Arch Virol (2015) 160:865–867
DOI 10.1007/s00705-014-2316-8

ANNOTATED SEQUENCE RECORD

Complete genome sequence of a filamentous bacteriophage, RS611, that infects the phytopathogen *Ralstonia solanacearum*

Truong Thi Bich Van · Shohei Yoshida · Kaito Miki · Akihiro Kondo · Kaeko Kamei

Received: 16 July 2014 / Accepted: 15 December 2014 / Published online: 7 January 2015

Abstract Filamentous bacteriophage RS611 (ϕRS611), which infects the phytopathogen *Ralstonia solanacearum*, had a circular single-stranded DNA genome that was characterized as an Ff-type phage belonging to the family *Inoviridae*. The ϕRS611 genome was composed of 6386 bases with a G + C content of 62.1 % and contained 11 putative open reading frames. The ϕRS611 genome showed high similarity to those of *Ralstonia* phages RSS0 and RSS1. However, approximately 900-nucleotide deletions were found in the region corresponding to open reading frames 10 and 11 of ϕRSS0 and ϕRSS1.

Ralstonia solanacearum is a soil-borne gram-negative bacterium that causes bacterial wilt disease in many important farm products such as tomato, potato, and eggplant. Bacterial wilt disease has resulted in severe economical damage to the agricultural industry. To replace chemical control methods, the use of virulent bacteriophages may have great potential for protection of plants from these infectious diseases. Phages may also be useful for specific and effective identification of *R. solanacearum* strains based on their high host specificities [1]. Several articles describing Ff-type *R. solanacearum* phages have been published. These include ϕRSM1 and ϕRSS1, with genomic single-stranded DNA (ssDNA) of 9.0 and 6.6 kb, respectively [2, 3]. The genomic ssDNA of another Ff-type phage, ϕPE226, is composed of 5475 nucleotides [4].

In this study, *R. solanacearum* phage ϕRS611 was isolated in 2010 from the soil of a tomato field (Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Center, Japan, 35.01655 north latitude, 135.563849 east longitude) that had been contaminated with bacterial wilt disease. A mixture of three *R. solanacearum* strains, MAFF 730138, 211556 and 106611, obtained from the National Institute of Agrobiological Sciences Genebank (Japan), was added to a soil suspension from a tomato field and then cultured overnight to propagate phages. The phage was purified from a crude phage solution by a conventional double agar overlay plate method using a single bacterial strain, *R. solanacearum* MAFF 106611, as a host. After repeats of plaque purification, phage ϕRS611 was isolated.

The phage was observed in the transmission mode of scanning transmission electron microscopy (HD-2700, Hitachi High Technologies Corp, Japan). It showed a flexible filamentous shape suggesting that it belongs to the family *Inoviridae* (Supplementary Fig. 1). The average size of the phage was ∼1120 nm in length and ∼8 nm in width. The morphology of ϕRS611 was similar to that of other *R. solanacearum* phages such as ϕRSS1 (1100 ± 100 nm in length and 10 ± 0.5 nm in width) [5] and ϕPE226 (1050 nm in length and 6-9 nm in width) [4].

The genomic DNA sequence of ϕRS611 was analyzed by the Biotechnology Center of Akita Prefectural University using a BigDye Terminator Version 3.1 Cycle

The nucleotide sequence accession number: The complete genome sequence of bacteriophage ϕRS611 is available in the DNA Data Bank of Japan (DDBJ) (http://www.ddbj.nig.ac.jp/) database under accession number AB931172.

Electronic supplementary material The online version of this article (doi:10.1007/s00705-014-2316-8) contains supplementary material, which is available to authorized users.

T. T. B. Van · S. Yoshida · K. Miki · K. Kamei (✉)
Department of Biomolecular Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan
e-mail: kame@kit.ac.jp

A. Kondo
Hiyoshi Corporation, 908 Kitanosho, Omihachiman, Shiga 523-8555, Japan

Sequencing Kit (Applied Biosystems). The first primer for DNA sequencing was designed from the genome sequence of *Ralstonia* phage RSS0 (accession number JQ408219, nt 7199-7220), and a full-length genomic DNA sequence of ϕRS611 was obtained via the primer-walking method using phage genome DNA as a template. The ϕRS611 genome was a circular ssDNA of 6386 nucleotides (nt) with a G + C content of 62.1 %. As shown in Fig. 1, the genome size of ϕRS611 was smaller than those of other filamentous *Ralstonia* phages such as ϕRSS0 and ϕRSS1 [3, 6], whose genomic DNA sequences were highly homologous to that of ϕRS611. The DNA sequence of nt 1-5251 of the ϕRS611 genome was 97 % identical to those of nt 1-5253 of ϕRSS0 (accession number JQ408219) and ϕRSS1 (accession number AB259124). However, ϕRS611 differed distinctly from these two phages, because the ϕRS611 genome had a deletion of about 900 nucleotides corresponding to nt 5254-6153 of ϕRSS0 and ϕRSS1.

