



Complete Genome Sequence of a Phycodnavirus, *Heterosigma* akashiwo Virus Strain 53

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We report the complete genome sequence of *Heterosigma akashiwo* virus strain 53. The virus is a member of the *Phycodnaviridae*, one of the families regarded as giant double-stranded DNA viruses. The 274,793-bp genome contained 246 protein-coding and 3 tRNA-coding sequences.

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Leterosigma akashiwo is a photosynthetic eukaryotic unicellular alga that belongs to the class Raphidophyceae. It is one of the bloom-causing algae, which is widely observed in Pacific Rim regions, including North and South America, eastern Asia, Oceania, and the Northern Atlantic region (1–12). *H. akashiwo* bloom is known to be terminated by algicidal bacteria (13–21) and viruses (22–24). *Heterosigma akashiwo* virus (HaV) was identified as one such bloom-terminating factor (24, 25). Its genome was characterized to be a linear double-stranded DNA (dsDNA), with an estimated size of ~290 kbp (25). It is a member of the *Phycodnaviridae*, one of the viral families regarded as giant dsDNA viruses that possess genomes larger than several hundred kilobase pairs in size (26).

Here, we report the complete genome sequence of HaV strain 53, originally isolated from the Itsukaichi Fishing Port in Hiroshima Bay, Japan (27). HaV53 was propagated on H. akashiwo, and viral particles were collected by adding polyethylene glycol 8000 at a final concentration of 6% to the culture medium containing lysed hosts, followed by centrifugation of the mixture at 21,000 \times g. The HaV53 DNA was extracted from the purified HaV53 particles by proteinase K digestion, followed by chloroform-isoamyl alcohol treatment and ethanol precipitation. A genomic DNA library was prepared using a Nextera XT DNA sample prep kit (Illumina), and 24 million reads were generated by HiSeq 2500 using the 100-bp paired-end mode. Reads with high-quality scores (>28) were assembled using Platanus (28), yielding five high-sequence-coverage contigs (73.2, 58.5, 57.6, 41.2, and 33.2 kb) derived from HaV and numerous low-coverage contigs derived from the host DNA. Gaps between the contigs were filled by sequencing of gap-spanning PCR products using ABI3130xl and Illumina MiSeq sequencers. The accuracy of assembly was confirmed by mapping the paired-end reads to the final assemblage using BWA (29).

The genome of HaV53 was 274,792 bp in size, and the A+T content was 69.6%. It was predicted to contain 247 open reading frames (ORFs) by GeneMarkS (30) and 3 tRNAs by tRNAscan-SE (31). Among the 246 ORFs, 105 had significant hits in the NCBI

nonredundant protein database (BlastX, searched with *E* value $<10^{-5}$); 4 had best hits to the sequences previously reported for HaV strain 01 (accession numbers BAE06835.1, BAE06251.1, BAB69884.1, and BAB69883.1) and 23 has best hits to other *Phycodnaviridae* members. The 105 ORFs coded for polypeptides with a variety of functions, including gene regulation, metabolism, signal transduction, and ubiquitin-related protein regulation. As the sequence of HaV53 reported here is the first complete genome sequence of HaV, it would help advance research on *Phycodnaviridae*.

Accession number(s). The annotated genome sequence of HaV53 has been deposited in DDBJ/EMBL/GenBank under the accession number KX008963.

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REFERENCES

- 1. Honjo T. 1993. Overview on bloom dynamics and physiological ecology of *Heterosigma akashiwo*, p 33–41. *In* Smayda TJ, Shimizu Y (ed), Toxic phytoplankton blooms in the sea. Elsevier, Amsterdam, The Netherlands.
- Chang FH, Anderson C, Boustead NC. 1990. 1st record of a *Heterosigma* (Raphidophyceae) bloom with associated mortality of cage-reared salmon in Big Glory Bay, New Zealand. N Z J Mar Freshwat Res 24:461–469. http://dx.doi.org/10.1080/00288330.1990.9516437.
- 3. Mackenzie L. 1991. Toxic and noxious phytoplankton in Big Glory Bay,

Stewart Island, New Zealand. J Appl Phycol 3:19–34. http://dx.doi.org/ 10.1007/BF00003916.

