Complete Genome Sequence Resource for the Necrotrophic Plant-Pathogenic Bacterium *Pectobacterium carotovorum* WPP14

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Abstract

Pectobacterium spp. are a major cause of loss in vegetable and ornamental plant production. One of these species, *Pectobacterium carotovorum*, can cause soft rot disease on many plants, particularly potato. These diseases lead to significant economic loss and pose food security threats by reducing crop yields in the field, in transit, and during storage. The Gram-negative enterobacterium *P. carotovorum* WPP14 is a particularly virulent strain for which there is no available closed genome, limiting the molecular research for this important pathogen. Here, we report a high-quality complete and annotated genome sequence of *P. carotovorum* WPP14. The 4,892,225-bp genome was assembled with Nanopore reads and polished with Illumina reads, yielding 394× and 164× coverage, respectively. This closed genome provides a resource for research on improved detection and biology of *P. carotovorum*, which could translate into improved disease management.

Pectobacterium spp. are necrotrophic bacterial pathogens that cause a wide range of diseases such as soft rot, stem wilt, and blackleg on a variety of plant hosts all over the world (Charkowski 2018; Kim and Jeon 2016; Lu et al. 2020; Voronina et al. 2019; Zlatković et al. 2019). Many species belong to the family Pectobacteriaceae. The species *Pectobacterium carotovorum* is divided into several subspecies. Recent genome analyses and taxonomic reassessments have resulted in some *P. carotovorum* strains of the subspecies being elevated to species rank and the designation of novel species of *Pectobacterium* (Oulghazi et al. 2019; Portier et al. 2019).

P. carotovorum WPP14 has been reported as one of the most virulent among different *P. carotovorum* (formerly *Erwinia carotovora* subsp. *carotovora*) strains when inoculated onto potato leaf petioles (Yap et al. 2004). A draft genome of *P. carotovorum* WPP14 is available with 731 contigs and 23.3-fold coverage (Glasner et al. 2008). Here, we report a de novo assembled, closed, and annotated genome sequence of *P. carotovorum* WPP14, which was originally isolated from diseased potato tubers and stems from Wisconsin during summer 2001 (Yap et al. 2004). This improved genome resource of *P. carotovorum* WPP14 could aid in population studies of *Pectobacterium*, advance understanding of pathogenicity, and facilitate the development of diagnostics tools of these widespread and destructive plant pathogens.

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Keywords

de novo assembly, *Pectobacterium*, soft rot, whole-genome sequencing

Table 1. Genome summary and average nucleotide comparisons of *Pectobacterium carotovorum* WPP14 to other *Pectobacterium* spp. type strains

<i>Pectobacterium</i> strains	Source	ANI (%) ^a	Genome size (bp)	Contigs
<i>P. carotovorum</i> WPP14 (this work)	NCBI: CP051652	NA	4,892,225	1
<i>P. carotovorum</i> WPP14 (previous)	NCBI: ASM17315v1	99.87	4,823,187	741
<i>P. carotovorum</i> ICMP 5702	NCBI: ASM103905v1	97.50	4,774,457	46
<i>P. versatile</i> CFBP 6051	NCBI: ASM429668v1	95.87	4,949,375	148
<i>P. odoriferum</i> NCPPB 3839	NCBI: ASM75476v1	95.27	5,066,211	156
<i>P. aquaticum</i> A212-S19-A16	NCBI: ASM338256v2	94.04	4,348,076	158
<i>P. polaris</i> NIBIO1006	NCBI: ASM230735v1	93.58	4,826,824	1
<i>P. actinidiae</i> KKH3	NCBI: ASM80331v1	93.23	4,922,167	3
<i>P. brasiliense</i> LMG21371	NCBI: ASM75469v1	93.20	4,831,535	109
<i>P. peruvienne</i> IFB5232	NCBI: ASM284734v1	89.85	4,785,880	60
<i>P. atrosepticum</i> ICMP 1526	NCBI: ASM103868v1	89.80	4,869,384	33
<i>P. betavascularum</i> NCPPB 2795	NCBI: ASM74984v1	89.55	4,685,213	93
<i>P. zantedeschiae</i> 9M	NCBI: ASM413779v1	89.29	5,054,916	18
<i>P. punjabense</i> SS95	NCBI: ASM302839v1	89.10	4,732,531	73
<i>P. wasabiae</i> CFBP3304	NCBI: ASM174218v1	89.00	5,043,228	1
<i>P. parmentieri</i> RNS08-42-1A	NCBI: ASM174214v1	88.83	5,030,841	1
<i>P. fontis</i> M022	NCBI: ASM80321v1	88.50	4,151,564	105

^a Average nucleotide identity (percent ANI) between the *P. carotovorum* WPP14 strain sequenced in this study compared with other type strains of *Pectobacterium* spp.

