Phycological Research

*Phycological Research* 2013; **61:** 27–36

# Isolation and characterization of a single-stranded RNA virus that infects the marine planktonic diatom *Chaetoceros* sp. (SS08-C03)

**Yuji Tomaru,**[1]* **Kensuke Toyoda,**[1,2] **Kei Kimura,**[1] **Yoshitake Takao,**[3] **Kiyonari Sakurada,**[4] **Natsuko Nakayama**[1] **and Keizo Nagasaki**[1]

[1]National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Hatsukaichi, [2]Research and Education Center for Natural Sciences, Keio University, Yokohama, [3]Department of Marine Bioscience, Fukui Prefectural University, Obama, and [4]Kumamoto Prefectural Fisheries Research Center, Kami-Amakusa, Japan

## SUMMARY

Diatoms are the major primary producers in the world's aquatic environment; hence, their dynamics are an important focus in current studies. Viruses, along with other physical, chemical, and biological factors, have recently been recognized as potential factors of diatom mortality. We isolated and characterized a new diatom virus (Csp03RNAV) that causes lysis of the marine planktonic diatom *Chaetoceros* sp. strain SS08-C03 isolated from Hiroshima Bay, Japan. Here, we present the physiology, morphology, and genome characteristics of this virus. Csp03RNAV was isolated from surface waters of Yatsushiro Sea, Japan. Virions were icosahedral and 32 nm in diameter, and accumulated in the cytoplasm of the host cells. The latent period was estimated to be <48 h. Csp03RNAV harbors a single-stranded RNA genome, which has 9417 bases encoding two open reading frames that code for putative replication-related proteins and putative structural proteins, respectively. The monophyly of Csp03RNAV and the other known diatom-infecting single-stranded RNA viruses (genus *Bacillarnavirus*), *Rhizosolenia setigera* RNA virus, *Chaetoceros socialis* f. *radians* RNA virus, and *Chaetoceros tenuissimus* RNA virus was strongly supported by phylogenetic analysis based on the amino acid sequence of the RNA-dependent RNA polymerase domain. On the basis of these results, Csp03RNAV is considered to be a new member of the genus *Bacillarnavirus*.

Key words: *Bacillarnavirus*, *Chaetoceros*, diatom, ssRNA, virus.

## INTRODUCTION

Diatoms (Bacillariophyta) account for a large part of marine primary production, with productivities of up to 35% in oligotrophic and 75% in nutrient-rich systems (Nelson *et al*. 1995). Among them, the genus *Chaetoceros* is a major taxonomic group in the ocean from the perspective of species diversity and ecological impact. At least 400 species have been described in this genus, and most of them play essential roles as primary photosynthetic producers in various marine environments (e.g. in the North Water; Booth *et al*. 2002), particularly during their bloom periods (Van-Landingham 1968; Rines & Hargraves 1988). Therefore, the dynamics of diatom blooms, including *Chaetoceros* blooms, is an important theme for marine ecology researchers. As many studies have suggested, diverse physical, chemical, and biological factors affect diatom dynamics (Tilstone *et al*. 2000; Sarthou *et al*. 2005). Furthermore, several recent studies have shown the potential significance of viruses infecting the diatoms (Bettarel *et al*. 2005; Tomaru *et al*. 2011a).

To date, at least nine different viruses that infect the species of the genera *Chaetoceros* and *Rhizosolenia* have been isolated and characterized to different degrees, and two virus genera have been newly established and approved by the International Committee on Taxonomy of Viruses (http://ictvonline.org/index.asp). Three viruses (CsfrRNAV, CtenRNAV, and RsetRNAV (previously reported as RsRNAV)) have been grouped into the genus *Bacillarnavirus*, which harbors a single-stranded RNA (ssRNA) genome (Nagasaki *et al*. 2004; Shirai *et al*. 2008; Tomaru *et al*. 2009; Tomaru & Nagasaki 2011). The other genus *Bacilladnavirus* comprises only one accepted species, CsalDNAV, and two tentative members, ClorDNAV and CtenDNAV, which

*To whom correspondence should be addressed.
Email: tomaruy@affrc.go.jp
Communicating Editor: S. Mayama.
Received 26 August 2011; accepted 29 September 2012.
doi: 10.1111/j.1440-1835.2012.00670.x

harbor circular single-stranded DNA (ssDNA) genomes (Nagasaki *et al*. 2005; Tomaru *et al*. 2011b,c). CdebDNAV, another orphan virus that infects *Chaetoceros debilis*, is also an ssDNA virus; however, its genome structure has not been identified (Tomaru *et al*. 2008). Two other diatom viruses, CspNIV and CwNIV, infect *Chaetoceros* cf. *gracilis* (Bettarel *et al*. 2005) and *Chaetoceros* cf. *wighamii* (Eissler *et al*. 2009), respectively, but their nucleic acid type is still unknown. All of these diatom viruses are 22–38 nm in diameter, and specifically lyse their respective host diatom species. For further understanding of diatom host-virus relationships in nature, however, more virus isolations and characterizations are necessary.

In the present study, we introduced a new ssRNA diatom virus that infects *Chaetoceros* sp. strain SS08-C03 isolated from Hiroshima Bay, Japan.

