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Isolation of a virus infecting the novel shellfish-killing dinoflagellate *Heterocapsa circularisquama*

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ABSTRACT: A virus infecting the novel shellfish-killing dinoflagellate *Heterocapsa circularisquama* (*H. circularisquama* Virus: HcV) was isolated from Japanese coastal waters in August 1999 during a *H. circularisquama* bloom. Transmission electron microscopy of ultrathin sections of infected *H. circularisquama* revealed the presence of intracellular virus-like particles 24 to 48 h after infection. The virus was icosahedral, lacking a tail, ca 180 to 210 nm (mean ± standard deviation = 197 ± 8 nm) in diameter and contained an electron-dense core. It was a double-stranded DNA virus, and the appearance of the virus particles was associated with a granular region (viroplasm) in the cytoplasm that did not appear within uninfected cells. The virus caused cell lysis of 18 strains of *H. circularisquama* isolated from various embayments throughout central and western Japan, but did not lyse 24 other phytoplankton species that were tested. To our knowledge, this is the first report of a virus infecting dinoflagellates which has been isolated and maintained in culture, and our results demonstrate that viruses which infect and cause lysis of dinoflagellates are a component of natural marine viral communities.

KEY WORDS: Dinoflagellate · Harmful algal bloom · HcV · *Heterocapsa circularisquama* · Viral infection

INTRODUCTION

Heterocapsa circularisquama Horiguchi sp. nov. is a small thecate dinoflagellate (20 to 29 µm in length, 14 to 20 µm in width), which was recently described from Ago Bay, central Japan (Horiguchi 1995). Since this dinoflagellate was first recorded in Uranouchi Bay in the western part of Japan in 1988, its distribution area has expanded rapidly into embayments throughout central and western Japan (Matsuyama 1999). Due to such distribution expansion, this species has often formed large-scale red tides and caused mass mortality of bivalves such as pearl oysters *Pinctada fucata*, oysters *Crassostrea gigas*, and short-necked clams *Tapes philippinarum* (Matsuyama et al. 1996, Nagai et al. 1996, Matsuyama 1999). The most notable case occurred in 1998 when a large bloom resulted in severe financial losses of approximately 4 billion yen to the culture oyster industry in Hiroshima Bay (Matsuyama 1999). Due to the severe damage caused to commercial fisheries, there is an urgent need to find a way of predicting blooms and establishing preventative measures. As a result, several physiological and ecological studies have been conducted in order to clarify the mechanism of bloom outbreaks and toxicity of this species (Uchida et al. 1995, 1999, Matsuyama et al. 1996, Nagai et al. 1996, Yamaguchi et al. 1997).

Viruses or virus-like particles have been observed in numerous phytoplankton species from most major classes (Dodds 1979, Van Etten et al. 1991, Reisser 1993, Zingone 1995, Proctor 1997). These observations have led to increased interest in the role of algal viruses in aquatic environments. Some reports suggest that viruses are significant agents of phytoplankton mortality, and directly control the population dynamics of phytoplankton by viral lysis (Suttle et al. 1990, Bratbak et al. 1993, Nagasaki et al. 1994). Also, as a result of the release of cell contents due to viral lysis of the

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host organisms, viruses may indirectly affect the carbon and nutrient flow in natural aquatic ecosystems (Gobler et al. 1997, Thingstad & Lignell 1997).

Despite the importance of the role of algal viruses in marine ecosystem, detailed information on the effect of viral infection on marine phytoplankton is still lacking. The main reason for this is that most studies have been based on ultrastructural observations of field-collected phytoplankton cells, while few algal host-virus systems have been successfully cultured and studied in the laboratory to date (Waters & Chan 1982, Cottrell & Suttle 1991, 1995, Van Etten et al. 1991, Suttle & Chan 1995, Bratbak et al. 1996, Jacobsen et al. 1996, Nagasaki & Yamaguchi 1997, Gastrich et al. 1998, Nagasaki et al. 1999a). In the present paper, we describe the isolation and general characteristics of a virus infecting and lysing *Heterocapsa circularisquama*. To our knowledge, this is the first report on a virus infecting a dinoflagellate which has been isolated and maintained in culture.

