Complete Genome Sequence of an Ictalurid Herpesvirus 1 Strain Isolated from Blue Catfish (Ictalurus furcatus)

Kuttichantran Subramaniam,a Arun Venugopalan,b,c,d,e Andrew J. Davison,f Matt J. Griffin,b,e Lorelei Ford,c Thomas B. Waltzek,a Larry Hansonc

a Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA
b Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Stoneville, Mississippi, USA
c Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Starkville, Mississippi, USA
d Department of Wildlife, Fisheries and Aquaculture, College of Forest Resources, Mississippi State University, Stoneville, Mississippi, USA
e Thad Cochran National Warmwater Aquaculture Center, Delta Research and Extension Center, Mississippi State University, Stoneville, Mississippi, USA
f MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom

ABSTRACT

The complete genome sequence of an alloherpesvirus isolated from blue catfish (Ictalurus furcatus) is reported. Genomic analyses revealed that this virus is a distinct strain of ictalurid herpesvirus 1, the first strain of which was isolated previously from a channel catfish (Ictalurus punctatus).

Ictalurid herpesvirus 1 (IcHV1) is classified in the species Ictalurid herpesvirus 1, genus Ictalurivirus, and was the first member of the family Alloherpesviridae to be sequenced (1). This virus causes acute mortality in young channel catfish (Ictalurus punctatus). Blue catfish (Ictalurus furcatus) are related closely to channel catfish, and channel × blue catfish hybrids are becoming increasingly popular in U.S. aquaculture. In 1998, a population of blue catfish fingerlings reared in an earthen pond exhibited clinical signs like those caused by IcHV1. Moreover, a virus referred to as blue catfish alloherpesvirus (BCAHV) was isolated in the channel catfish ovary (CCO) cell line (ATCC CRL-2772) and exhibited cytopathic effects like those of IcHV1. The partial nucleotide sequence of the gene encoding the DNA polymerase catalytic subunit showed the highest identity (98%, 428/438) to IcHV1 (2).

Clarified CCO cell lysate was centrifuged at 20,000 × g for 30 min, and DNA was isolated from the pellet using a Gentra Puregene tissue kit (Qiagen). A DNA sequencing library was prepared using a Nextera XT kit (Illumina) and sequenced on a MiSeq (Illumina) instrument using a v3 600-cycle kit. The 2,027,408 reads were depleted of host (Ictalurus punctatus) sequences (GenBank accession no. LBML00000000) using Kraken v2.0 (3) with default parameters. De novo assembly of the resulting 1,955,567 reads using SPAdes 3.11 (4) with default settings generated two contigs closely related to the IcHV1 Auburn strain sequence (GenBank accession no. M75136). These contigs were joined into a genome sequence by manually incorporating reads at their ends until they overlapped. The genome termini were identified from large sets of reads commencing at the same positions. The integrity of the sequence was verified by mapping the reads using Bowtie 2.1.0 (5) and inspecting the alignment in Tablet 1.17.08.17 (6) with default settings. The average coverage was 3,883 reads/nucleotide.

The linear BCAHV genome (134,493 bp) consists of a unique region (97,333 bp) flanked by terminal direct repeats (18,580 bp). A total of 91 protein-coding open reading frames (ORFs) were predicted, with 63 in the unique region and 14 in each terminal repeat. These ORFs were identified initially by transferring the IcHV1 annotations to the BCAHV genome using GATU (https://4virology.net/virology-ca-tools/gatu/) with default settings. The annotation was improved by comparisons with other mem-
bers of genus Ictalurivirus, resulting in the substitution of ORF31 and ORF32 by ORF32A, the substitution of ORF40 by ORF40A, and the addition of ORF51A. Three ORFs, namely, ORF16A, ORF57, and ORF62, were predicted from sequence comparisons to be spliced and encode a chloride channel CLIC-like membrane protein, the DNA polymerase catalytic subunit, and DNA packaging terminase subunit 1, respectively.

The BCAHV and IcHV1 sequences are 93.9% identical and colinear except for the absence of ORF16A from IcHV1. An analysis of genetic distances among BCAHV and 11 members or potential members of family Alloherpesviridae performed using SDT v1.2 (7), with the MAFFT alignment option implemented, confirmed that BCAHV is most closely related to IcHV1 (Fig. 1). These results indicate that BCAHV may be a novel IcHV1 strain and further expand the known host range of IcHV1.

Data availability. The genome sequence of BCAHV (IcHV1 strain S98-675) and sequence read data have been deposited in NCBI GenBank and the Sequence Read Archive under accession no. MK392382 and SRX5493855, respectively.

ACKNOWLEDGMENTS

This research was supported by the U.S. Department of Agriculture (USDA) Agricultural Research Service (project no. 58-6402-2729), the USDA Catfish Health Research Initiative (CRIS 6402-31320-002-02), the Mississippi State University College of Veterinary Medicine, the Mississippi Agricultural and Forestry Experiment Station, and the Medical Research Council (MC_UU_12014/3). A.V. received support by a Netaji Subhas-Indian Council of Agricultural Research (NS-ICAR) international fellowship.

REFERENCES

1. Davison AJ. 1992. Channel catfish virus: a new type of herpesvirus. Virology 186:9–14. https://doi.org/10.1016/0042-6822(92)90056-U.

2. Hanson LA, Rudis MR, Vasquez-Lee M, Montgomery RD. 2006. A broadly applicable method to characterize large DNA viruses and adenoviruses based on the DNA polymerase gene. Virol J 3:28. https://doi.org/10.1186/1743-422X-3-28.

3. Wood DE, Salzberg SL. 2014. Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15:R6. https://doi.org/10.1186/1748-7188-15-3-R6.

4. Bankiewicz A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prijibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.

5. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923.

6. Milne I, Stephen G, Bayer M, Cock PJA, Pritchard L, Cardle L, Cardle L, Shaw PD, Marshall D. 2013. Using Tablet for visual exploration of second-generation sequencing data. Brief Bioinform 14:193–202. https://doi.org/10.1093/bib/bbs012.

7. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/molbev/msu300.