## MATERIALS AND METHODS

### Algal cultures and growth conditions

The axenic clonal algal strain *Chaetoceros* sp. SS08-C03 (Fig. 1) was isolated from surface water in front of the National Research Institute of Fisheries and Environment of Inland Sea (FEIS) (34°27.525′N, 132°26.653′E) in Hiroshima Bay, Japan on 27 April 2008. Algal cultures were grown at 15°C in modified SWM3 medium enriched with 2 nM $Na_2SeO_3$ (Chen *et al*. 1969; Itoh & Imai 1987) under a 12:12 h LD (light : dark) cycle of approximately 110 to 150 μmol of photons $m^{-2}$ $s^{-1}$ by using cool white fluorescent illumination. The species of *Chaetoceros* sp. strain SS08-C03 is considered to be different from the diatom host species of the viruses reported to date, according to analyses of PCR-restriction fragment length

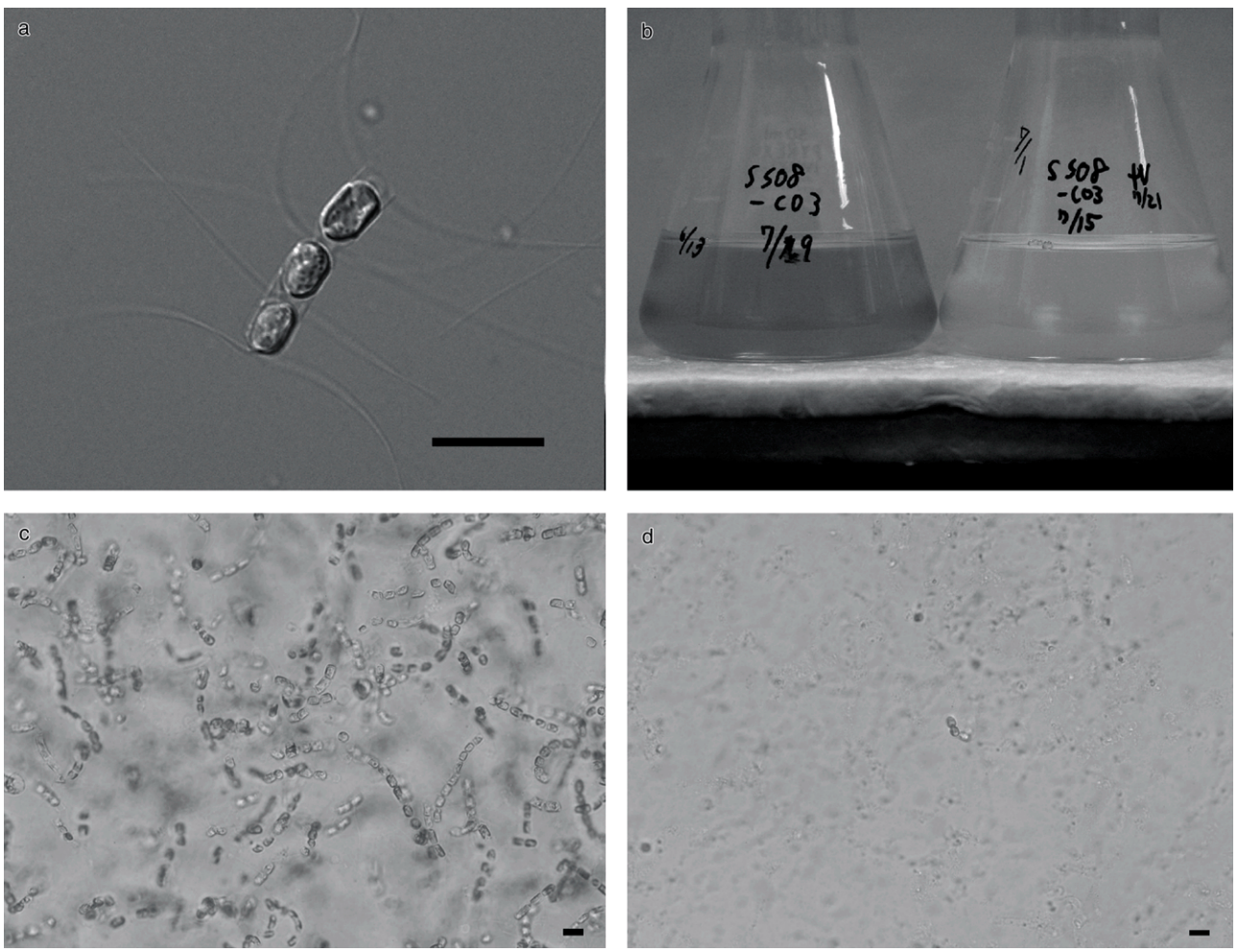


**Fig. 1.** *Chaetoceros* sp. strain SS08-C03 isolated from surface water in Hiroshima Bay, Japan. (a) Optical micrograph of intact cells. (b) Cultures without (left) and with inoculation of Csp03RNAV (right) at 7 days post-inoculation (dpi). (c) Optical micrograph of the intact culture. (d) Optical micrograph of the Csp03RNAV-inoculated culture at 7 dpi. Bars, 20 μm.

polymorphisms targeting the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene of chloroplast DNA (Toyoda *et al*. 2011). The species to which this strain belongs has not yet been determined based on its morphological features.