MATERIALS AND METHODS

Algal cultures and growth conditions. The main strains of *Heterocapsa circularisquama*, HU9433-P and HA92-1, used throughout this study were isolated from Uranouchi Bay (Kochi Prefecture, Japan) in 1994 and Ago Bay (Mie Prefecture, Japan) in 1992, respectively (Table 1). Both strains were extracellularly axenic, but HA92-1 harbors bacteria within the cell probably in a symbiotic relationship. In contrast, HU9433-P was obtained by selecting a non-bacteria-harboring cell from the original strain isolated from Uranouchi Bay (HU9433), most of whose cells harbor some bacteria within them, and was thus intracellularly axenic. Other *H. circularisquama* strains used for host range study were isolated from various embayments throughout central and western Japan (Table 1). In addition, 24 algal species listed in Table 2 were also used as potential host organisms. All algal strains used in this study were maintained in the National Research Institute of Fisheries and Environment of Inland Sea, Japan. Cultures were grown in modified SWM3 medium (Chen et al. 1969) enriched with 2 nM Na_2SeO_3 under a 12:12 h light:dark cycle of ca 50 µmol photons m^{-2} s^{-1} with cool white fluorescent illumination. All experiments were performed at 20°C, with the exceptions of *Alexandrium tamarense*, *Chaetoceros didymum*, *Chattonella verruculosa*, *Ditylum brightwellii*, *Skeletonema costatum* and *Thalassiosira* sp., which were grown at 15°C.

Table 1. List of *Heterocapsa circularisquama* strains used in this study and susceptibility of these strains against HcV03 infection. +: lysed

Isolate	Isolation		Susceptibility
	Locality	Date	
AG978	Ago Bay	Aug 1997	+
HA92-1	Ago Bay	Dec 1992	+
HI9428	Imari Bay	Sep 1994	+
HK9903-1	Hakata Bay	Mar 1999	+
HK9903-2	Hakata Bay	Mar 1999	+
HK9903-3	Hakata Bay	Mar 1999	+
HK9903-8	Hakata Bay	Mar 1999	+
HO-2	Obama Bay	Sep 1997	+
HU9433	Uranouchi Bay	Mar 1994	+
HU9433-P	Uranouchi Bay	Mar 1994	+
HY9423	Yatsushiro Kai	Sep 1994	+
MZ-1	Maizuru Bay	Dec 1998	+
MZ-2	Maizuru Bay	Dec 1998	+
MZ-3	Maizuru Bay	Dec 1998	+
MZ-4	Maizuru Bay	Dec 1998	+
MZ-5	Maizuru Bay	Dec 1998	+
UN979	Unoshima Port	Sep 1997	+
WK-2	Wakinoura Fisheries Port	Aug 1999	+

Virus isolation. A surface water sample was collected in a small fishing port (Wakinoura Fishing Port) in Fukuoka Prefecture, Japan, during a bloom of *Heterocapsa circularisquama* (11 August 1999) and sent to the laboratory within 24 h of sampling. This sample was gently filtered through a 0.2 µm pore size polycarbonate membrane filter (Nuclepore). Aliquots (1 ml) of filtrate were inoculated into 1 ml of exponentially growing *H. circularisquama* HA92-1 cultures and were incubated under the conditions described above. Control cultures were inoculated with the 0.2 µm filtrate heat-treated at 100°C for 15 min.

The clonal pathogen was obtained through 2 cycles of the extinction dilution procedure (Cottrell & Suttle 1991, Nagasaki & Yamaguchi 1997). Briefly, each culture lysate was diluted with modified SWM3 medium in a series of 10-fold dilution steps. Aliquots (100 µl) of each dilution were added to 8 wells in cell-culture plates with 96 round bottom wells (NUNC), mixed with 150 µl of exponentially growing culture of *Heterocapsa circularisquama* HA92-1 strains, and incubated under the conditions described above. Lysed cultures were removed from the most diluted wells in which lysis occurred and the above entire procedure was repeated. The lysate in the most diluted wells of the second assay was sterilized by filtration through 0.2 µm pore size polycarbonate membrane filters and transferred into an exponentially growing culture of *H. circularisquama* HU9433-P. The resultant lysate was centrifuged at 7000 rpm (4490 × *g*) for 2 min at 4°C to remove cell debris, and the supernatant was used as the clonal pathogen suspension.

