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Note

Isolation and characterization of a novel virus infecting *Teleaulax amphioxeia* (Cryptophyceae)

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Abstract: *Teleaulax amphioxeia* (Conrad) Hill is a marine free-living cryptophyte. Here, we report the basic characteristics of the cryptophyte-infecting virus "TampV (*Teleaulax amphioxeia* virus)", the host of which is *T. amphioxeia*, as the first such virus to be successfully cultured. TampV strain 301 (TampV301) is a polyhedral large virus (ca. 203 nm in diameter), propagating in its host's cytoplasm. Because of the virion size, thin-section view and propagation characteristics, TampV301 was assumed to harbor a large double-stranded (ds) DNA genome; i.e., TampV is most likely one of the "nucleo-cytoplasmic large DNA viruses (NCLDVs)" belonging to the family *Phycodnaviridae*. Its infectivity was 'strain-specific' rather than 'species-specific' as is the case in other algal viruses. The burst size and latent period were roughly estimated to be 430–530 infectious units cell^{-1} and $<$24 h, respectively. Considering the uniqueness of cryptophytes' evolutionary position and the host's unique role within the complicated food chain involving kleptoplastid acquisition (composed of *T. amphioxeia*, the ciliate *Myrionecta rubra* and a dinoflagellate *Dinophysis* species), TampV is of much interest from the viewpoints of both eukaryotic host-virus coevolution and marine microbial ecology.

Key words: algal virus, cryptophyte, NCLDV, *Teleaulax amphioxeia*

Viruses or virus-like particles (VLPs) have been found in more than 50 species in 12 classes of eukaryotic algae (Brussaard 2004, Van Etten et al. 1991, Zingone 1995). Among them, intensive studies on both DNA and RNA viruses infecting various algal families have been conducted within the last two decades (Brussaard 2004, Nagasaki 2008). Cryptophytes are cosmopolitan algae distributed throughout freshwater and marine environments. However, viruses of the division Cryptophyta have rarely been examined so far. There is only one report of VLPs in cryptophytes by Pienaar (1976) where two distinct types of VLP were found in a *Cryptomonas* sp.; one was accumulated within the degenerating nuclear membrane, and averaged 99 nm in diameter; and the other was found external to the nucleus possessing a stalk-like region that terminated in a swollen head (240 nm in entire length). Further characterization of these two VLPs was not conducted, presumably due to cultivation being unsuccessful. Later, a virus-like agent, filterable through 0.22-μm-pore-sized membrane and showing lytic activity against a cryptophyte *Rhodomonas* sp., was screened by Suttle et al. (1991); however, it has not been further checked whether the virus-like agent was a lytic virus or not. To this end, the relationships between cryptophytes and their viruses are only poorly understood. In the present study, we report on the first virus infecting cryptophytes that has been cultured and characterized. In view of its possible ecological significance as mentioned below, its basic features are quickly reported in this note prior to its formal description as a species.

Five clonal strains (TA0704Hama01-05) of *Teleaulax amphioxeia* (Conrad) Hill (Cryptophyceae) isolated from Lake Hamana, Shizuoka Prefecture, Japan (34°45′N, 137°37′E) were cultured in modified SWM3 medium (Chen et al. 1969, Itoh & Imai 1987) enriched with 2 nM Na_2SeO_3 under a 12 hL : 12 hD cycle of 130 to 150 μmol photons m^{-2} s^{-1} with cool white fluorescent illumination at 20°C; they were all shown to be *T. amphioxeia* based upon D1/D2 and 18S rDNA sequence identities (data not shown). Unfortunately, none of the *T. amphioxeia* cultures were free from bacterial contamination, in spite of the authors' intensive efforts; thus, in addition to viruses causing lysis of *T. amphioxeia*, smaller phages infecting the accompanying bacteria were also screened and entered the culture unintentionally (data not shown). This has been a serious obstacle for genome analysis of the target algal virus.