## Virus isolation

Surface water samples were obtained from Yatsushiro Sea (32°51.551′N, 130°49.835′E) in July 2009. The samples were sent to the laboratory (FEIS) within 24 h of sampling, and stored at 4°C until analysis. The water sample was filtered through 0.2-μm Dismic-25 cs filters (Advantec Toyo, Tokyo, Japan) to remove eukaryotic microorganisms and most bacteria. An aliquot (0.5 mL) of the filtrate was inoculated into an exponentially growing *Chaetoceros* sp. SS08-C03 culture (1 mL) and incubated at 15°C by using the lighting conditions described above. Algal cultures inoculated with SWM3 served as controls. A *Chaetoceros* sp. SS08-C03 culture inoculated with the filtrate exhibited inhibition of algal growth (Fig. 1b) at 4 days post-inoculation (dpi). Cell conditions were observed using inverted optical microscope (TE-300, Nikon, Tokyo, Japan), without fixation of the samples. We cloned the responsible pathogen through two extinction dilution cycles (Suttle 1993; Tomaru *et al*. 2004) by using lysed cultures of *Chaetoceros* sp. SS08-C03. Briefly, the algal lysate was diluted in modified SWM3 medium in a series of 10-fold dilution steps. Aliquots (100 μL) of each dilution step were added to eight wells of a 96-well flat-bottom plate (Falcon; Becton, Dickinson and Company, NJ, USA) containing 150 μL of an exponentially growing host culture. Thereafter, the algal lysate in the most diluted well in the first assay was carried over to the second extinction dilution cycle. Finally, the resultant lysate in the final end-point dilution was used as a clonal lysate, in which the probability of the occurrence of two or more viruses (i.e. failure in cloning) was estimated as <0.0106. Bacterial contamination was removed from each lysate in the highest dilution well of the second assay by filtration through a 0.1-μm polycarbonate membrane filter (Nuclepore, Whatman, Kent, UK), and was transferred to an exponentially growing host culture. The bacterial contamination of each lysate was examined using epifluorescence microscopy after staining with SYBR-Gold (Molecular Probes, Life Technologies, Carlsbad, CA, USA). Briefly, the lysate was fixed with 1% glutaraldehyde, and a $1.0 \times 10^{-4}$ dilution of the commercial SYBR-Gold stock was added to each fixed sample. The stained samples were filtered through a 0.2-μm pore size polycarbonate membrane filter (Nuclepore); subsequently, 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 (excitation, 470–490 nm; emission, 510–550 nm; dichroic mirror, 505 nm). The resultant axenic lysate was referred to as a clonal virus, Csp03RNAV, suspension.

## Host range

Inter-species host specificity of the virus, Csp03RNAV, was tested by addition of 5% (v/v) aliquots of fresh lysate passed through 0.2-μm filters (Nuclepore) into duplicate cultures of 28 exponentially growing clonal algal strains: *Chaetoceros debilis*, *Chaetoceros salsugineum*, *C. socialis* f. *radians*, *Chaetoceros tenuissimus*, *Chaetoceros* sp. SS08-C03, *Chaetoceros* cf. *affinis*, *Chaetoceros lorenzianus*, *Chaetoceros* cf. *pseudocurvisetus*, *Detonula pumila*, *Ditylum brightwellii*, *Eucampia zodiacus*, *Rhizosolenia setigera*, *Skeletonema* sp., *Stephanopyxis* sp. (Bacillariophyta), *Nannochloropsis* sp. (Eustigmatophyceae), *Teleaulax amphioxeia* (Cryptophyceae), *Alexandrium catenella*, *Gymnodinium catenatum*, *Heterocapsa circularisquama*, *Heterocapsa triquetra*, *Karenia mikimotoi*, *Prorocentrum micans*, *Scrippsiella* sp. (Dinophyceae), *Chattonella antiqua*, *Chattonella marina*, *Chattonella ovata*, *Fibrocapsa japonica*, and *Heterosigma akashiwo* (Raphidophyceae). Diatoms were cultured at 15°C under the conditions described above, and the other cultures were maintained at 20°C. Growth and evidence of lysis were monitored in each algal culture by optical microscopy, and were compared with those in control cultures inoculated with SWM3. Cultures that were not lysed at 14 dpi were considered unsuitable hosts for the viral pathogen.

## Virus purification

A 450-mL exponentially growing *Chaetoceros* sp. SS08-C03 culture was inoculated with 5 mL of the virus suspension and lysed. The lysate was passed through 0.4-μm polycarbonate membrane filters (Nuclepore) to remove cellular debris. Polyethylene glycol 6000 (Wako Pure Chemical Industries, Osaka, Japan) was added to the filtrate to achieve a final concentration of 10% (w/v), and the suspension was stored at 4°C in the dark overnight. After centrifugation at 57 000 *g* at 4°C for 1.5 h, the pellet was washed with 10 mM phosphate buffer (pH 7.2) and added to an equal volume of chloroform. After vigorous vortexing, the suspension was centrifuged at 2200 *g* for 20 min at room temperature to remove the chloroform. The water phase was collected and ultracentrifuged at 217 000 *g* for 4 h at 4°C to collect the virus particles. The resultant viral pellets were used for genome analysis. The virus particles resuspended in 600 μL of ultrapure water, that is, virus suspension, were used for viral

protein analysis and negative staining observations by transmission electron microscopy.