Aliquots (5% v/v) of the pathogen suspension were added to triplicate exponentially growing cultures of

Table 2. List of phytoplankton strains used in this study and susceptibility of these strains against HcV03 infection. –: not lysed

Species (strain)	Isolation		Susceptibility
	Locality	Date	
DINOPHYCEAE			
Alexandrium catenella (UJm)	Uwajima Bay (sediments)		–
Alexandrium tamarense (KR-6)	Kure Bay	Apr 1998	–
Gymnodinium catenatum (1ax)	Inokushi Bay	Apr 1996	–
Gymnodinium mikimotoi (G303-ax2)	Suo Nada	Jul 1985	–
Gymnodinium sanguineum (UR974)	Uranouchi Bay	Apr 1997	–
Heterocapsa triquetra (H9104)	Hiroshima Bay	Apr 1991	–
Prorocentrum micans (8304)	Hiroshima Bay	Jul 1983	–
Prorocentrum triestinum (H9109)	Hiroshima Bay	Sep 1991	–
Scrippsiella trochoidea (KR)	Kure Bay	Apr 1997	–
BACILLARIOPHYCEAE			
Chaetoceros didymum (Ch-4)	Hiroshima Bay	Mar 1989	–
Ditylum brightwellii (Di)	Hiroshima Bay	Mar 1989	–
Skeletonema costatum (SK-1)	Hiroshima Bay	Mar 1989	–
Thalassiosira sp. (Th-2)	Hiroshima Bay	Feb 1989	–
CHLOROPHYCEAE			
Oltmannsiellopsis viridis	Osaka Bay	Oct 1993	–
CRYPTOPHYCEAE			
Rhodomonas ovalis	Off Fukuyama	Jun 1967	–
EUGLENOPHYCEAE			
Eutreptiella sp. (Eut-ax01)	Hiroshima Bay (sediments)		–
EUSTIGMATOPHYCEAE			
Nannnochloropsis sp. (SFBB)	Unknown		–
PRYMNESIOPHYCEAE			
Isochrysis galbana	Unknown		–
Pavlova lutheri	Unknown		–
RAPHIDOPHYCEAE			
Chattonella antiqua (HBG8)	Hiuchi Nada (sediments)		–
Chattonella marina (UR976)	Uranouchi Bay	Jun 1997	–
Chattonella verruculosa (Misuji)	Hiroshima Bay	May 1993	–
Fibrocapsa japonica (Fib-1)	Harima Nada	Oct 1985	–
Heterosigma akashiwo (H93616)	Hiroshima Bay	Jun 1993	–

Heterocapsa circularisquama HU9433-P. Control cultures received no addition. Growth of *H. circularisquama* was monitored as *in vivo* chlorophyll fluorescence using a Turner Designs fluorometer (Brand et al. 1981). Serial transfers of lysed culture to triplicate exponentially growing cultures of *H. circularisquama* were performed twice to propagate any pathogen that was present. Moreover, the pathogen suspension was also tested for infectivity following filtration through 0.1 µm (Anotop^{TM}25, Anotec) and 0.2 µm pore size filters (DISMIC-25, Advantec) or treatment at 100°C for 15 min.

Transmission electron microscopy. Duplicate *Heterocapsa circularisquama* HU9433-P cultures were inoculated with 5% (v/v) of clonal pathogen suspension. Control cultures received no addition. Subsamples were withdrawn at 0, 24, and 48 h, fixed with 1% glutaraldehyde, and harvested by centrifugation (2000 rpm [860 × *g*], 10 min, 4°C). The cell pellets were post-fixed for 3 h in 2% osmic acid (in 0.1 M phosphate buffer, pH 7.2 to 7.4), dehydrated in a graded ethanol series, and embedded in Quetol 653 resin (NISSHIN EM Co., Ltd). Thin sections were stained with 4% uranyl acetate, 3% lead citrate, and observed at 80 kV using a JEOL JEM-1010 transmission electron microscope.

The suspension was absorbed onto carbon-coated copper grids, stained with 4% uranyl acetate, and observed at 80 kV using a JEOL JEM-1010 transmission electron microscope. Particle diameters were estimated from negatively stained images.

