja ZK, Dossett M, Gerbrandt E. Understanding the root rot and wilting complex of raspberry: current research advances and future perspectives. *Canadian Journal of Plant Pathology*. 2022;44: 323-344. doi:10.1080/07060661.2021.2011420
- Sapkota S, Burlakoti RR, Lubberts M, Lamour K. Genome resources and whole genome resequencing of *Phytophthora rubi* isolates from red raspberry. *Front Plant Sci*. 2023;14: 1161864. doi:10.3389/fpls.2023.1161864
- Sapkota S, Burlakoti RR, Lamour K, Lubberts M, Punja ZK. Development and application of multiplex targeted-sequencing approaches to identify *Phytophthora* species associated with root rot and wilting complex of red raspberry. Pandey AK, editor. *PLoS ONE*. 2022;17: e0275384. doi:10.1371/journal.pone.0275384
- Burlakoti RR, Sapkota S, Lubberts M, Lamour K. First report of *Phytophthora gonapodyides* causing root rot on raspberry in Canada. *Plant Disease*. 2023;107: 2268. doi:10.1094/PDIS-08-22-1940-PDN
- Wilcox WF. Identity, virulence, and isolation frequency of seven *Phytophthora* spp. causing root rot of raspberry in New York. *Phytopathology*. 1989;79: 93. doi:10.1094/Phyto-79-93

6. Wilcox WF, Latorre BA. Identities and geographic distributions of *Phytophthora* spp. causing root rot of red raspberry in Chile. *Plant Disease*. 2002;86: 1357-1362. doi:10.1094/PDIS.2002.86.12.1357
7. Pribelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A. Using SPAdes De Novo Assembler. CP in *Bioinformatics*. 2020;70: e102. doi:10.1002/cpbi.102
8. Jones RAC. Plant virus emergence and evolution: Origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. *Virus Research*. 2009;141: 113-130. doi:10.1016/j.virusres.2008.07.028
9. Teufel F, Almagro Armenteros JJ, Johansen AR, Gíslason MH, Pihl SI, Tsigirios KD, et al. SignalP 6.0 predicts all five types of signal peptides using protein language models. *Nat Biotechnol*. 2022;40: 1023-1025. doi:10.1038/s41587-021-01156-3
10. Manni M, Berkeley MR, Seppely M, Simão FA, Zdobnov EM. BUSCO Update: Novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Kelley J, editor. *Molecular Biology and Evolution*. 2021;38: 4647-4654. doi:10.1093/molbev/msab199
11. Engelbrecht J, Duong TA, Prabhu SA, Seedat M, Van Den Berg N. Genome of the destructive oomycete *Phytophthora cinnamomi* provides insights into its pathogenicity and adaptive potential. *BMC Genomics*. 2021;22: 302. doi:10.1186/s12864-021-07552-y
12. McGowan J, O'Hanlon R, Owens RA, Fitzpatrick DA. Comparative Genomic and Proteomic Analyses of Three Widespread *Phytophthora* Species: *Phytophthora chlamydospora*, *Phytophthora gonapodyides* and *Phytophthora pseudosyringae*. *Microorganisms*. 2020;8: 653. doi:10.3390/microorganisms8050653