## Transmission electron microscopy

An exponentially growing culture of *Chaetoceros* sp. SS08-C03 was inoculated with Csp03RNAV suspension (5% v/v). As the control, a *Chaetoceros* sp. SS08-C03 culture was inoculated with autoclaved culture medium SWM3. An aliquot of cell suspension was sampled at 2 dpi. *Chaetoceros* sp. SS08-C03 cells were harvested by centrifugation at 860 *g* for 10 min at 4°C, and fixed with 1% glutaraldehyde in SWM3 for 4 h at 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 (50 to 100%), and embedded in Quetol 812 resin (Nisshin EM, Tokyo, Japan). Ultrathin sections were stained with 4% uranyl acetate and 3% lead citrate, and observed at 80 kV by using a JEOL JEM-1010 transmission electron microscope (TEM). Csp03RNAV particles negatively stained with uranyl acetate were also observed using TEM. Briefly, the purified virus suspension was mounted on a grid (no. 780111630; JEOL Datum, Tokyo, Japan) for 30 s; and excess water was removed using a filter paper (no. 1; Toyo, Tokyo, Japan). Thereafter, 4% uranyl acetate was applied for 10 s, and excess dye was removed using a filter paper. After the grid was dried in a desiccator for 3 h, negatively stained Csp03RNAV particles were observed under a TEM at an acceleration voltage of 80 kV. Particle diameters were estimated from the negatively stained images.

## Csp03RNAV proteins

The virus suspension was mixed with four volumes of denaturing sample buffer (62.5 mM Tris-HCl, 5% 2-mercaptoethanol, 2% sodium dodecyl sulfate (SDS), 20% glycerol, 0.005% bromophenol blue) and boiled for 5 min. The proteins were then separated by SDS-PAGE (polyacrylamide gel electrophoresis) ($80 \times 40 \times 1$ mm, 12.5% polyacrylamide, 150 V) using the XV Pantera System (DRC, Tokyo, Japan). Proteins were visualized using Coomassie Brilliant Blue stain. Protein molecular mass standards (Bio-Rad Laboratories, Tokyo, Japan) ranging from 6.5 to 200 kDa were used for size calibration.

## Nucleic acids

Nucleic acids of the virus were extracted from the viral pellet by using the RNeasy Mini Kit (Qiagen, Tokyo, Japan). Aliquots (7 μL) of the nucleic acids solution were digested with 0.025 μg $\mu L^{-1}$ RNase A (Nippon Gene, Tokyo, Japan) in $0.01 \times$ saline sodium citrate buffer (SSC) or $2 \times$ SSC ($1 \times$ SSC = 0.15 M NaCl and 0.015 M Na-citrate, pH 7.0) at 37°C for 1 h (Tai *et al.* 2003), and incubated with 0.5 U $\mu L^{-1}$ DNase I (Takara Bio, Otsu, Shiga, Japan) at 37°C for 1 h. Nucleic acid extracts held on ice without any treatment served as controls. The nucleic acid samples thus prepared were electrophoresed on denatured agarose gels (1.5%; SeaKem Gold Agarose; Lonza, Tokyo, Japan) at 50 V for 1 h. Nucleic acids were visualized using SYBR-Gold staining (Molecular Probes).

## Genome sequencing

Sequencing of the viral genome was performed as follows: The RNA purified from the virus pellet by using RNeasy Mini Kit (Qiagen) was treated with Poly(A) Tailing Kit (Ambion, Life Technologies, Carlsbad, CA, USA) to add poly(A) to the 3′ end of the ssRNA genome. For sequencing analysis, the cDNA library was prepared using GS FLX (Roche, Basel, Switzerland), according to the manufacturer's protocol.

The sequence data were automatically assembled using GS De Novo Assembler v2.3 (Roche) and manually reassembled with Sequencher v4.9 (Hitachi Soft, Tokyo, Japan). Putative open reading frames were identified using open reading frame (ORF) Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Automated comparisons of the Csp03RNAV sequence to genetic databases were performed using the BLAST program (Basic Local Alignment Research Tool).

## Phylogenetic analysis

We identified a conserved RNA-dependent RNA polymerase (RdRp) domain in the assembled contiguous sequence using BLAST. The deduced amino acid sequence of the corresponding region was compared with RdRp domains of other viruses. They were automatically aligned using ClustalW (Thompson *et al.* 1994) and manually refined. Phylogenetic trees based on the deduced amino acid sequences of the RdRp domain were constructed using the neighbor-joining (NJ) and maximum likelihood (ML) methods with the Jones-Taylor-Thornton matrix (JTT model) packaged in the Phylip 3.65 Program (Jones *et al.* 1992). Amino acid sequences used for comparison, scientific names of the organisms with abbreviations in parentheses, and the database accession numbers (referring to National Center for Biotechnology Information (NCBI) unless otherwise stated) are as follows: Aichi virus (AiV), AB010145; bovine enteric calicivirus (BoCV), AJ011099; bean pod mottle virus (BPMV), NC_003496; black queen cell virus (BQCV), NC_003784; *Chaetoceros socialis* f. *radians* RNA virus (CsfrRNAV), AB469874; *Chaetoceros* sp. number03 RNA virus (Csp03RNAV), AB639040; *Chaetoceros tenuissimus* RNA virus (CtenRNAV), AB375474; cowpea