Epifluorescence microscopy. The clonal pathogen was also observed using epifluorescence microscopy following staining with DAPI (4′,6-diamidino-2-phenylindole) as described in Suttle (1993) and Weinbauer & Suttle (1997). Briefly, the clonal pathogen suspension was fixed with glutaraldehyde at a final concentration of 1%, and DAPI solution was added to each fixed sample at a final concentration of 1 µg ml^{-1}. The stained samples were filtered onto 0.02 µm pore size Anodisk filters (Whatman) under a low vacuum. The filters were mounted on a glass slide with a drop of

low-fluorescence immersion oil and covered with another drop of immersion oil and a cover slip. The slides were viewed at a magnification of 1000× with an Olympus BX50 epifluorescence microscope.

Viral DNA extraction and restriction enzyme analysis. An aliquot (2% v/v) of fresh viral suspension was added to a 900 ml culture of exponentially growing *Heterocapsa circularisquama* HU9433-P. After 5 d the lysed culture was prefiltered through a glass fiber filter (Whatman GF/F). The viruses were collected from the filtered lysate by centrifuging at 22000 rpm (56800 and 85900 × *g*, respectively) for 50 min in a P42A-974 rotor (Hitachi) and P40ST-1400 rotor (Hitachi).

The viral DNA was extracted almost according to the procedure of Maniatis et al. (1982). Briefly, the resultant pellet was treated with Proteinase K (100 µg ml^{-1}) in 0.15 M NaCl, 0.01 M Tris-HCl (pH 8.0), 0.01 M EDTA, 0.1% sodium dodecylsulfate at 55°C for 1 h and 37°C for 16 h to disrupt the viral proteins, and then extracted with the same volume of Tris buffer-saturated phenol, phenol-chloroform-isoamyl alcohol (25:24:1), and chloroform-isoamyl alcohol (24:1). The DNA from the resultant aqueous layer was precipitated by the additions of 100 and 70% ethanol, and the pellet was resuspended in Tris-HCl EDTA (TE) buffer.

The viral DNA was digested with restriction endonucleases (*Eco*RI, *Bgl*II, *Hind*III, *Bam*HI) under the conditions recommended by the manufacturers. The DNA fragments were separated on a 0.7% agarose gel in Tris-acetate EDTA (TAE) buffer.

Viral susceptibility of surviving cells. In order to characterize the immotile *Heterocapsa circularisquama* cells surviving viral infection (see 'Results'), 4 cells were randomly picked from the culture lysate using capillary pipettes and were inoculated into fresh SWM3 medium separately. The viral susceptibility of the clonal subisolates thus obtained was tested by adding 5% (v/v) aliquots of fresh viral suspension to triplicate of exponentially growing cultures. Cultures were monitored for evidence of lysis by optical microscopic observation and *in vivo* chlorophyll fluorescence measurement.

Host range. The host range of the isolated virus was tested by adding 5% (v/v) aliquots of fresh viral suspension to triplicate cultures of the exponentially growing strains listed in Tables 1 & 2. The cultures were incubated under the same conditions of light and temperature used for growth of each algal culture and monitored for evidence of lysis by optical microscopic observation and *in vivo* chlorophyll fluorescence measurement. These observations and measurements were compared to control cultures to which aliquots of viral suspension treated at 100°C for 15 min were added. Cultures that were not lysed after 14 d were considered to be unsuitable hosts for this virus.

RESULTS

Virus isolation

Within 7 d of receiving an inoculum from the natural seawater sample, cell lysis was detected in the *Heterocapsa circularisquama* HA92-1 culture. The resultant lysate contained numerous small particles stainable with DAPI. In contrast, in the control culture, cell lysis did not occur and the small DAPI-positive particles were not observed.

In *Heterocapsa circularisquama* HU9433-P cultures to which the clonal pathogen suspension was added, cell lysis occurred approximately 3 d after the inoculation (Figs. 1A & 2). Serial transfers from cultures that showed cell lysis into exponentially growing *H. circularisquama* HU9433-P cultures consistently resulted in cell lysis (Fig. 2). Moreover, the pathogen retained algicidal activity after filtration through a 0.2 µm pore size filter, although the algicidal response lagged behind that of the non-treated pathogen suspension

