

## Complete Genome Sequence of Le Blanc Virus, a Third Caenorhabditis Nematode-Infecting Virus

Carl J. Franz, Guoyan Zhao, Marie-Anne Félix, b and David Wang

Departments of Molecular Microbiology and Pathology & Immunology, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA, and Institute of Biology of the Ecole Normale Supérieure (IBENS), Paris, France<sup>b</sup>

Orsay virus and Santeuil virus, the first known viruses capable of naturally infecting the nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae*, respectively, were recently identified by high-throughput sequencing of wild *Caenorhabditis* strains. By similar analysis of another wild *C. briggsae* isolate, we have now discovered and sequenced the complete genome of a third novel virus, Le Blanc virus, that is distantly related to Orsay and Santeuil viruses. All three viruses are positive-sense RNA viruses with bipartite genomes that are most closely related to nodaviruses. Identification of a third virus capable of infecting *Caenorhabditis* nematodes enables comparative analysis of this clade of viruses and strengthens this model for investigating virus-host interactions.

rsay and Santeuil viruses were recently discovered in wild strains of Caenorhabditis nematodes with abnormal intestinal morphology (2). High-throughput sequencing of RNA isolated from the wild nematode isolate JU1498, a Caenorhabditis briggsae strain obtained from a rotting peach in an orchard near Le Blanc, France, yielded 764 unique sequence reads with limited similarity to sequence of Orsay and Santeuil viruses. From these sequences, 3 contiguous sequences (contig1, contig2, and contig3) were assembled. contig1, upon translation to predicted amino acid sequence, aligned to the RNA1 segment of Santeuil virus with 51.3% identity when BLASTx was used (1). Similarly, contig2 and contig3 aligned to the RNA2 segment of Santeuil virus with 58.2% and 59.2% amino acid identities, respectively. The gap between contig2 and contig3 was spanned by reverse transcription-PCR (RT-PCR) using primers designed from the contigs that flanked the gap. For both segments, the termini were defined by 5' and 3' rapid amplification of cDNA ends, yielding complete RNA1 and RNA2 segments of 3,473 and 3,001 nucleotides (nt), respectively.

The genomic organization of Le Blanc virus paralleled those of Orsay and Santeuil viruses. The RNA1 segment encoded a large open reading frame (ORF) of 3,057 nt. The predicted protein shared 34% amino acid identity with the RNA-dependent RNA polymerase of Orsay virus as determined by BLASTx. The RNA2 segment of Le Blanc virus contained two predicted ORFs. The first ORF encoded a predicted 349-amino-acid sequence with 69% identity to the capsid protein of Orsay virus. The second ORF encoded a 398-amino-acid sequence with detectable similarity only to the ORF  $\delta$  proteins of Orsay and Santeuil viruses.

Orsay virus can infect strains of *Caenorhabditis elegans* but not *C. briggsae*. Santeuil virus can infect strains of *C. briggsae* but not

C. elegans. Similar to Santeuil virus, Le Blanc virus could infect C. briggsae strains but could not infect strains of C. elegans. With the discovery of Le Blanc virus, there are now two distantly related viruses capable of infecting C. briggsae, enabling comparative analysis of the host responses to infection by these two viruses. Further comparisons can be made to the response of C. elegans to infection by Orsay virus to define highly conserved and unique responses to viral infection in Caenorhabditis nematodes. The identification of a third nematode-infecting virus augments the robustness of this model infection system.

Nucleotide sequence accession numbers. The complete sequences of the Le Blanc virus RNA1 and RNA2 segments have been deposited in GenBank under the accession numbers JQ943579 and JQ943580.

## **ACKNOWLEDGMENT**

D.W. holds an Investigator in the Pathogenesis of Infectious Diseases award from the Burroughs-Wellcome Fund.

## **REFERENCES**

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

  –410.
- Felix MA, et al. 2011. Natural and experimental infection of Caenorhabditis nematodes by novel viruses related to nodaviruses. PLoS Biol. 9:e1000586. doi:10.1371/journal.pbio.1000586.

Received 7 August 2012 Accepted 8 August 2012 Address correspondence to David Wang, davewang@borcim.wustl.edu. Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JVI.02025-12