Isolation and Physiological Characterization of a New Algicidal Virus Infecting the Harmful Dinoflagellate *Heterocapsa pygmaea*

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Dinoflagellates are considered one of the most abundant and diverse groups of marine microplankton and viruses are recognized as one of the significant factors affecting the plankton dynamics. Here, we report basic characteristics of a new dinoflagellate-infecting virus, *Heterocapsa pygmaea* DNA virus (HpygDNAV) which infects a toxic dinoflagellate, *H. pygmaea*. HpygDNAV is a polyhedral large virus (ca. 160–170 nm in diameter) propagating in its host’s cytoplasm. Because of the virion size, appearance in thin sections, and propagation characteristics, HpygDNAV is assumed to harbor a large double-stranded DNA genome; i.e., HpygDNAV is most likely a nucleocytoplasmic large DNA virus (NCLDV) belonging to the family Phycodnaviridae. Its infectivity is strain-specific, rather than species-specific, as is the case for other algal viruses. The burst size and latent period are estimated to be roughly 100–250 infectious units cell⁻¹ and < 96 h, respectively.

**Keywords**: algal virus, dinoflagellate, *Heterocapsa pygmaea*, NCLDV

Sea water contains diverse organisms and viruses that infect their host. It is assumed that there are 10³ to 10⁶ virus particles in one milliliter of sea water. Most of these viruses infect bacteria, cyanobacteria, archaea and other eukaryotic organisms but some portions of these viruses infect macro or micro algae. These algal viruses are known to play an important role in regulating the population dynamics of their phytoplankton hosts (Bratbak et al., 1993; Suttle et al., 1990; Tarutani et al., 2000). Over 50 different viruses or virus like particles (VLPs) infecting marine eukaryotic algae have been isolated and characterized during the last two decades (Nagasaki, 2008; Willson et al., 2009). To date, many algal host-virus systems were brought into *in vitro* and studied to different extents (Nagasaki et al., 2008; Van Etten and Meint, 1999, 2001; Van Etten et al., 1991). Among these species, the first and second cultured dinoflagellate-infecting viruses reported were characterized as a large icosahedral double-stranded (ds) DNA virus, *Heterocapsa circularisquama* virus (HeV, Tarutani et al., 2001; Nagasaki et al., 2003), and a small icosahedral single-stranded (ss) RNA virus, *Heterocapsa circularisquama* RNA virus (HcRV, Tomaru et al., 2004), infecting *Heterocapsa circularisquama*. Prior to the isolation of these viruses, viral infection in dinoflagellate has been scarcely investigated; there were only three reports concerning the VLPs observed in dinoflagellate by transmission electron microscope in the 1970’s (Franca, 1976; Sicko-Goad and Walker, 1979; Soyer, 1978). In this study, we present the new dinoflagellate virus *Heterocapsa pygmaea* DNA virus, HpygDNAV, which infects *Heterocapsa pygmaea* and is newly isolated from Korean coastal waters.

Dinoflagellates are single-celled aquatic organisms with two dissimilar flagella, and are thought to be some of the most abundant and diverse phytoplankton and net primary producers (Graham and Wilcox, 2000). The genus *Heterocapsa* comprises small, marine, gymnodinioid dinoflagellates (Loeblich et al., 1981). *Heterocapsa pygmaea* has been isolated from Hong Kong to Japan (Iwataki, 2008), but this species is newly identified from Korean coastal waters. In the present study, the isolation, growth in culture, life-cycle, stability, and gene content of HpygDNAV, a novel DNA virus, are described. This is the second dinoflagellate-infecting virus isolated from Korean coastal waters.