On the other hand, seawater samples were collected at a station in the Yatsushiro Sea in Kumamoto Prefecture, Japan (32°21′N, 130°15′E) from Aug. 2007 through Jan. 2008. The

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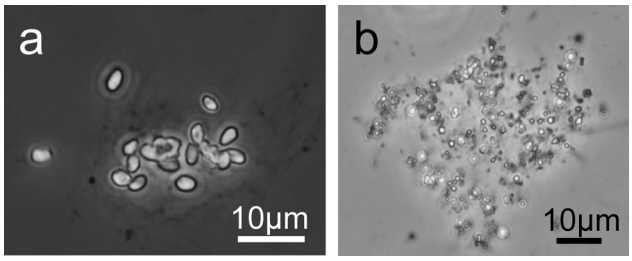

Fig. 1. Optical micrographs of a *Teleaulax amphioxeia* culture 0 day (a) and 2 days post-inoculation of TampV301 (b).

logarithmic phase cultures of *T. amphioxeia* (Fig. 1a) were inoculated with seawater samples filtered through 0.2 μm-pore-size Dismic-25cs filters (Advantec) and incubated under the conditions mentioned above. The screening and cloning procedure was almost exactly the same as the method of Tarutani et al. (2001). As a result, twelve clonal viral agents that caused lysis of *T. amphioxeia* were isolated (Fig. 1b) and nine of them are now maintained in our laboratory. Out of the nine, one typical strain (TampV301) was selected and intensively investigated in this study.

TampV301 was not lytic towards any other microalgal species that was tested, other than towards *T. amphioxeia*; indeed, it was not even lytic to all of the tested strains of *T. amphioxeia* (data not shown). Hence, the infectivity of TampV301 is considered to be not only 'species-specific' but also 'strain-specific' as observed in the case of other algal viruses (Tomaru et al. 2004a, 2004b, 2008).

On the basis of the thin section observation of TampV301-infected host cells by TEM, the intracellular virus particles were shown to be icosahedral and accumulated in the cytoplasm, being estimated at about 0.2 μm in diameter (Fig. 2a, b). No typical crystalline array formation was observed. In order to obtain more precise morphological details of TampV301, negatively-stained lysate sample was also examined under TEM. Based on the size and morphology of the virions, TampV301 was carefully distinguished (excluding smaller tailed virions [phage-like particles]) and minutely observed by TEM; then, it was estimated to be 203±19 nm in diameter (average±standard deviation; n=30); no outer membrane or tail-like structure was observed (Fig. 2c).

The replication parameters of TampV301 were roughly estimated through growth experiments. A logarithmic phase host culture (*T. amphioxeia* strain TA0704Hama03) was inoculated with TampV301 at a multiplicity of infection (m.o.i.) of 0.13; then, cell density and virus titer (most probable number [MPN]) were respectively measured by optical microscopy and the extinction dilution method (Tarutani et al. 2001) every 12 h until 60 h post-inoculation (hpi) (Fig. 3). Consequently, increase in virus titer was detected from 12 hpi over 24 hpi; thus, the lytic cycle of TampV301 was predicted to be shorter than 24 h. Further, by comparing the decrease in host cell abundance and increase in virus titer between 24–48 hpi, the burst size was approximated at 430–530 infectious units cell^{-1}. Based on the thin-section view of a TampV301-infected cell

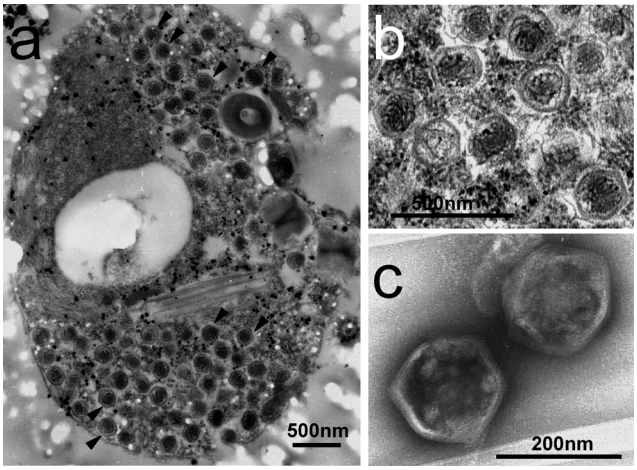

Fig. 2. Electron micrographs of TampV301. (a) thin section of a TampV301-infected cell harboring virus particles (arrowhead); (b) higher magnification of a virus assembly in the host cytoplasm; (c) negatively-stained virions.

