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ISOLATION AND CHARACTERIZATION OF A VIRUS INFECTING *PHAEOCYSTIS POUCHETII* (PRYMNESIOPHYCEAE)[1]

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ABSTRACT

A virus infecting the haptophyte Phaeocystis pouchetii *(Hariot) Lagerheim was isolated from Norwegian coastal waters in May 1995 at the end of a bloom of this phytoplankter. The virus was specific for* P. pouchetii *because it did not lyse 10 strains of* P. globosa *Scherffel,* Phaeocystis *sp., and* P. antarctica *Karsten. It was a double-stranded DNA virus, and the viral particle was a polyhedron with a diameter of 130–160 nm. The virus had a main polypeptide of about 59 kDa and at least five minor polypeptides between 30 and 50 kDa. The latent period of the virus when propagated in cultures of* P. pouchetii *was 12–18 h, and the time required for complete lysis of the cultures was about 48 h. The burst size was estimated to be 350–600 viral particles per lysed cell.*

Key index words: Phaeocystis pouchetii; phytoplankton; population control; Prymnesiophyceae; virus; virus-like particles

The marine haptophyte genus *Phaeocystis* Lagerheim has a worldwide distribution (Lancelot et al. 1994) and is also considered to be one of the most intriguing of the marine genera. It alternates between a free-living flagellated phase (3–9 μm) and a gelatinous colony phase of several millimeters in diameter (Kornmann 1955, Verity et al. 1988, Rousseau et al. 1994). *Phaeocystis* forms dense, nearly monospecific blooms in many nutrient-rich areas of the world (Davidson and Marchant 1992, Lancelot et al. 1994). During the last few decades, dense blooms of *Phaeocystis* have increased in North European waters, especially in the southern part of the North Sea. This increase in abundance of *Phaeocystis* is related to increased input of nutrients to this area (Lancelot et al. 1987, 1994). Studies have also shown that *Phaeocystis* may play an important role in ocean climate control by the emission of dimethylsulfide to the atmosphere (Liss et al. 1994). This volatile compound has an impact on marine cloud formation and climate regulation (Charlson et al. 1987). Low sedimentation may occur during some *Phaeocystis* blooms (Wassmann 1994), which indicates that cell lysis may occur in the water column. Brussard et al. (1995) reported that cell lysis was the major loss factor for *Phaeocystis* during the decline of a bloom

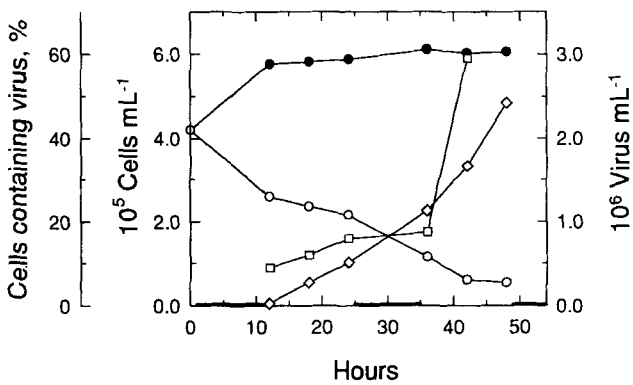

FIG. 1. Growth of *Phaeocystis pouchetii* in a noninfected control culture and in a culture infected with ca. 10 PpV01 virus particles per cell. Bars on the X-axis indicate light/dark cycles. *P. pouchetii* in control culture (●), *P. pouchetii* in infected culture (○), % *P. pouchetii* containing visible viral particles (□), and free PpV01 virus in infected culture (◇).

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TABLE 1. *List of* Phaeocystis *strains used in the host range experiment.* − = *not lysed;* + = *lysed.*