Eleven putative open reading frames (ORFs) were found in the ϕRS611 genome by searching with GeneMarkS (http://exon.gatech.edu/GeneMark/genemarks.cgi) and ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), and by comparison of genome sequences of ϕRS611, ϕRSS0 and ϕRSS1. The ORFs were organized in a manner similar to those of ϕRSS0 and ϕRSS1 (Fig. 1, Supplementary Table 1). The deduced ORFs, their putative functions, and identities with those of ϕRSS0 and ϕRSS1 are summarized in Supplementary Table 1. The amino acid sequences of ORFs 1, 3, 5, 6 and 8, were identical to ORFs 1, 3, 5, 6 and 8 of ϕRSS0 and ϕRSS1, respectively, and ORFs 2, 4, 7 and 9 were also highly homologous to ORFs 2, 4, 7 and 9 of ϕRSS0 and ϕRSS1 (identity, >98 %). However, ϕRS611 ORF 2 (343 aa) lacked 30 and 2 amino acids in the N-terminal and C-terminal regions, respectively, in comparison to ORF 2 of ϕRSS0 and ϕRSS1. ORF 4 (66 aa) of ϕRS611 lacked 23 amino acids in the N-terminal region compared with ORF 2 (89 aa) of ϕRSS0 and ϕRSS1. The common modules found in the genomic DNA of filamentous phages—the replication module, the structural module, and the assembly and secretion module—were encoded by ORF 1-ORF 3, ORF 4-ORF 8, and ORF 9, respectively, in ϕRS611, and the region of nt 5398-5779 was deduced to be an intergenic region. The ϕRS611 genome also possessed an *att*P sequence corresponding to the *dif* sequence of *R. solanacearum* GMI1000 [8].

The ϕRS611 genome had a deletion of approximately 900 nucleotides from inside the ORF 10 coding region, which corresponded to nt 5253-6154 of ϕRSS0 and ϕRSS1. The N-terminal region (aa 1-66), encoded by nt 5053-5251 of ϕRS611 ORF 10, was highly homologous to the corresponding regions of ϕRSS0 and ϕRSS1. In contrast, the C-terminal region (aa 67-108) of ϕRS611 ORF 10 had no homology to them. Furthermore, ϕRS611 lacked the ORF corresponding to ORF 11 of ϕRSS0 and ϕRSS1. The effects of these differences in the genome sequences should be studied further in order to determine the function of these ORFs.

The host specificity of ϕRS611 was examined using *R. solanacearum* strains obtained from the National Institute of Agrobiological Sciences Genebank (Japan). All examined strains of race 1, biovar 4 (MAFF 730139, 211556, 106611) were sensitive to ϕRS611. In addition, ϕRS611 infected *R. solanacearum* MAFF 211272 (race 4, biovar 4). Ff-like filamentous phages are known to recognize their host cells through a minor coat protein (pIII) located on one edge of the filament, which specifically interacts with pilus