P. carotovorum WPP14 was cultured in Luria-Bertani medium at 28°C (10 g of tryptone, 5 g of yeast extract, and 10 g NaCl per 1 liter of medium) (Bertani 1951). High molecular weight DNA was extracted from overnight bacterial cultures using a previously described method (Miller et al. 1988). DNA quantity was assessed using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, U.S.A.) and a Qubit 3.0 Fluorometer (Thermo Fisher Scientific). DNA integrity was assessed using the Agilent 2100 BioAnalyzer system (Agilent, U.S.A.).

A complete scaffold of the genome was obtained using long reads from Oxford Nanopore Technologies (U.K.). Illumina (U.S.A.) short reads were used to polish the assembled genome. All software systems were run with their default settings unless otherwise noted. For long-read sequencing, the DNA library was prepared using the Nanopore Rapid Sequencing Kit SQK-RAD004 on a R9.4.1 SpotON flow cell with a MinION Mk1B device. This generated 242,453 reads with a total length of 1,987,696,323 bp and a genome coverage of 394x. The reads were base called using Guppy v3.5.1 and assembled using Flye v2.5 (Kolmogorov et al. 2019). For short-read sequencing, the DNA library was prepared using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina with the NEBNext Multiplex Oligos (New England Biolabs, U.S.A.). AMPure XP beads (Beckman Coulter, U.S.A.) were used for DNA size selection and purification. The DNA library was sequenced on a MiSeq instrument (Illumina, U.S.A.) with the 2 × 250-bp mode, yielding 2,812,428 paired-end reads. Raw reads were processed to remove the adapter and low-quality sequences using Trimmomatic v0.39 (Bolger et al. 2014) (quality value ≤ 20), ILLUMINACLIP:NEBNext.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:20 MINLEN:50). Duplicate read pairs were removed using a customized Perl script (https://github.com/Sunhh/NGS_data_processing/blob/master/drop_dup_both_end.pl) (Sun et al. 2017). BWA v0.7.17 (Li and Durbin 2009) was used to align the resulting 2,535,566 high-quality cleaned Illumina read pairs to the draft genome, with a genome coverage of 164x. Pilon v1.22 (Walker et al. 2014) was used for four rounds to correct the draft genome and yield the final polished genome. Circlator v1.5.5 was used with the “fix start” argument to set the start of the genome at the *dnaA* gene (Hunt et al. 2015).

The final polished *P. carotovorum* WPP14 genome is 4,892,225 bp long with 52.07% GC content. It was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP v4.11) (Tatusova et al. 2016). The resulting annotation contains 4,380 genes in total, with 4,201 protein-coding genes; 72 pseudogenes; 8, 7, and 7 ribosomal RNA (rRNA)-coding sequences (5S, 16S, and 23S rRNAs), 76 tRNAs, and 9 noncoding RNAs; and 5 clustered regularly interspaced short palindromic repeat (CRISPR) arrays. Whole-genome comparison of *P. carotovorum* WPP14 and other *Pectobacterium* type strains based on average nucleotide identity (ANI) can be found in Table 1, including their genome sizes and number of contigs. Genome sequences were retrieved from publicly available sources (Glasner et al.

2008; NCBI Research Coordinators 2016). ANI was calculated using FastANI (Jain et al. 2018). Based on ANI, *P. carotovorum* WPP14 showed the closest similarity to the *P. carotovorum* type strain ICMP 5702.

Data availability. The *P. carotovorum* WPP14 genome sequence has been deposited at NCBI GenBank under accession number CP051652. The Nanopore and Illumina raw reads have been deposited in the Sequence Read Archive under accession numbers SRR11435198 and SRR11435197, respectively.

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