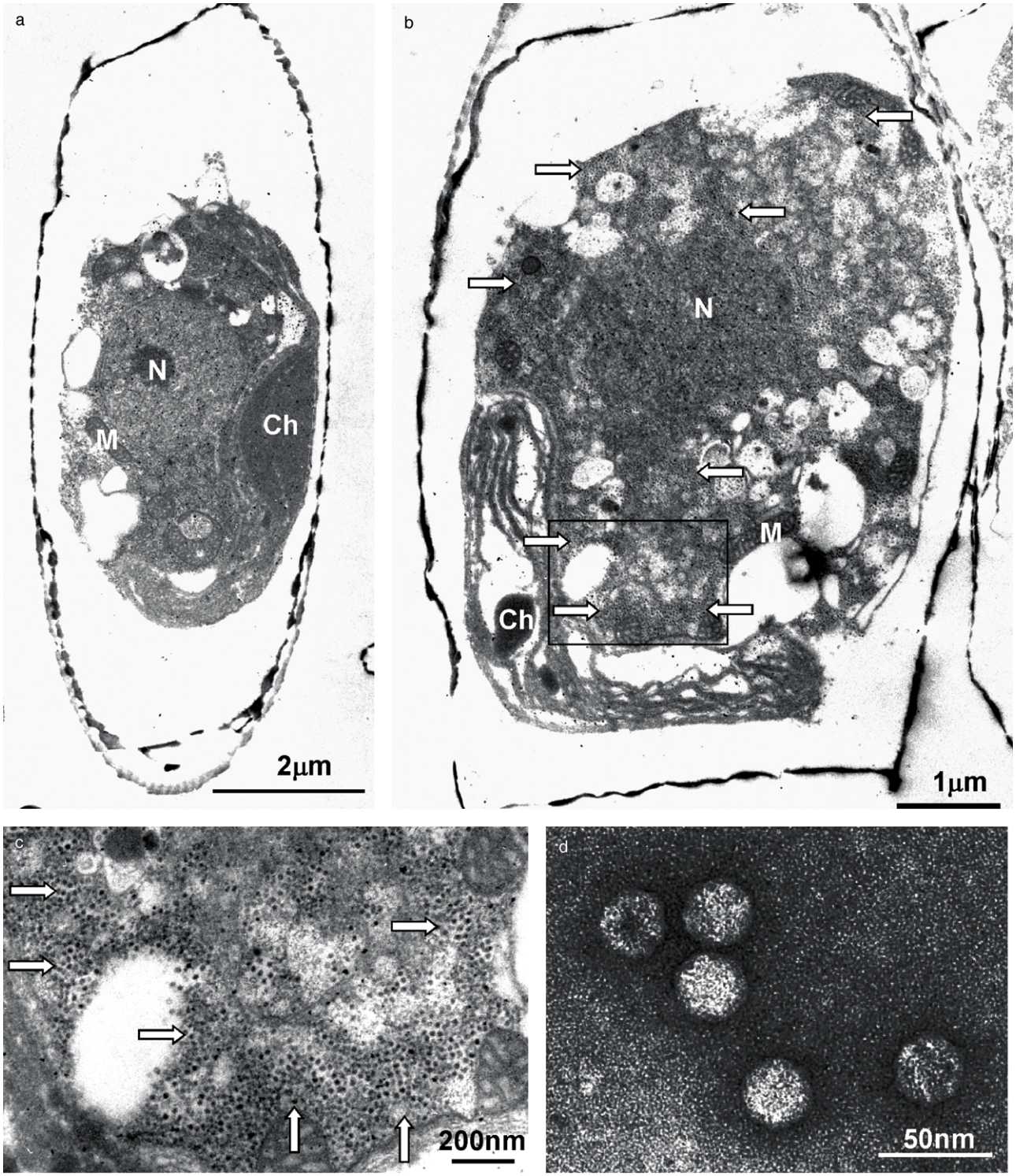


**Fig. 2.** Transmission electron micrographs of ultrathin sections of *Chaetoceros* sp. strain SS08-C03 (a–c) and negatively stained Csp03RNAV particles (d). (a) Healthy cell. (b, c) Cells infected with Csp03RNAV at 2 days post-inoculation (dpi). (b) Degraded host cytoplasm and chloroplast. (c) Higher magnification of virus-like particles (VLPs) in the cytoplasm, rectangular frame in panel b. Arrows denote the accumulation of VLPs. (d) Negatively stained Csp03RNAV particles in culture lysate. N, nucleus; Ch, chloroplast; M, mitochondrion.

severe mosaic virus (CPSMV), M83830; cricket paralysis virus (CrPV), NC_003924; *Drosophila* C virus (DCV), NC_001834; deformed wing virus (DWV), NC_004830; *Heterosigma akashiwo* RNA virus (HaRNAV), NC_005281; Norwalk virus (NV), M87661; human poliovirus 1 Mahoney (PV), V01149; parsnip yellow fleck virus (PYFV), D14066; *Rhizosolenia setigera* RNA virus (RsetRNAV), AB243297; rice turgo spherical virus (RTSV), AAA66056; Sacbrood virus (SBV), NC_002066; *Aurantiochytrium* single-stranded RNA virus (AuRNAV), BAE47143; Triatoma virus (TrV), NC_003783; and Taura syndrome virus (TSV), NC_003005.

## Growth experiment

An exponentially growing culture of *Chaetoceros* sp. SS08-C03 (50 mL) was inoculated with Csp03RNAV at a multiplicity of infection of 582. A host culture inoculated with an autoclaved culture medium served as the control. An aliquot of the cell suspension was sampled from each culture at 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 dpi, and the number of host cells and lytic agents were estimated. This experiment was a single trial. Cell counts were carried out with Fuchs-Rosenthal hemocytometer using optical microscopy (Nikon TE-300), without fixation of the samples. The viral titer (= the number of viral infectious units) was determined using the extinction dilution method (Suttle 1993). Briefly, the samples used for estimation of viral titer were passed through 0.8-μm polycarbonate membrane filters (Nuclepore) to remove cellular debris. These filtrates were diluted with modified SWM3 medium in a series of 10-fold dilution steps. Aliquots (100 μL) of each dilution were added to eight wells of a 96-well flat-bottom plate containing 150 μL of an exponentially growing culture of host algae. The cell culture plates were incubated at 15°C under a 12:12 h LD cycle of 130 to 150 μmol photons $m^{-2}$ $s^{-1}$ with cool white fluorescent illumination, and were monitored by optical microscopy (TE-300, Nikon) over 14 days for the occurrence of culture lysis. Culture lysis due to virus infection was usually observed as near complete destruction of the host cells in a well. Using a BASIC program, we calculated viral titer from the number of wells in which algal lysis occurred (Nishihara *et al*. 1986).

