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Complete Genome Sequence of Phytopathogenic Pectobacterium carotovorum subsp. carotovorum Bacteriophage PP1

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GENOME ANNOUNCEMENT

Complete Genome Sequence of Phytopathogenic *Pectobacterium carotovorum* subsp. *carotovorum* Bacteriophage PP1

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***Pectobacterium carotovorum* subsp. *carotovorum* is a phytopathogen causing soft rot disease on diverse plant species. To control this plant pathogen, *P. carotovorum* subsp. *carotovorum*-targeting bacteriophage PP1 was isolated and its genome was completely sequenced to develop a novel biocontrol agent. Interestingly, the 44,400-bp genome sequence does not encode any gene involved in the formation of lysogen, suggesting that this phage may be very useful as a biocontrol agent because it does not make lysogen after host infection. This is the first report on the complete genome sequence of the *P. carotovorum* subsp. *carotovorum*-targeting bacteriophage, and it will enhance our understanding of the interaction between phytopathogens and their targeting bacteriophages.**

Pectobacterium carotovorum subsp. *carotovorum* (formerly *Erwinia carotovora* subsp. *carotovora*) is a phytopathogenic Gram-negative bacterium causing soft rot on various plant species (5, 7, 10). This pathogen causes serious loss of produce quality during its growth, transit, and even storage. Recently, a novel bacteriocin, carocin D, was isolated from *P. carotovorum* subsp. *carotovorum* strain Pcc21 to develop a biocontrol agent to control this phytopathogen (9). Furthermore, to increase this antibacterial activity, an additional biocontrol agent such as bacteriophage treatment is needed. This approach could be one of the best biocontrol agents to maximize the growth inhibition of this pathogen in plants. Here, we isolated *P. carotovorum* subsp. *carotovorum*-targeting bacteriophage PP1 and fully sequenced its genome to understand its inhibition mechanism against this pathogen.

The genome of phage PP1 was isolated using the alkaline lysis and phenol extraction method (11). Physically shared DNA was cloned to T vector, and libraries were completely sequenced using ABI3730 (GGBio, South Korea). The open reading frames (ORFs) were bioinformatically predicted using Glimmer3 (4), GeneMarkS (2), and FgenesB (Softberry, Inc., Mount Kisco, NY) and confirmed by RBS finder (J. Craig Venter Institute, Rockville, MD). The functions of ORFs were predicted by BLASTP (1) and InterProScan with protein motif databases (12). The complete genome sequence and annotation information were edited and handled using Artemis14 (3).

The genome of *P. carotovorum* subsp. *carotovorum*-targeting phage PP1 consisted of a length of 44,400 bp with a GC content of 49.74%, encoding 48 ORFs with no tRNA. Although six phage genomes are available in the GenBank database infecting *Erwinia amylovora*, *E. tasmaniensis*, and *E. pyrifoliae* to date (6, 8), there is no report on the complete genome sequence of the *P. carotovorum* subsp. *carotovorum*-infecting phage. Therefore, more than 50% of the annotated ORFs (26 of 48) encode hypothetical proteins due to insufficient genome annotation information on this phage. The genes encoded in this genome are categorized into five groups: DNA replication/manipulation (DNA-directed RNA polymerase, DNA primase, DNA polymerase, HNH endonuclease, 5′-3′ exonuclease, and DNA ligase), phage structure (head-tail connector, scaffolding protein, major capsid protein, tail tubular proteins, and internal virion proteins), phage packaging (large and small subunits of terminase), host lysis (lysozyme domain protein and holin), and host specificity (tail fiber protein). Interestingly, this genome has only one gene encoding tail fiber protein for host specificity, suggesting that this gene may be important to recognize and to infect the host. After infection of this host, the genes encoding lysozyme and holin may play important roles in the host lysis. However, this genome does not encode proteins involved in lysogen formation, suggesting that this phage probably does not make lysogen after phage infection. The absence of genes involved in lysogen formation may be very useful to develop an effective biocontrol agent with this phage. This is the first report on the complete genome sequence of the *P. carotovorum*-targeting bacteriophage, and it should enhance our understanding of bacterial pathogenesis to plants and its control between *P. carotovorum* subsp. *carotovorum* and its infecting phages.

Nucleotide sequence accession number. The complete genome sequence of *P. carotovorum* subsp. *carotovorum*-targeting bacteriophage PP1 is available under GenBank accession number JQ837901.

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REFERENCES

1. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** 1990. Basic local alignment search tool. J. Mol. Biol. **215**:403–410.
2. **Besemer J, Lomsadze A, Borodovsky M.** 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res. **29**:2607–2618.
3. **Carver T, et al.** 2008. Artemis and ACT: viewing, annotating and comparing sequences stored in a relational database. Bioinformatics **24**:2672–2676.

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4. **Delcher AL, Bratke KA, Powers EC, Salzberg SL.** 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics **23**:673–679.
5. **Gardan L, Gouy C, Christen R, Samson R.** 2003. Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavasculorum* sp. nov. and *Pectobacterium wasabiae* sp. nov. Int. J. Syst. Evol. Microbiol. **53**:381–391.
6. **Lehman SM, Kropinski AM, Castle AJ, Svircev AM.** 2009. Complete genome of the broad-host-range *Erwinia amylovora* phage ΦEa21-4 and its relationship to *Salmonella* phage Felix O1. Appl. Environ. Microbiol. **75**:2139–2147.
7. **Marquez-Villavicencio MDP, Weber B, Witherell RA, Willis DK, Charkowski AO.** 2011. The 3-hydroxy-2-butanone pathway is required for *Pectobacterium carotovorum* pathogenesis. PLoS One **6**:e22974. doi: 10.1371/journal.pone.0022974.
8. **Müller I, Kube M, Reinhardt R, Jelkmann W, Geider K.** 2011. Complete genome sequences of three *Erwinia amylovora* phages isolated in North America and a bacteriophage induced from an *Erwinia tasmaniensis* strain. J. Bacteriol. **193**:795–796.
9. **Roh E, et al.** 2010. Characterization of a new bacteriocin, Carocin D, from *Pectobacterium carotovorum* subsp. *carotovorum Pcc21*. Appl. Environ. Microbiol. **76**:7541–7549.
10. **Toth IK, Bell KS, Holeva MC, Birch PRJ.** 2003. Soft rot erwiniae: from genes to genomes. Mol. Plant Pathol. **4**:17–30.
11. **Wilcox SA, Toder R, Foster JW.** 1996. Rapid isolation of recombinant lambda phage DNA for use in fluorescence in situ hybridization. Chromosome Res. **4**:397–398.
12. **Zdobnov EM, Apweiler R.** 2001. InterProScan—an integration platform for the signature-recognition methods in InterPro. Bioinformatics **17**: 847–848.