JVI
Journals.ASM.org

Complete Genome Sequence of *Pectobacterium carotovorum* subsp. *carotovorum* Bacteriophage My1

Dong Hwan Lee,[a] Ju-Hoon Lee,[b] Hakdong Shin,[c] Samnyu Ji,[a] Eunjung Roh,[a] Kyusuk Jung,[a] Sangryeol Ryu,[c] Jaehyuk Choi,[a] and Sunggi Heu[a]

Division of Microbial Safety, National Academy of Agricultural Science, Rural Development Administration, Suwon, Republic of Korea[a]; Department of Food Science and Biotechnology, Kyung Hee University, Yongin, Republic of Korea[b]; and Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Research Institute for Agriculture and Life Sciences, and Center for Agricultural Biomaterials, Seoul National University, Seoul, Republic of Korea[c]

***Pectobacterium carotovorum* subsp. *carotovorum*, a member of the *Enterobacteriaceae* family, is an important plant-pathogenic bacterium causing significant economic losses worldwide. *P. carotovorum* subsp. *carotovorum* bacteriophage My1 was isolated from a soil sample. Its genome was completely sequenced and analyzed for the development of an effective biological control agent. Sequence and morphological analyses revealed that phage My1 is a T5-like bacteriophage and belongs to the family *Siphoviridae*. To date, there is no report of a *Pectobacterium*-targeting siphovirus genome sequence. Here, we announce the complete genome sequence of phage My1 and report the results of our analysis.**

The plant-pathogenic bacterium *Pectobacterium carotovorum* subsp. *carotovorum* (formerly known as *Erwinia carotovora* subsp. *carotovora*) produces numerous plant cell wall-degrading enzymes to cause soft rot diseases in diverse plants (5, 6, 13, 19) and, consequently, causes severe economic losses. Bacteriophages have been considered novel natural biocontrol agents for bacterial plant diseases (8, 14). Several phages infecting *Erwinia carotovora* subsp. *carotovora* (14, 18) or other *Erwinia* spp. have been isolated (4, 11, 12), and a few of these species have been sequenced. Although we have announced the complete sequence of *P. carotovorum* subsp. *carotovorum Podoviridae* phage PP1 (10), a genome sequence of a *Pectobacterium*-targeting phage belonging to the *Siphoviridae* family has not been reported to date. In this study, we isolated the *P. carotovorum* subsp. *carotovorum*-targeting *Siphoviridae* named My1 and completely sequenced its genome.

Genomic DNA extraction of bacteriophage My1 was carried out using SDS-proteinase K and phenol-chloroform extraction methods (15, 21). Purified DNA was sequenced using a Genome Sequencer FLX (GS-FLX) Titanium by Macrogen Inc. (Seoul, Republic of Korea), with 52-fold coverage. Quality filtered reads were assembled using a 454 Newbler 2.3 assembler. The open reading frames (ORFs) were predicted using the Glimmer v3.02 (3), GeneMarkS (2), and FgenesB (Softberry, Inc., Mount Kisco, NY) and then confirmed by the RBSfinder (17). Transfer RNAs were predicted using the tRNAscan-SE program (16). Functional analysis of ORFs was conducted using the BLASTP (1) and InterProScan (22) programs.

In silico complete genome sequence analysis of bacteriophage My1 revealed that it consists of 122,024 bp in length, with an average GC content of 40.61%, 149 ORFs, and 20 tRNA genes. The predicted ORFs were highly homologous to those of T5 (20) or T5-like phages (7, 9) in the *Siphoviridae* family. Interestingly, this genome contains redundant 12,854-bp terminal repeats at both ends.

Of the 149 predicted ORFs, 51 (34.23%) have been annotated and categorized into five functional groups as follows: phage structure and packaging (portal protein, prohead protease, tail protein, terminase large subunit), DNA replication/modification (DNA helicase, DNA primase, DNA polymerase, endonuclease, NAD-dependent DNA ligase, RNase H), signal transduction and regulatory functions (PhoH-like protein, serine/threonine protein phosphatase, single-stranded DNA [ssDNA]-binding transcriptional regulator), nucleotide metabolism (thymidylate synthase, dihydrofolate reductase, thioredoxin), and host lysis (endolysin, holin, cell wall hydrolase). However, lysogeny-related genes were not detected. Two lysis proteins and no detection of lysogeny-related genes suggest that this phage could be a useful biocontrol agent. YadA domain-containing protein showed high similarity to the L-shaped tail fiber protein among T5 phage genes but low coverage, suggesting that it probably contributes to host-phage-specific interaction. The others were annotated as hypothetical proteins, probably due to insufficient information about *Pectobacterium* phage genes.