# RESULTS AND DISCUSSION

## Isolation of the viral pathogen and its host range

The isolated virus retained its lytic activity (Fig. 1), and was serially transferable to exponentially growing *Chaetoceros* sp. SS08-C03 cultures. The host range of the virus was tested using 28 phytoplankton strains, including 14 diatom strains. Csp03RNAV caused lysis in only its single host strain and not any other microalgal species tested, indicating that the virus had high infection specificity.

## Morphological features

Thin sections of healthy *Chaetoceros* sp. SS08-C03 cells exhibited the cytoplasmic organization and frustules that are typical of diatom cells (Fig. 2a). In contrast, electron micrographs of thin-sectioned *Chaetoceros* sp. SS08-C03 cells with virus at 2 dpi exhibited the presence of virus-like particles (VLPs) randomly assembled in the host cytoplasm (Fig. 2b,c).

No VLPs were detected in healthy control cultures (Fig. 2a). Further, the VLPs were observed in culture lysates by using negative staining. They were icosahedral, 32 ± 1 nm (average ± standard deviation (SD), $n = 40$), and lacked a tail and an outer membrane (Fig. 2d). Because the algacidal pathogen was transferable to a fresh algal culture, and the VLPs were observed in the lysed culture and not in healthy cultures, Koch's postulates were fulfilled. We concluded that the VLP observed in the infected cells and in the algal lysates is a previously undescribed virus pathogenic to *Chaetoceros* sp. strain SS08-C03.

## Proteins

The sizes and numbers of structural proteins of the virus particles were determined using SDS-PAGE. Csp03RNAV expressed at least three major polypeptides at 42.0, 34.0 and 28.0 kDa (Fig. 3). The number

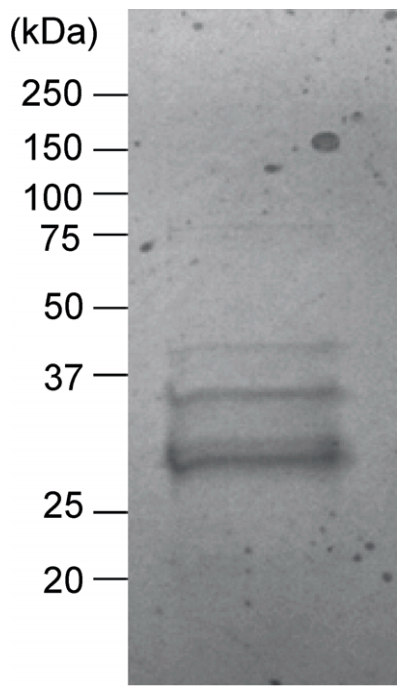


**Fig. 3.** Structural proteins of Csp03RNAV.

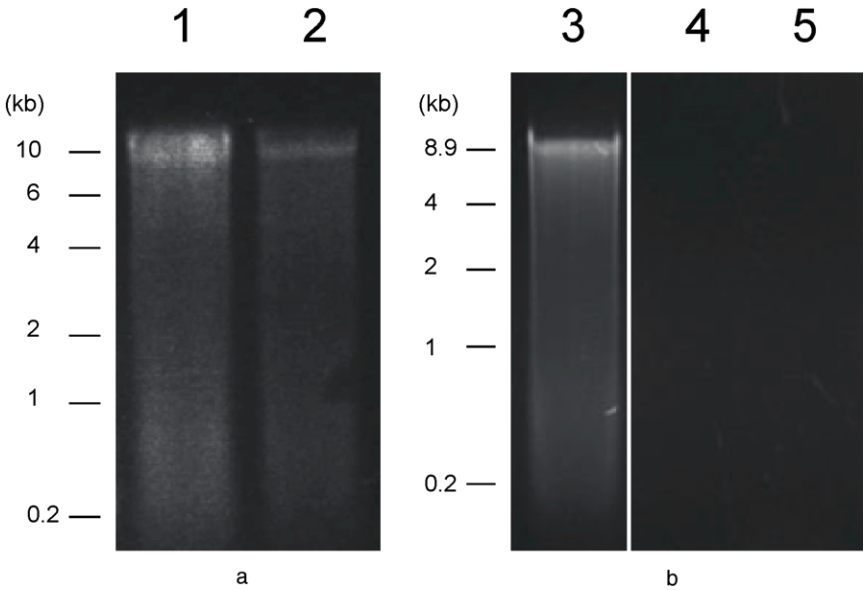


**Fig. 4.** Nucleic acids of Csp03RNAV without treatment (lanes 1, 3), treated with DNase I (lane 2), RNase A in low (lane 4), and high salt buffer (lane 5). Samples were electrophoresed on a formaldehyde agarose gel with RNA molecular size markers (0.2–10.0 kb) (panels a and b).

and sizes of major proteins of Csp03RNAV were similar to those of ssRNA diatom viruses RsetRNAV, CtenRNAV, and CsfrRNAV, which have major polypeptides of 41.5, 41.0, and 29.5 kDa; 33.5, 31.5, and 30.0 kDa; and 32.0, 28.5, and 25.0 kDa; respectively (Nagasaki *et al*. 2004; Shirai *et al*. 2008; Tomaru *et al*. 2009). The genome of RsetRNAV is composed of polyprotein genes and multiple major polypeptides of the viral structural proteins are observed in SDS-PAGE (Shirai *et al*. 2006). Csp03RNAV genome would be also composed of polyprotein genes.