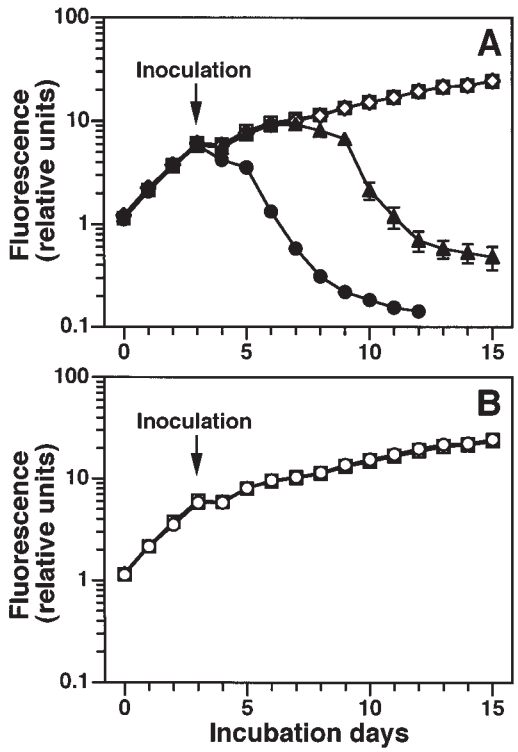

Fig. 1. Growth curves of *Heterocapsa circularisquama* HU9433-P as measured by *in vivo* chlorophyll fluorescence, showing the response following additions of the clonal pathogen suspension with (A) no treatment (●), filtered through a 0.2 µm pore size filter (▲), a 0.1 µm pore size filter (◇) and (B) no treatment (○), filtered through a 0.2 µm pore size filter (△) with further heat treatment at 100°C for 15 min relative to a no-addition control (□). Values are mean ± SD (n = 3). Note that several symbols overlap

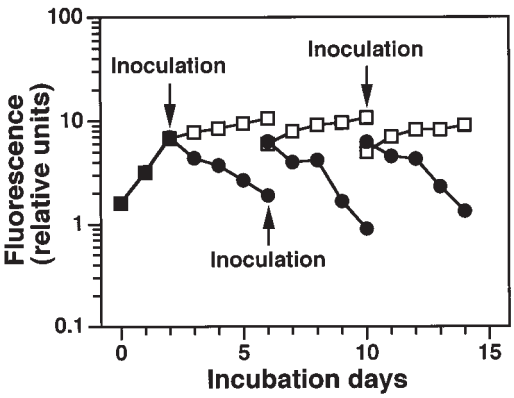

Fig. 2. Growth curves of *Heterocapsa circularisquama* HU9433-P after serial transfer of lysed cultures (●) relative to a no-addition control (□). Values are mean ± SD (n = 3)

addition, probably because of the decrease in the multiplicity of infection caused by the partial retention of pathogens onto filters (Fig. 1A). In contrast, algicidal activity was lost by filtration through a 0.1 µm pore size filter and also by heat treatment at 100°C for 15 min (Fig. 1), indicating that the pathogen is about 0.1 to 0.2 µm in size and is heat-labile.

Thin sections of healthy *Heterocapsa circularisquama* cells in control cultures indicated that the cytoplasmic organization is diagnostic of dinoflagellates (Fig. 3A). In contrast, an electron micrograph of *H. circularisquama* cells inoculated with the pathogen revealed the presence of intracellular virus-like particles (Fig. 3B–E). No trace of these particles was evident in healthy cells in the control cultures. Moreover, particles similar in appearance were observed in culture lysates (Fig. 3F).

These results demonstrated that (1) the algicidal pathogen was transferable to a fresh algal culture, (2) virus-like particles were present in the lysed culture, and (3) virus-like particles were not present in healthy culture, fulfilling Koch's postulates. Therefore, it was concluded that the virus-like particles observed within infected cells and in the culture lysates were the algicidal pathogen, which was both morphologically and physiologically a lytic virus. This virus was then termed HcV03 (*Heterocapsa circularisquama* Virus type 03) after its host species.