- Black EA, Whyth JNC, Bagshaw JW, Ginther NG. 1991. The effects of *Heterosigma akashiwo* on juvenile *Oncorhynchus tshawytscha* and its implications for fish culture. J Appl Ichthyol 7:168–175. http://dx.doi.org/ 10.1111/j.1439-0426.1991.tb00523.x.
- Rensel J, Homer RA, Postel LE. 1989. Effects of phytoplankton blooms on salmon aquaculture in Puget Sound, Washington: initial research. Northwest Environ J 5:53–59.
- 6. **Taylor FJR**. 1993. Current problems with harmful phytoplankton blooms in British Colombia waters, p 699–703. *In* Smayda TJ, Shimizu Y (ed), Toxic phytoplankton blooms in the sea. Elsevier, Amsterdam, The Netherlands.
- O'Halloran C, Silver MW, Holman TR, Scholin CA. 2006. *Heterosigma akashiwo* in central California waters. Harmful Algae 5:124–132. http:// dx.doi.org/10.1016/j.hal.2005.06.009.
- Lackey JB, Lackey EW. 1963. Microscopic algae and protozoa in the waters near Plymouth in August 1962. J Mar Biol Ass 43:797–805. http:// dx.doi.org/10.1017/S0025315400025698.
- 9. Throndsen J. 1969. Flagellates of Norwegian coastal waters. Nytt Magasin for Botanikk 16:161–216.
- Rojas de Mendiola B. 1979. Red tide dong the Peruvian coast, p 183–190. In Taylor DL, Seliger HH (ed), Toxic dinofagellate bloom. Elsevier, Amsterdam, The Netherlands.
- 11. Park JS. 1991. Recent approaches on red tides. Agency KNFRaD, Pusan.
- Tseng CK, Zhou MJ, Zou JZ. 1993. Toxic phytoplankton studies in China, p 347–352. In Smayda TJ, Shimizu Y (ed), Toxic phytoplankton blooms in the sea. Elsevier, Amsterdam, The Netherlands.
- 13. Skerratt J, Bowman J, Hallegraeff G, James S, Nichols P. 2002. Algicidal bacteria associated with blooms of a toxic dinoflagellate in a temperate Australian estuary. Mar Ecol Prog Ser 244:1–15. http://dx.doi.org/ 10.3354/meps244001.
- 14. Imai I, Sunahara T, Nishikawa T, Hori Y, Kondo R, Hiroishi S. 2001. Fluctuations of the red tide flagellates *Chattonella* spp. (Raphidophyceae) and the algicidal bacterium *Cytophaga* sp. in the Seto Inland Sea, Japan. Mar Biol 138:1043–1049. http://dx.doi.org/10.1007/s002270000513.
- Kim J, Kim B, Lee C. 2007. Alga-lytic activity of *Pseudomonas fluorescens* against the red tide causing marine alga *Heterosigma akashiwo* (Raphidophyceae). Biol Contr 41:296-303. http://dx.doi.org/10.1016/ j.biocontrol.2007.02.010.
- Liu J, Lewitus AJ, Kempton JW, Wilde SB. 2008. The association of algicidal bacteria and raphidophyte blooms in South Carolina brackish detention ponds. Harmful Algae 7:184–193. http://dx.doi.org/10.1016/ j.hal.2007.07.001.
- 17. Liu J, Lewitus AJ, Brown P, Wilde SB. 2008. Growth-promoting effects of a bacterium on raphidophytes and other phytoplankton. Harmful Algae 7:1–10. http://dx.doi.org/10.1016/j.hal.2007.04.009.
- Kim JD, Kim JY, Park JK, Lee CG. 2009. Selective control of the Prorocentrum minimum harmful algal blooms by a novel algal-lytic bacterium

Pseudoalteromonas haloplanktis AFMB-008041. Mar Biotechnol 11: 463–472. http://dx.doi.org/10.1007/s10126-008-9167-9.

- Kim YS, Lee DS, Jeong SY, Lee WJ, Lee MS. 2009. Isolation and characterization of a marine algicidal bacterium against the harmful Raphidophyceae *Chattonella* marina. J Microbiol 47:9–18. http://dx.doi.org/ 10.1007/s12275-008-0141-z.
- 20. Park J, Yoshinaga I, Nishikawa T, Imai I. 2010. Algicidal bacteria in particle-associated form and in free-living form during a diatom bloom in the Seto Inland Sea, Japan. Aquat Microb Ecol 60:151–161. http://dx.doi.org/10.3354/ame01416.
- 21. Cho JY. 2012. Algicidal activity of marine *Alteromonas* sp. KNS-16 and isolation of active compounds. Biosci Biotechnol Biochem **76**:1452–1458. http://dx.doi.org/10.1271/bbb.120102.
- 22. Tomaru Y, Shirai Y, Nagasaki K. 2008. Ecology, physiology and genetics of a phycodnavirus infecting the noxious bloom-forming raphidophyte *Heterosigma akashiwo*. Fish Sci 74:701–711. http://dx.doi.org/10.1111/j.1444-2906.2008.01580.x.
- 23. Nagasaki K, Tarutani K, Yamaguchi M. 1999. Growth characteristics of *Heterosigma akashiwo* virus and its possible use as a microbiological agent for red tide control. Appl Environ Microbiol **65**:898–902.
- Nagasaki K, Yamaguchi M. 1997. Isolation of a virus infectious to the harmful bloom causing microalga *Heterosigma akashiwo* (Raphidophyceae). Aquat Microb Ecol 13:135–140. http://dx.doi.org/10.3354/ ame013135.
- Nagasaki K, Shirai Y, Tomaru Y, Nishida K, Pietrokovski S. 2005. Algal viruses with distinct intraspecies host specificities include identical intein elements. Appl Environ Microbiol 71:3599–3607. http://dx.doi.org/ 10.1128/AEM.71.7.3599-3607.2005.
- Van Etten JL, Graves MV, Müller DG, Boland W, Delaroque N. 2002. Phycodnaviridae—large DNA algal viruses. Arch Virol 147:1479–1516. http://dx.doi.org/10.1007/s00705-002-0822-6.
- Tarutani K, Nagasaki K, Yamaguchi M. 2006. Virus adsorption process determines virus susceptibility in *Heterosigma akashiwo* (Raphidophyceae). Aquat Microb Ecol 42:209–213. http://dx.doi.org/10.3354/ ame042209.
- Kajitani R, Toshimoto K, Noguchi H, Toyoda A, Ogura Y, Okuno M, Yabana M, Harada M, Nagayasu E, Maruyama H, Kohara Y, Fujiyama A, Hayashi T, Itoh T. 2014. Efficient *de novo* assembly of highly heterozygous genomes from whole-genome shotgun short reads. Genome Res 24: 1384–1395. http://dx.doi.org/10.1101/gr.170720.113.
- Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760. http:// dx.doi.org/10.1093/bioinformatics/btp324.
- Borodovsky M, Lomsadze A. 2014. Gene identification in prokaryotic genomes, phages, metagenomes, and EST sequences with GeneMarkS suite. Curr Protoc Microbiol 32:Unit 1E.7.
- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25: 955–964. http://dx.doi.org/10.1093/nar/25.5.0955.