## Genomic and phylogenetic analyses

Denaturing agarose gel electrophoresis revealed that the nucleic acid extracted from Csp03RNAV was approximately 9–10 kb (Fig. 4). The bands were not sensitive to DNase I, but were digested by RNase A under both high and low salt conditions (Fig. 4), suggesting that the genome is ssRNA. This virus is the third ssRNA virus that infects genus *Chaetoceros*. Therefore, this new virus was designated as *Chaetoceros* sp. number 03 RNA virus (Csp03RNAV).

The total length of the Csp03RNAV genome was estimated to be 9417 nt, excluding the poly-A tail. The adenine and uracil (AU) richness of the Csp03RNAV genome is 59.7%, which is comparable to that of CsfrRNAV (60.4%), CtenRNAV (61.1%), and RsetRNAV (63.7%) (Table 1). Compared to these RNA viruses, the AU richness of other stramenopile-infecting viruses, HaRNAV (family Marnaviridae) and AuRNAV (genus *Labyrnavirus*), are much lower at 53.1% and 50.2%, respectively. The Csp03RNAV genome includes two ORFs encoding putative replication-related proteins (ORF-1; 1709 amino acids) and putative capsid proteins (ORF-2; 791 amino acids). The genome structure of Csp03RNAV is similar to that of *Bacillarnavirus* members. BLASTP analysis also revealed that the ORF-1 amino acid sequence of Csp03RNAV is highly similar to that of ssRNA diatom viruses, with *e*-values = $7.0e^{-140}$ – $5.0e^{-153}$ (Table 1).

Here, both NJ and ML methods were used to assess the phylogenetic relationship among the positive-sense ssRNA viruses, including Csp03RNAV. Similar topologies were obtained using the two methods; hence, only the ML phylogenetic tree is shown in Figure 5. The monophyly of Csp03RNAV, CsfrRNAV, CtenRNAV, and RsetRNAV was supported by a 100% bootstrap value (Fig. 5), suggesting the existence of a diatom-infecting ssRNA virus clade within the positive-sense ssRNA viruses. Taken together, the results of the morphological, genomic, and phylogenetic analyses indicate that Csp03RNAV is the fourth member of the genus *Bacillarnavirus*.

## Replication

In the growth experiment, a significant increase in viral titer was observed after 2 dpi (Fig. 6); thus, the latent period of Csp03RNAV was estimated to be <48 h. The host cell number in the virus-inoculated culture continuously increased until 3 dpi, with a simultaneous and rapid increase in viral titer. An increase in viral titer during exponential host growth has been commonly observed in *Chaetoceros* host-virus systems, including CtenRNAV, CtenDNAV, and ClorDNAV (Shirai *et al*. 2008; Tomaru *et al*. 2011b,c). A possible explanation for this phenomenon has been previously suggested (Tomaru *et al*. 2011c). During early exponential growth, only a fraction of the host cells are virus sensitive. On

**Table 1.** General features of single-stranded RNA viruses infecting stramenopile organisms

| Host | Virus | Particle diameter (nm) | Genome size (b)† | AU richness (%) | Poly-A | Number of ORFs | Virion assemblage site in host cells | BLASTP comparison for ORF-1 of Csp03RNAV | | | BLASTP comparison for ORF-2 of Csp03RNAV | | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | E-value | No. identical/total (%) | No. similar/total (%) | E-value | No. identical/total (%) | No. similar/total (%) | |
| *Chaetoceros* sp. (SS08-C03) | Csp03RNAV | 32 | 9417 | 59.7 | ND | 2 | Cytoplasm | – | – | – | – | – | – | This study |
| *Chaetoceros socialis* f. *radians* | CsfrRNAV | 22 | 9467 | 60.4 | Yes | 2 | Cytoplasm | 7.0E-140 | 434/1520 (29%) | 701/1520 (46%) | 5.0E-65 | 209/740 (28%) | 343/740 (46%) | Tomaru *et al.* (2009) |
| *Chaetoceros tenuissimus* | CtenRNAV | 31 | 9431 | 61.1 | Yes | 2 | Cytoplasm | 7.0E-151 | 449/1530 (29%) | 710/1530 (46%) | 5.0E-89 | 218/654 (33%) | 339/654 (52%) | Shirai *et al.* (2008) |
| *Rhizosolenia setigera* | RsetRNAV | 32 | 8877 | 63.7 | Yes | 2 | Cytoplasm | 5.0E-153 | 440/1488 (30%) | 683/1488 (46%), | 4.0E-87 | 215/635 (34%) | 326/635 (51%) | Shirai *et al.* (2006) |
| *Aurantiochytrium* sp. | AuRNAV | 25 | 9018 | 50.2 | Yes | 3 | Cytoplasm | 5.0E-28 | 179/725 (25%) | 289/725 (40%) | 2.0E-45 | 152/479 (32%) | 237/479 (49%) | Takao *et al.* (2006) |
| *Heterosigma akashiwo* | HaRNAV | 25 | 8587 | 53.1 | Yes | 4 | Cytoplasm | 1.0E-28 | 159/707 (22%) | 290/707 (41%) | 1.0E-77 | 227/704 (32%) | 342/704 (49%) | Tai *et al.* (2003) |

†Excluding pol-A. ND, not determined.