Seawater samples were collected at Jaran Bay, Korea, between April and October 2009 and filtered through 0.2 µm pore-size Dismic-25cs filters (Advantec, Charlotte, NC). The host organism, labeled as *Gymnodinium* sp. (NF-F-GYM-SP-1 strain), was obtained from the National Fisheries Research and Development Institute. However, recent
A taxonomic study on *Gymnodinium* sp. established a new genus, *Heterocapsa* (Iwataki et al., 2008; Pennick and Clarke, 1977; Tamura et al., 2005). Therefore, the host alga was identified by the sequence analysis of the large-subunit RNA gene (D1-D2 region) and the 18S rDNA region and transmission electron microscopic observation. Sequences of the D1-D2 region and the 18S rDNA region showed 100% sequence identity to those of *Gymnodinium* sp. USA29-9 (accession no. AF201747) which had been reclassified as *Heterocapsa* spp. (Iwataki et al. 2008). The host was further identified as *H. pygmaea* based on the morphological observation showing the typical characteristics of the species including the cell size, body scale size, and the number of pyrenoid among them. The host alga was cultured in modified f/2 medium (Gillard, 1973) under a 12L:12D cycle of 80 to 90 µmol photons/m²/s with cool white fluorescent illumination at 20°C. Five hundred milliliter of logarithmic-phase cultures of *H. pygmaea* were inoculated with 20 ml of filtered sea water and incubated at the same condition as above. Cultures and cells of *H. pygmaea* lysed by the filtrate and became pale in color, presumably due to the loss or degradation of photosynthetic pigments. Incubation with the viral lysate caused complete lysis of host cultures within 1 week, in contrast to controls, which remained healthy (Fig. 1A, B). Further cloning of a virus strain was performed according to the method of Tarutani et al. (2001), and the isolated virus was named as HpygDNA V.

The host range of HpygDNA V was examined by adding 50 µl of the lysate to each 1 ml culture of exponentially growing algal strains listed in Table 1. Each culture was incubated under the culture conditions described above and observed by light microscopy. HpygDNA V was not lytic to any microalgal species tested other than *H. pygmaea*; moreover, it was not lytic to all strains of *Gymnodinium* sp. tested (Table 1). The infectivity of HpygDNA V is therefore considered not only ‘species-specific’ but also ‘strain-specific’, as observed in the case of other algal viruses (Tomaru et al., 2004a, 2004b, 2008).

The replication parameters of HpygDNA V were determined by growth experiments. Cultures containing 500 ml of exponentially growing host cells were inoculated with 20 ml of lysate containing HpygDNA V at a viral titer of 7.0 × 10^4 estimated by most probable number (MPN); cell density and virus titer were then respectively measured by light microscopy and the extinction dilution method (Tarutani et al., 2001) every 24 h until 140 hours post-inoculation (hpi; Fig. 2). There was a gradual decrease of host cell numbers from 20 to 72 hpi followed by a remarkable decline. In accordance with the changes in host cell number, there was slight increase of virus titer until 72 hpi followed by sharp increase. Therefore, the lytic cycle of HpygDNA V was predicted to be shorter than 36 h. The slow increase of the virus titer from 36 to 72 hpi could be related to low infection efficiency of the virus and relatively small burst size, which was estimated as 100–250 infectious units/cell. The latent period of HpygDNA V is between those of two previously reported dinoflagellate infecting viruses, HeV and HeRNA V, which were 40–56 h and 24–48 h, respectively and longer than those of other microalgal viruses (Nagasaki et al., 2003). However, the estimated burst size is much smaller than those of HeV and HeRNA V, 1,800–2,440 and 3,400–21,000, respectively.

The morphology of HpygDNA V was observed by using a transmission electron microscope. *H. pygmaea* cultures were inoculated with HpygDNA V and samples (10 ml) were collected at 0, 24, 48, 72, 80, and 92 hours post-inoculation, fixed with 1% glutaraldehyde in f/2 for 2 h at 4°C. Cells were harvested by centrifugation at 3,000 rpm for 20 min, then post-fixed for an additional 1 h with 2% osmium tetroxide.

![Fig. 1. Images of healthy culture and *Heterocapsa pygmaea* DNA Virus (HpygDNA V)-inoculated culture. Light micrographs of a *H. pygmaea* culture at 0 day (A) and 4 days (B) post-inoculation with HpygDNA V. Scale bars represent 20 µm.](image-url)