Virus characteristics

When exponentially growing cultures of *Heterocapsa circularisquama* HU9433-P (Fig. 4A) were inoculated with HcV03, the infected cells lost mobility and settled to the bottom of the incubation vessel. Thereafter, the settled cells became roundish, and were lysed within 2 to 3 d after the inoculation, indicating that the HcV03 virus has a latent period of 48 to 72 h (Figs. 1A & 4B). However, viral lysis was not complete and some cells survived in the culture lysate. Most of the cells that survived were immotile and lost their theca. These immotile cells recovered to the motile form within several days when isolated and cultured in fresh medium. Their growth rates during the exponential growth phase ranged from 0.69 to 0.72 d^{-1}, and were not significantly different from that of the source strain *H. circularisquama* HU9433-P (ANOVA; $p > 0.2$, Table 3). Moreover, the regrowth cultures were also lysed by an addition of HcV03 as well as the source strain (Table 3).

Thin-sectioned cells revealed that 24 h after inoculation some cells exhibited formation of finely granular viroplasm in the cytoplasm near the nucleus (Fig. 3B). Numerous viral capsids were observed inside the viroplasm. Further disruption of the organelles progressed 48 h after inoculation (Fig. 3C). The viruses were pentagonal or hexagonal in cross section, suggesting icosahedral symmetry (Fig. 3D), and 180 to 210 nm (mean ± standard deviation = 197 ± 8 nm, n = 30) in diameter (Fig. 3F). They were tailless with a multi-layered capsid and an electron-dense core in the center (Fig. 3E). More than 200 viral particles were counted within a thin section of an infected cell 48 h after inoculation. Using geometric analysis, the whole cell was estimated to contain over 1300 viral particles.

The viral particles could be stained with the fluorochrome DAPI, which stains double-stranded DNA preferentially (data not shown). The viral DNA was resistant to RNase but was degraded by DNase, and was cleaved by all the restriction endonucleases that were tested (data not shown). These results demonstrated that the viral genome consists of double-stranded DNA.

Table 3. Specific growth rates during exponential growth phase (mean ± SD) and viral susceptibility against HcV03 infection of *Heterocapsa circularisquama* HU9433-P strain and 4 clonal subisolates. The clonal subisolates were established from the immotile cells of *H. circularisquama* HU9433-P strains surviving viral infection. +: lysed

Strain or subisolates	Growth rate (d^{-1})	Susceptibility
HU9433-P	0.715 ± 0.024	+
Subisolates		
1	0.705 ± 0.013	+
2	0.690 ± 0.021	+
3	0.720 ± 0.016	+
4	0.703 ± 0.030	+