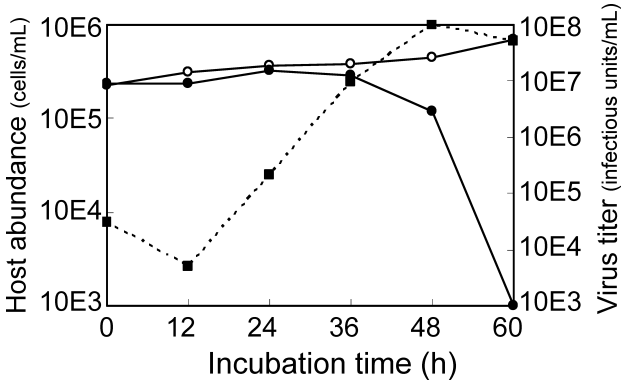

Fig. 3. Changes in abundance of *Teleaulax amphioxeia* cells with (●) or without inoculation of TampV301 on Day 0 (○), and viral titer (MPN: most probable number) enumerated by extinction dilution method (■).

(Fig. 2a), the number of virus particles in the cell was estimated to be about 760 assuming that they were distributed at the same concentration throughout the virus-synthesizing three-dimensional zone. This value is comparable to the above-mentioned roughly-calculated burst size (430–530 infectious units cell^{-1}). This parameter should probably be recalculated by means of the one-step growth experiment as the m.o.i. was as low as 0.13 in the present experiment. The highest yield was 9.8×10^7 infectious units mL^{-1} (at 48 hpi). The titer at 60 hpi (5.1×10^7 infectious units mL^{-1}) might be an underestimate, considering the decrease in host cell number between 48–60 hpi; this is presumably due to virion aggregation with host cell debris.

A number of VLPs stainable with DAPI (4′,6-diamidino-2-phenylindole) were observed in the algal lysate (data not shown); however, this cannot be regarded as direct evidence of TampV301 having a double-stranded DNA genome because of bacterial contamination and the coexistence of bacteriophages

in the culture, as verified by TEM observations (data not shown). Considering the virion size, shape, and form of accumulation in the cytoplasm (Fig. 2), TampV is most likely one of the NCLDV members (Iyer 2006); still, direct evidence supporting this supposition is essential.

Effect of temperature on TampV was examined according to the method of Tomaru et al. (2005). A TampV301 suspension containing 2.1×10^6 infectious units mL^{-1} was subjected to a storage test. Then, the infectious titers of virus suspension after 28 days of storage at 5, 10, 15, 20, 25 and 30°C in the dark were estimated at 1.2×10^4 (0.6%), 7.0×10^4 (3.3%), 7.0×10^4 (3.3%), 3.9×10^4 (1.8%), 2.3×10^3 infectious units mL^{-1} (0.1%), and less than the detection limit ($<3.0\times10^1$ infectious units mL^{-1}), respectively. These results suggest that there may be a significant loss of infectivity in natural environments also. The cryopreservation conditions for TampV were optimized along the lines of Nagasaki & Yamaguchi (1999) and Tomaru et al. (2005). The highest remaining titer (2.0×10^6 infectious units mL^{-1} [93%]) after 28 days of storage was recorded when the viral suspension (TampV301) was preserved in liquid nitrogen (−196°C) with the addition of a commercial cryoprotectant 'Cell Banker-2' (final concentration=50% [v/v], Juji Field Inc.).

As far as we are aware, this is the first report describing features of a virus infecting cryptophytes. Up to the present time, within the supergroup Chromalveolata, large dsDNA viruses infecting raphidophytes (Stramenopiles), dinoflagellates (Alveolata) and haptophytes (Haptophyta) have been isolated (Brussaard 2004, Nagasaki 2008). Considering that Cryptophyta is one of the principal members of the "Chromalveolata" along with the Stramenopiles, Alveolata and Haptophyta (Adl et al. 2005), and is apparently a key unicellular organism group for study of the endosymbiotic theory of chloroplast evolution (Stiller & Hall 1997). A genome analysis of TampV would be interesting from the viewpoints of eukaryotic evolution and host-virus coevolution; in particular, possible functions of TampV as a vector are of great interest.

The cryptophyte *T. amphioxeia* is known to function as an endosymbiont when fed on by the bloom-forming ciliate *Myrionecta rubra*; further, the plastid in *M. rubra*, originally derived from *T. amphioxeia*, is taken up by the toxic dinoflagellate *Dinophysis* species (Nagai et al. 2008, Nishitani et al. 2008, Park et al. 2006). Considering the lytic activity of TampV against the plastid-donor organism *T. amphioxeia*, the ecological impact of TampV on this complicated food chain also needs further study.

Integrating several fields of study, TampV may invite interest of researchers in the fields of algal virology, algology and eukaryotic evolutionary studies.

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