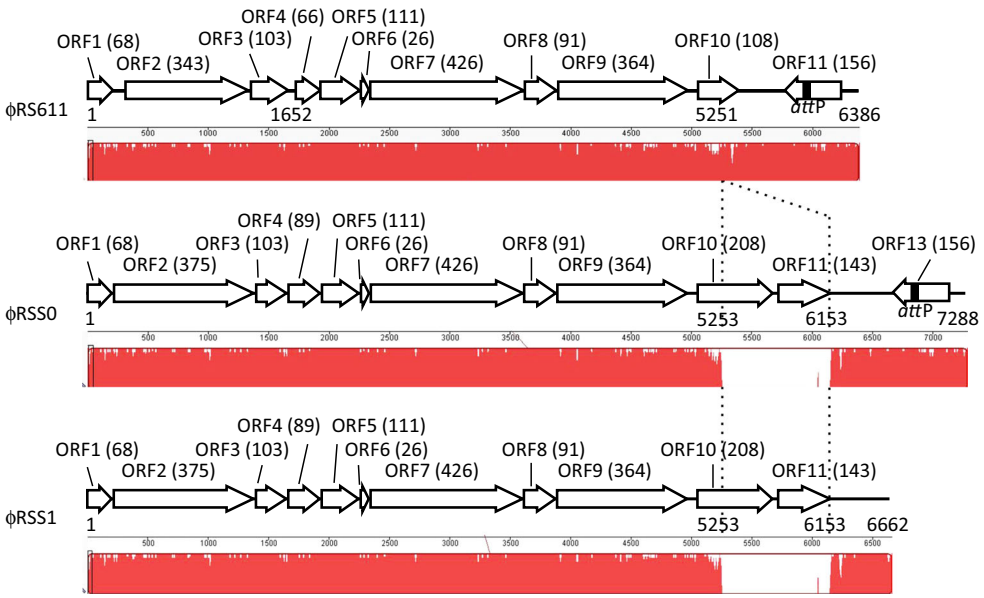

Fig. 1 Genomic organization and alignment of ϕRS611, ϕRSS0, and ϕRSS1. Linear ORF maps of ϕRSS0 and ϕRSS1 are from articles by T. Yamada and coworkers [3, 8], and that of ϕRSS1 was constructed with ORF Finder software. The numbers in parentheses indicate the length (aa) of the ORF. The arrows represent the direction of the transcription of ORFs or genes. The region corresponding to nt 5254-6153 of ϕRSS0 and ϕRSS1 was deleted in the ϕRS611 genome. The linear genome alignment results were generated using the MAUVE program with default settings [9]. The color indicates identity

structures on bacterial cells. Therefore, the host range of a bacteriophage is determined by the pilus structure, and according to Askora *et al.* [2], ϕRSS1 also recognizes a specific pilus structure on *R. solanacearum* cells [2, 3]. The genomic data shown in Supplementary Table 1 indicated that the amino acid sequences of pIII (ORF7) are exactly identical in ϕRS611 and ϕRSS1, suggesting that the host range of the two phages should be the same. However, ϕRS611 showed different host specificity from that of ϕRSS0 and ϕRSS1, which has been reported by Yamada *et al.* [7]. For instance, MAFF106603 was sensitive to ϕRSS0 and ϕRSS1, but not to ϕRS611. By contrast, MAFF211272 was sensitive to ϕRS611, but not to ϕRSS0 or ϕRSS1. The reason for this discrepancy in these two cases should be clarified.

Acknowledgments We thank the Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Center, for providing the soil from a tomato field.

Conflict of interest The authors declare that none of the authors has any conflict of interest associated with this study.

References

1. Jones JB, Jackson LE, Balogh B, Obradovic A, Iriarte FB, Momol MT (2007) Bacteriophages for plant disease control. Annu Rev Phytopathol 45:245–262
2. Askora A, Kawasaki T, Usami S, Fujie M, Yamada T (2009) Host recognition and integration of filamentous phage phiRSM in the phytopathogen, *Ralstonia solanacearum*. Virology 384:69–76
3. Kawasaki T, Nagata S, Fujiwara A, Satsuma H, Fujie M, Usami S, Yamada T (2007) Genomic characterization of the filamentous integrative bacteriophages ϕRSS1 and ϕRSM1, which infect *Ralstonia solanacearum*. J Bacteriol 189:5792–5802
4. Murugaiyan S, Bae JY, Wu J, Lee SD, Um HY, Choi HK, Chung E, Lee JH, Lee SW (2011) Characterization of filamentous bacteriophage PE226 infecting *Ralstonia solanacearum* strains. J Appl Microbiol 110:296–303
5. Yamada T, Kawasaki T, Nagata S, Fujiwara A, Usami S, Fujie M (2007) New bacteriophages that infect the phytopathogen *Ralstonia solanacearum*. Microbiology 153:2630–2639
6. Addy HS, Askora A, Kawasaki T, Fujie M, Yamada T (2012) The filamentous phage varphiRSS1 enhances virulence of phytopathogenic *Ralstonia solanacearum* on tomato. Phytopathology 102:244–251
7. Yamada T (2012) Bacteriophages of *Ralstonia solanacearum*: their diversity and utilization as biocontrol agents in agriculture. In: Kurtböke I (ed) Bacteriophages, 1st edn. Croatia, InTech, pp 113–138
8. Yamada T (2013) Filamentous phages of *Ralstonia solanacearum*: double-edged swords for pathogenic bacteria. Front Microbiol 4:325. doi:10.3389/fmicb.2013.00325
9. Darling AC, Mau B, Blattner FR, Perna NT (2004) Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 14:1394–1403. doi:10.1101/gr.2289704