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VIRUSES

Complete Genome Sequence of *Rathayibacter toxicus* Phage NCPPB3778

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ABSTRACT The phage NCPPB3778 was isolated from *Rathayibacter toxicus* strain CS14, and the genomic DNA was sequenced. The genome is similar to siphoviruses, consisting of 44,520 bases including 77 predicted open reading frames. Portions of the genome are annotated as typical phage proteins, but much of the genome sequence is unique from other bacteriophages.

Received 25 May 2017 **Accepted** 19 September 2017 **Published** 19 October 2017

Citation Schneider WL, Sechler AJ, Rogers EE. 2017. Complete genome sequence of *Rathayibacter toxicus* phage NCPPB3778. Genome Announc 5:e00671-17. https://doi.org/10.1128/genomeA.00671-17.

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Rathayibacter toxicus is a phytobacterium of forage grasses and related monocots (1). The bacterium has only been found in Australia, with minor exceptions when infected hay was inadvertently shipped out of the country (2–4). *R. toxicus* is the causal agent for annual ryegrass toxicity (ARGT), which results from bacterial production of a toxin (tunicamycin) in infested seeds. ARGT is a significant threat to livestock production, with losses in the millions of dollars per year (5). The production of tunicamycin by *R. toxicus* is most frequently associated with infection by an *R. toxicus* specific bacteriophage, NCPPB3778 (6), although toxin production without the presence of phage has been reported (7). A complete genome sequence for NCPPB3778 would be useful for determining the role of bacteriophage in *R. toxicus* biology and ARGT.

Particles of bacteriophage NCPPB3778 were isolated from a phage-lyzed infected culture of *R. toxicus* as in Riley and Gooden (8). DNA was isolated from purified phage particles using a modified Marmur method (9). Restriction analysis indicated the genome was linear. A shotgun library was made for Roche 454 Jr. according to manufacturer specifications (Roche, Indianapolis, IN). The reads (111,500 reads, 49,079,740 bp, average length 440 bp, median length 487 bp, >1000× coverage) were assembled using SeqMan NGen 12 (DNAStar, Madison, WI) into a single contig. Open reading frames (ORFs) were predicted and annotated using RAST (10). Additional manual annotation was done using BLASTp against the nonredundant protein sequence database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST_SPEC=BioassayBlast&PAGE_TYPE=BlastSearch&PROG_DEFAULTS=on).

In total, the complete NCPPB3778 linear genome was 44,520 nucleotides in length, with a GC content of 52.9%. A total of 77 predicted ORFs were identified. Twenty-three (~30%) of the ORFs were similar to previously described phage genes (21 with putative functions), including replication proteins, structural virion proteins, and exonucleases. The majority of these genes were grouped together near the 3′ end of the genome. Approximately half of the ORFs (~51%) were unique to the NCPPB3778 genome, with no significant matches among functionally annotated genes. Another 19% of the ORFs were predicted to encode hypothetical proteins, but with no similarity ascribed to bacteriophage ORFs. A number of other predicted ORFs had some degree of similarity to bacterial transcription factors. Only a single ORF from the phage had high similarity to an ORF from *R. toxicus*, a DNA polymerase III subunit epsilon. Two ORFs were found with similarity to *R. tritici*, a muramidase and a hypothetical protein. No other similarities to ORFs in *Rathayibacter* sp. were found. There were no obvious genes associated with tunicamycin production found in the NCPPB3778 genome.

Accession number(s). The NCPPB3778 genome is available from GenBank under the accession number KX911187.

ACKNOWLEDGMENTS

We thank Ian Riley, University of Adelaide, for assistance in obtaining purified NCPPB3778.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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