

A Narnavirus in the Trypanosomatid Protist Plant Pathogen *Phytomonas serpens*

Natalia S. Akopyants,^a Lon-Fye Lye,^a Deborah E. Dobson,^a Julius Lukeš,^{b,c}  Stephen M. Beverley^a

Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri, USA^a; Biology Centre and Faculty of Sciences, Institute of Parasitology, University of South Bohemia, České Budějovice, Czech Republic^b; Canadian Institute for Advanced Research, Toronto, Ontario, Canada^c

We describe here a new RNA virus (PserNV1) from the plant protist parasite *Phytomonas serpens* (family Trypanosomatidae, Kinetoplastida, supergroup Excavata). The properties of PserNV1 permit assignment to the genus *Narnavirus* (*Narnaviridae*), the first reported from a host other than fungi or oomycetes.

Received 30 May 2016 Accepted 10 June 2016 Published 28 July 2016

Citation Akopyants NS, Lye L-F, Dobson DE, Lukeš J, Beverley SM. 2016. A *Narnavirus* in the trypanosomatid protist plant pathogen *Phytomonas serpens*. *Genome Announc* 4(4):e00711-16. doi:10.1128/genomeA.00711-16.

Copyright © 2016 Akopyants et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Stephen M. Beverley, beverley@wusm.wustl.edu.

Viruses within the family *Narnaviridae* are composed of a positive single-stranded RNA segment (2.3 to 3.6 kb) encoding a single protein, the RNA-dependent RNA polymerase (RDRP) (1–3). *Narnavirus* derives from “naked RNA,” reflecting the absence of a capsid or envelope, and narnaviruses do not form infectious viral particles. Two genera are recognized: *Mitovirus*, found in the mitochondrion of fungi and translated using the mitochondrial genetic code, and cytoplasmic *Narnavirus*, comprising the 20S/23S elements of *Saccharomyces cerevisiae* and one from the oomycete *Phytophthora infestans* (3–5). Here, we report the discovery and complete genome sequence (including termini) of *Phytomonas serpens* narnavirus 1 (PserNV1).

Total cellular RNA from *P. serpens* isolate 9T (6) was extracted using TRIzol reagent (Thermo Fisher), treated with DNase I (Thermo Fisher) at 37°C for 45 min, and purified with RNA Clean & Concentrator-25 (Zymo Research). Replicative viral double-stranded RNAs (dsRNAs) were visualized following treatment with S1 nuclease (Thermo Fisher), separation by agarose gel electrophoresis, and visualization by ethidium bromide staining (7). A prominent dsRNA band of about 4 kb was observed, eluted, and used as a template to generate a cDNA library, with fragment sizes ranging from 200 to 600 nucleotides (nt). A multiplexed TruSeq RNA library was sequenced (2 × 101 cycles, paired-end reads) on the HiSeq 2500 (Illumina). A total of 64,373 reads were obtained, of which 47,690 assembled into a single contig, which was confirmed by reverse transcriptase PCR and sequencing. The adaptor (5′-PO₄-CCCCTCGTTTGCTGGCTCTTT-NH₂) was added to the eluted RNA band with T4 RNA ligase (NEB), amplified using primers complementary to the adapter and virus, and sequenced to determine the termini.

The PserNV1 RNA genome is 3,782 nt in length, with a G+C composition comparable to that of the *P. serpens* nuclear genome (48.2 versus 46.6%, respectively) (6). As in other narnaviruses, short hairpins are evident at both the 5′ and 3′ termini. From the first AUG (nt 83), translation with the nuclear genetic code revealed a single open reading frame (ORF) encoding a 1,208-amino-acid protein exhibiting motifs seen in narnaviral RNA-dependent RNA polymerases (1). Phylogenetic comparisons with

accepted members of the *Narnaviridae* placed it firmly within the monophyletic genus *Narnavirus*. As the overall RDRP amino acid sequence difference was >80%, this element warrants status as a new *Narnavirus* species.

Members of the genus *Phytomonas* affect >100 plant species, including economically important plants, such as coffee, coconut, cassava, and oil palms (8), which may bear relatives of PserNV1. Classification as a narnavirus suggests that PserNV1 differs from the virus-like particles described previously in other *Phytomonas* species. (9). Some data suggest that PserNV1 may be unstable during culture, as reverse transcriptase PCR tests of this strain acquired independently from another source did not reveal PserNV1 (the authenticity of this strain was confirmed by sequence of two nuclear genes, *GAPDH* and *PTR1*). In the future, studies of NV1-positive and -negative lines of *Phytomonas* may establish potential roles of this viral element in plant pathogenicity (1).