This first report of the complete genome sequence of *Pectobacterium carotovorum* subsp. *carotovorum* bacteriophage My1 will extend our knowledge about the interaction between the *Pectobacterium* host and its phages and provide a novel potential as a biocontrol agent to prevent plant diseases caused by *Pectobacterium*.

Nucleotide sequence accession number. The complete genome sequence of *P. carotovorum* subsp. *carotovorum* bacteriophage My1 has been deposited in GenBank under the accession number JX195166.

ACKNOWLEDGMENT

This work was supported by the Rural Development Administration (RDA) fund (grant PJ008615).

REFERENCES

1. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** 1990. Basic local alignment search tool. J. Mol. Biol. **215**:403–410.

Received 31 July 2012 Accepted 31 July 2012
Address correspondence to Sunggi Heu, sunggiheu@korea.kr.
D.H.L. and J.-H.L. contributed equally to this article.

doi:10.1128/JVI.01987-12

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. **Delcher AL, Bratke KA, Powers EC, Salzberg SL.** 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics **23**:673–679.
4. **Gill JJ, Svircev AM, Smith R, Castle AJ.** 2003. Bacteriophages of *Erwinia amylovora*. Appl. Environ. Microbiol. **69**:2133–2138.
5. **Gnanamanickam SS.** 2006. Plant-associated bacteria. Springer, Dordrecht, The Netherlands.
6. **Hauben L, et al.** 1998. Phylogenetic position of phytopathogens within the *Enterobacteriaceae*. Syst. Appl. Microbiol. **21**:384–397.
7. **Hong J, et al.** 2008. Identification of host receptor and receptor-binding module of a newly sequenced T5-like phage EPS7. FEMS Microbiol. Lett. **289**:202–209.
8. **Jones JB, et al.** 2007. Bacteriophages for plant disease control. Annu. Rev. Phytopathol. **45**:245–262.
9. **Kim M, Ryu S.** 2011. Characterization of a T5-like coliphage, SPC35, and differential development of resistance to SPC35 in *Salmonella enterica* serovar Typhimurium and *Escherichia coli*. Appl. Environ. Microbiol. **77**: 2042–2050.
10. **Lee JH, et al.** 2012. Complete genome sequence of phytopathogenic *Pectobacterium carotovorum* subsp. *carotovorum* bacteriophage PP1. J. Virol. **86**:8899–8900.
11. **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.
12. **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.
13. **Pérombelon MCM.** 2002. Potato diseases caused by soft rot erwinias: an overview of pathogenesis. Plant Pathol. **51**:1–12.
14. **Ravensdale M, Blom T, Gracia-Garza J, Svircev A, Smith R.** 2007. Bacteriophages and the control of *Erwinia carotovora* subsp. *carotovora*. Can. J. Plant Pathol. **29**:121–130.
15. **Sambrook J, Russell DW.** 2001. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
16. **Schattner P, Brooks AN, Lowe TM.** 2005. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. Nucleic Acids Res. **33**:W686–W689.
17. **Suzek BE, Ermolaeva MD, Schreiber M, Salzberg SL.** 2001. A probabilistic method for identifying start codons in bacterial genomes. Bioinformatics **17**:1123–1130.
18. **Toth I, Perombelon M, Salmond G.** 1993. Bacteriophage ΦKP mediated generalized transduction in *Erwinia carotovora* subspecies *carotovora*. J. Gen. Microbiol. **139**:2705–2709.
19. **Toth IK, Bell KS, Holeva MC, Birch PR.** 2003. Soft rot erwiniae: from genes to genomes. Mol. Plant Pathol. **4**:17–30.
20. **Wang J, et al.** 2005. Complete genome sequence of bacteriophage T5. Virology **332**:45–65.
21. **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.
22. **Zdobnov EM, Apweiler R.** 2001. InterProScan—an integration platform for the signature-recognition methods in InterPro. Bioinformatics **17**: 847–848.