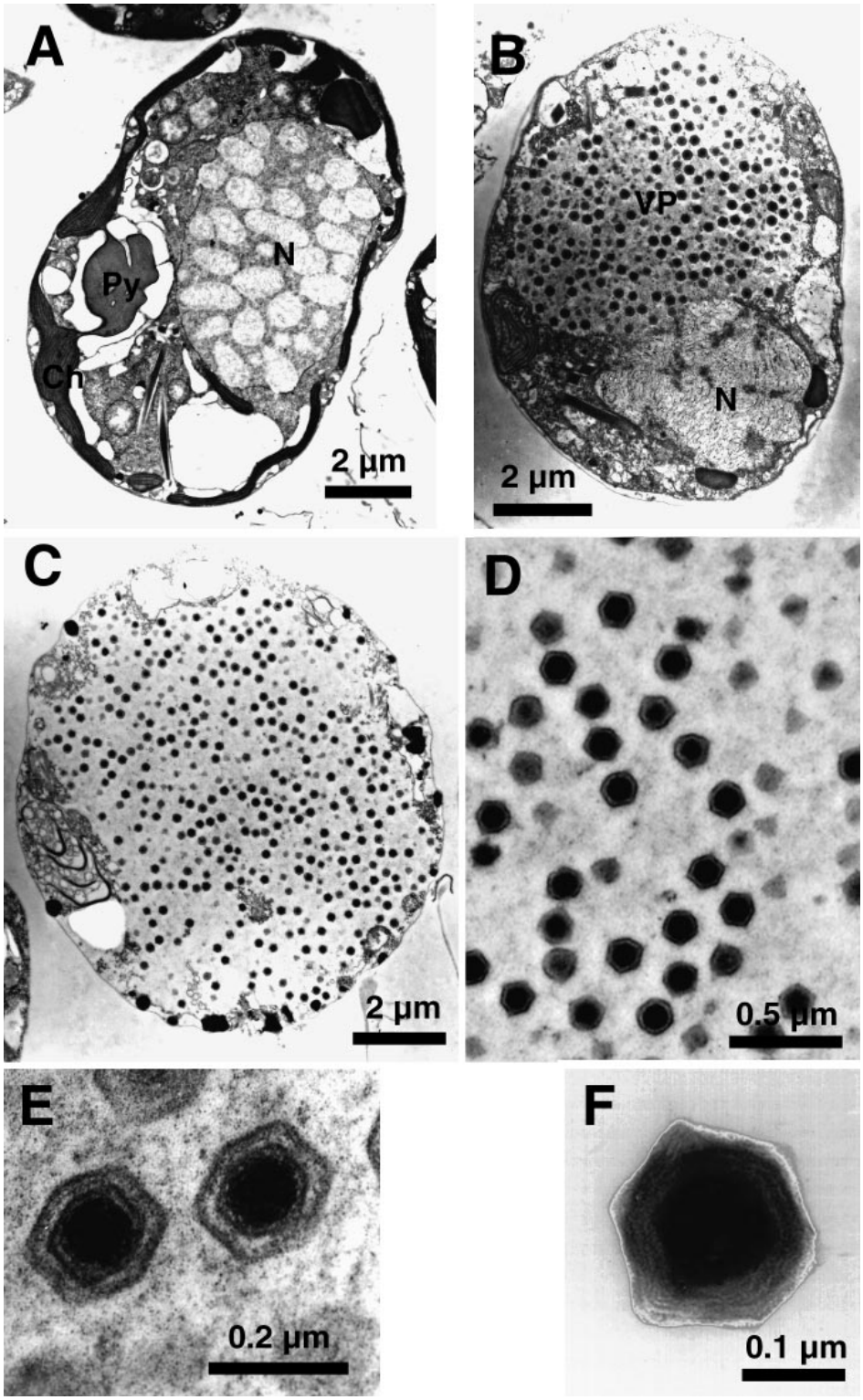

Fig. 3. Transmission electron micrographs of *Heterocapsa circularisquama* cultures. (A) Thin section of a healthy cell showing the nucleus (N), chloroplasts (Ch) and pyrenoids (Py). (B) Thin section of a cell 24 h after adding a clonal pathogen showing the development of the viroplasm (VP) in the cytoplasm. (C) Thin section of a cell 48 h after adding the clonal pathogen. Note that virus-like particles occupied a great part of the thin section. (D, E) Close-up of intracellular virus-like particles. (F) A positively stained virus-like particle that was present in the culture lysate

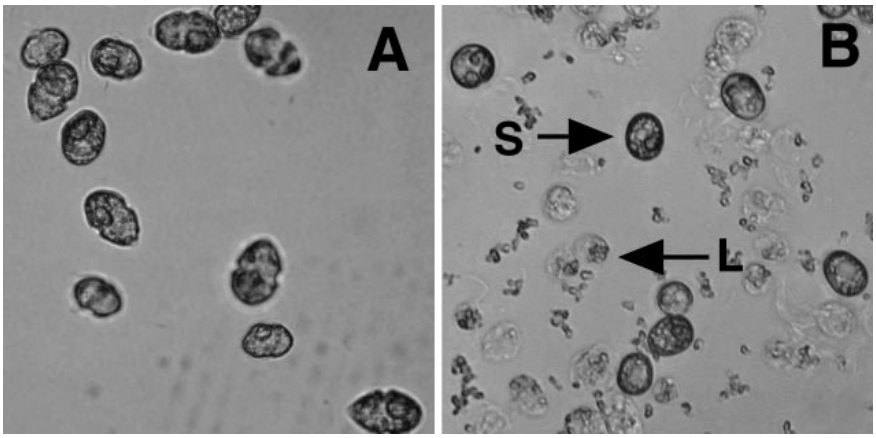

Fig. 4. Optical microphotograph of *Heterocapsa circularisquama* HU9433-P culture (A) before and (B) after HcV03 inoculation. Due to virus inoculation, a high proportion of the cells have been lysed (L) but some cells survived in a roundish and immotile form (S)

Host range

The host range of the virus (HcV03) was tested on 25 phytoplankton species, including 18 strains of *Heterocapsa circularisquama* isolated from various embayments throughout central and western Japan. The virus infected and lysed all *H. circularisquama* strains that were tested (Table 1), but did not cause lysis in any of the other 24 phytoplankton species (Table 2).