Nucleotide sequence accession number. The full-length viral genomic sequence of PserNV1 from *Phytomonas serpens* 9T strain was deposited in GenBank under the accession no. [KU882057](https://www.ncbi.nlm.nih.gov/nuclot/KU882057).

ACKNOWLEDGMENTS

We thank members of our laboratory, Nicolas Fasel and Vyacheslav Yurchenko, for discussions, and colleagues who provided *Phytomonas* strains that had been cultured independently.

This work was supported by NIH AID R56 AI099364 and AI29646 to S.M.B. Next-generation sequencing was performed at the Washington University School of Medicine, Department of Genetics Genome Technology Access Center (partially supported by grants NCI Cancer Center Support P30 CA91842 and NCCR ICTS/CTSA UL1 TR000448).

FUNDING INFORMATION

This work, including the efforts of Natalia S. Akopyants, Lon-Fye Lye, Deborah E. Dobson, and Stephen M. Beverley, was funded by HHS | NIH | National Cancer Institute (NCI) (P30-CA91842). This work, including the efforts of Natalia S. Akopyants, Lon-Fye Lye, Deborah E. Dobson, and Stephen M. Beverley, was funded by HHS | National Institutes of Health (NIH) (R01-AI29646, R56-

AI099364). This work, including the efforts of Natalia S. Akopyants, Lon-Fye Lye, Deborah E. Dobson, and Stephen M. Beverley, was funded by HHS | NIH | National Center for Research Resources (NCRR) (UL1-TR000448).

REFERENCES

- Hillman BI, Cai G. 2013. The family *Narnaviridae*: simplest of RNA viruses. *Adv Virus Res* 86:149–176. <http://dx.doi.org/10.1016/B978-0-12-394315-6.00006-4>.
- Hillman BI, Esteban R. 2012. Family *Narnaviridae*, p 1055–1060. In King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (ed), *Virus taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses*. Elsevier, San Diego, CA.
- Wickner RB, Fujimura T, Esteban R. 2013. Viruses and prions of *Saccharomyces cerevisiae*. *Adv Virus Res* 86:1–36. <http://dx.doi.org/10.1016/B978-0-12-394315-6.00001-5>.
- Hong Y, Dover SL, Cole TE, Brasier CM, Buck KW. 1999. Multiple mitochondrial viruses in an isolate of the Dutch elm disease fungus *Ophiostoma novo-ulmi*. *Virology* 258:118–127. <http://dx.doi.org/10.1006/viro.1999.9691>.
- Cai G, Myers K, Fry WE, Hillman BI. 2012. A member of the virus family *Narnaviridae* from the plant pathogenic oomycete *Phytophthora infestans*. *Arch Virol* 157:165–169. <http://dx.doi.org/10.1007/s00705-011-1126-5>.
- Kořený L, Sobotka R, Kovářová J, Gnipová A, Flegontov P, Horváth A, Oborník M, Ayala FJ, Lukeš J. 2012. Aerobic kinetoplastid flagellate *Phytomonas* does not require heme for viability. *Proc Natl Acad Sci USA* 109:3808–3813.
- Beiting DP, Peixoto L, Akopyants NS, Beverley SM, Wherry EJ, Christian DA, Hunter CA, Brodsky IE, Roos DS. 2014. Differential induction of TLR3-dependent innate immune signaling by closely related parasite species. *PLoS One* 9:e88398. <http://dx.doi.org/10.1371/journal.pone.0088398>.
- Jaskowska E, Butler C, Preston G, Kelly S. 2015. *Phytomonas*: trypanosomatids adapted to plant environments. *PLoS Pathog* 11:e1004484. <http://dx.doi.org/10.1371/journal.ppat.1004484>.
- Marché S, Roth C, Manohar SK, Dollet M, Baltz T. 1993. RNA virus-like particles in pathogenic plant trypanosomatids. *Mol Biochem Parasitol* 57:261–268. [http://dx.doi.org/10.1016/0166-6851\(93\)90202-9](http://dx.doi.org/10.1016/0166-6851(93)90202-9).