**Fig. 5.** Maximum likelihood (ML) tree based on deduced amino acid sequences of the entire RNA-dependent RNA polymerase (RdRp) domain. Neighbor joining (NJ) bootstrap values (%) from 100 samples are shown at the nodes, followed by bootstrap values based on the ML analysis (%) from 100 samples. The ML distance scale bar is shown. AIV, Aichi virus; AuRNAV, *Aurantiochytrium* single-stranded RNA virus; BoCV, bovine enteric calicivirus; BPMV, bean pod mottle virus; BQCV, black queen cell virus; CPSMV, cowpea severe mosaic virus; CrPV, cricket paralysis virus; CsfrRNAV, *Chaetoceros socialis* f. *radians* RNA virus; Csp03RNAV, *Chaetoceros* sp. number03 RNAV; CtenRNAV, *Chaetoceros tenuissimus* RNA virus; DCV, *Drosophila* C virus; DWV, deformed wing virus; HaRNAV, *Heterosigma akashiwo* RNA virus; NV, Norwalk virus; PV, human poliovirus 1 Mahoney; PYFV, parsnip yellow fleck virus; RsetRNAV, *Rhizosolenia setigera* RNA virus; RTSV, rice turgo spherical virus; SBV, Sacbrood virus; TrV, *Triatoma* virus; TSV, Taura syndrome virus.

the other hand, during late log phase, the percentage of virus-sensitive cells rapidly increases (resulting in clash); thus, virus sensitivity of *Chaetoceros* might be associated with its physiological conditions related to growth phases. Eissler *et al.* (2009) concluded that lytic rates of CwNIV have no relationships with its host growth rates. Effects of host growth rates on viral susceptibilities are important for understanding their relationships in nature. Future studies should reveal it

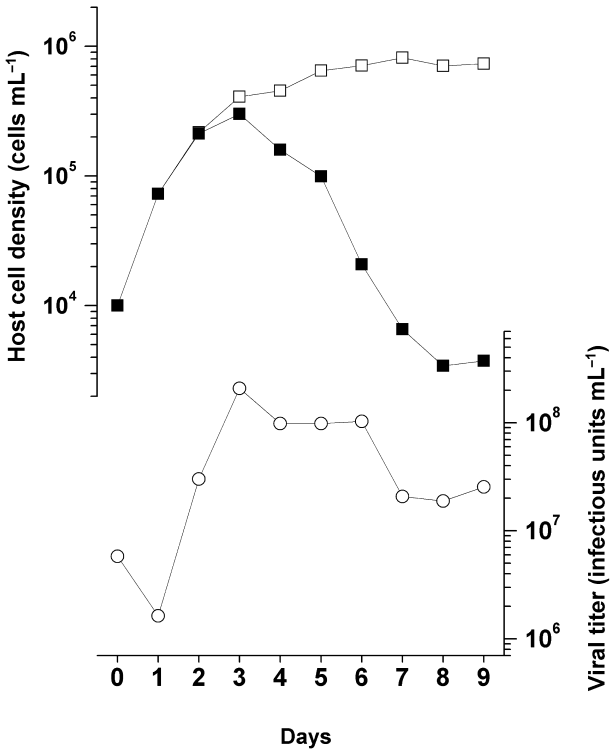


**Fig. 6.** Changes in the cell density of *Chaetoceros* sp. strain SS08-C03 with (■) or without virus inoculation (□), and viral titer (○). Virus inoculation was performed at 0 d in an exponentially growing host culture at a multiplicity of infection of 582.

based on more sensitive and accurate experiments, such as using a continuous culture system.

The host cell number in the virus-inoculated culture rapidly decreased from 4 dpi (Fig. 6). Increase in viral titer with a concomitant decrease in host cell abundance is usually observed in algal host-virus systems; however, this pattern was not detected in our study. Therefore, the burst size of this virus was not calculated. Viral titer was estimated using an extinction dilution method (see Materials and Methods) after the filtrate was passed through a 0.8-μm pore-size membrane filter. Most of the virus particles may be adsorbed to the host cell debris, resulting in fewer free virus particles in the lysate.

## IMPLICATIONS

The available knowledge on the relationships between diatoms and their infectious viruses is not extensive. A recent study showed diatom-virus dynamics in nature and suggested their ecological relationships based on several years' field surveys: (i) given that diatom viruses in water columns rapidly increase during host blooms, the viruses play important roles in regulating diatom population dynamics; (ii) elimination of virus-infected cells from the water column due to sedimentations and differences in host strain susceptibility to a virus may be important strategies of diatom populations to reduce the impact of viruses (Tomaru *et al*. 2011a). To elucidate the ecological relationships between *Chaetoceros* sp. strain SS08-C03 and Csp03RNAV, a quantitative detection system of the host cells as well as the viral particles should be established.

Diatom host-virus system studies are still in their infancy. It is well known that diatom blooms often include multiple species of *Chaetoceros* (Rines & Hargraves 1988). Hence, to understand viral impact on diatoms and their ecological relationships in nature, further isolation and characterization of diatom-infecting viruses are necessary.

## ACKNOWLEDGMENTS

This study was supported by Grants-in-Aid for Young Scientists (A) (22688016) from the Ministry of Education, Science, and Culture of Japan.

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