DISCUSSION

This is the first report on the successful isolation and cultivation of a lytic virus infectious to dinoflagellates. Previous ultrastructural investigations of field-collected samples have demonstrated that virus and virus-like particles are intracellular in many phytoplankton species from most major classes (Dodds 1979, Van Etten et al. 1991, Reisser 1993, Zingone 1995, Proctor 1997). However, there are very few reports on the presence of dinoflagellate cells containing virus-like particles (e.g. *Blastodinium* sp.: Soyer 1978; *Gymnodinium uberrimum*: Sicko-Goad & Walker 1979; and *Gyrodinium resplenders*: Franca 1976). Note that all these species lack a readily visible, multiplate cellulosic wall, the theca (and are hence called athecate dinoflagellates). Zingone (1995) proposed a hypothesis that the cell covering could contribute to reducing the probability of viral infection in thecate dinoflagellates. In contrast, our investigation demonstrates that viruses which infect and cause lysis of thecate dinoflagellates are present in nature, suggesting that the presence of a theca does not necessarily prevent viral infection.

The HcV03 virus has many common characteristics with other isolated viruses infectious to eucaryotic microalgae. Similarities include virus size, morphology, and genome type. This finding indicates that the HcV03 virus belongs to the newly defined family Phycodnaviridae, which infects unicellular algae (Van Etten et al. 1991). Clearly, further characterizations at molecular levels (e.g. Chen & Suttle 1996) will be required in order to make a definite decision about its taxonomic position.

On the other hand, there are some significant differences between this virus and other algal viruses. The addition of the virus to an exponentially growing culture of *Heterocapsa circularisquama* resulted in cell lysis within 2 to 3 d, indicating that the HcV03 virus has a latent period of 48 to 72 h. Also, the burst size was estimated to be >1300 viral particles per lysed cell, based on the geometric analysis of single thin sections from infected cells. Compared with other algal viruses, the latent period and the burst size of this virus are longer and larger, respectively. This could be the result of the large size of the host cells. In addition to the fact that our estimations are only rough ones, however, these parameters may be affected by the growth and physiological condition of the cells (Bratbak et al. 1998), so these differences should be interpreted with care.

A more noteworthy difference is that viral lysis was not complete and some cells survived in a roundish and immotile form despite the fact that this is a clonal strain. One possible explanation for this is the development of resistance to viral infection. Actually, the incidence of resistance has been shown in marine cyanobacteria and eucaryotic phytoplankton (Waterbury & Valois 1993, Nagasaki & Yamaguchi 1998, Nagasaki et al. 1999b). However, these immotile cells which survived viral infection recovered to the motile form within several days when isolated and cultured in fresh medium (Table 3). Moreover, the motile cells were infected and lysed by an addition of HcV03 (Table 3). Another possible explanation is derived from the morphological characteristics of the cells that survived, which were immotile and round in shape. *Heterocapsa circularisquama* often transforms into immotile and roundish forms under unfavorable growth

conditions (e.g. Uchida et al. 1999). They have been considered to be temporary cysts as described previously for some other dinoflagellates (Pfiester & Anderson 1987). Although it is unknown how they survived and succeeded in regrowth, these dinoflagellates might have some mechanism to avoid viral infection such as the formation of a thick-layered envelope as is observed in temporary cysts (Nagasaki et al. 2000).

The isolation and cultivation of HcV demonstrates that viruses which infect and cause lysis of dinoflagellates are a component of natural marine viral communities. Dinoflagellates are among the most important groups of phytoplankton in the sea, especially in coastal waters; they are global in distribution, contain a large number of species, and include various harmful bloom-forming species. Therefore, the host-virus system obtained in this study is expected to be important material for understanding the role of viruses in natural marine environments. Furthermore, HcV has the potential for use as a microbiological agent for eliminating the noxious *Heterocapsa circularisquama* blooms, because it can be produced in the lab at a relatively low cost, it has been found to be species-specific and is not likely to be harmful to other organisms. In addition, the HcV03 virus originates from natural seawater and has not been genetically manipulated. Intensive studies on this virus from the viewpoint of scale, cost, and safety must of course be assessed in more detail prior to its application to the natural environment.

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