Species	Strain	Origin	Colonies present in culture	Lysed by the PpV01 virus
P. globosa	P159 (CCMP 1528)	Galapagos	No	−
P. globosa	P181 (Big 677-3)	W. Atlantic (6°45'N, 53°19'W)	Yes	−
P. globosa	P182 (NIOZ 1)	North Sea (52°5'N, 4°45'E)	No	−
P. globosa	P184 (PCC 540)	E. Atlantic (47°37'N, 8°53'W)	No	−
P. globosa	P185 (Big 1209)	Gulf of Mexico (29°15'N, 85°54'W)	No	−
Phaeocystis sp.	P334 (OL1 26SA)	Equatorial Pacific (7°S, 150°W)	No	−
P. antarctica	P161 (CCMP 1374)	Antarctic (McMurdo station)	Yes	−
P. antarctica	P281 (D4-5)	Antarctic (68°47.5'S, 73°30.2'W)	Yes	−
P. antarctica	P287 (T4-2)	Antarctic (68°39.0'S, 72°21.2'W)	Yes	−
P. antarctica	P289 (DE2)	Antarctic (68°33.3'S, 77°51.5'W)	Yes	−
P. pouchetii	AJ 01	Norwegian coast (60°16'N, 5°7'E)	No	+
P. pouchetii	AJ 10	Svalbard (78.3°N, 15°E)	No	+

in the North Sea, accounting for 75% of the decrease in cell abundance.

Virus and virus-like particles are common in the marine environment and are intracellular in phytoplankton from all major classes (Dodds 1979, Cottrell and Suttle 1991, Proctor and Fuhrman 1991, van Etten et al. 1991, Bratbak et al. 1993). Viruses collected from natural seawater may also infect and lyse phytoplankton under experimental conditions (Suttle et al. 1990, 1991). Bratbak et al. (1993, 1995) described the importance of viruses in bloom dynamics of the coccolithophorid *Emiliania huxleyi*. Their results show that lytic viruses may play a key role in termination of algal blooms. Microalgae host–virus systems brought into culture to date number less than five, and viruses of only two hosts, *Chlorella* sp. and *Micromonas pusilla*, have been studied to any extent. Infection by viruses or virus-like particles has, as far as the authors are aware, never been described for any *Phaeocystis* species. In the present paper we describe a virus infecting and lysing *Phaeocystis pouchetii* and its isolation, host range, and lytic cycle.

MATERIALS AND METHODS

The *Phaeocystis pouchetii* cultures used in this study were obtained from the culture collection at the University of Bergen, Norway (AJ01 and AJ10), and grown at 5° C in f/2 medium (Guillard 1975). Cultures of *P. globosa* Scherffel, *Phaeocystis* sp., and *P. antarctica* Karsten were obtained from L. Medlin (Alfred Wegener Institute for Polar and Sea Research; for further details see Table 1) and maintained at 15° C (*P. globosa*, *Phaeocystis* sp.) and 5° C (*P. antarctica*) in K medium (Keller et al. 1987). All cultures were grown under a 14:10 h LD cycle of about 30 μmol photons·m^{-2}·s^{-1} white light. Daily growth was monitored by cell counts in a Fuchs Rosenthal hematocytometer.

Isolation of the virus. A water sample of 100 L was collected in Raunefjorden (60°16'N, 5°7'E, 6-m depth) at the end of a bloom of *Phaeocystis pouchetii* (4 May 1995) and brought back to the laboratory within 3 h of sampling. The plankton community was concentrated by continuous flow centrifugation in a Beckman J2-HS centrifuge with a JCF-Z rotor. At 5000 rpm and a flow rate of 600 mL·min^{-1}, particles larger than 100,000 S were retained in a final volume of about 800 mL. Aliquots (50 mL) of the cell concentrate were poured into a glass petri dish (15 cm diameter), exposed to ultraviolet (UV) radiation (254-nm wavelength, Philips fluorescent tube, type 57415 P/40 A6 T UV 15W, 40 cm distance) for 15, 30, 60, and 120 s, and then stored overnight in the dark at 4° C. Exponentially growing *P. pouchetii* cultures (100 mL) were inoculated with 1 mL from the UV-treated concentrates and were incubated for 3 days at the conditions already described. Lysed cultures (1 mL) were used to inoculate a new set of exponentially growing *P. pouchetii* cultures (100 mL), and this was later repeated once a week to maintain the virus isolate.

The host range of the virus was tested by adding 1 mL of lysate to triplicate cultures (100 mL) of 12 different exponentially growing strains of *Phaeocystis* (Table 1). The cultures were monitored daily for evidence of lysis by cell counts. Measurements were compared to control cultures to which viruses were not added. Cultures that were not lysed after 14 days were considered to be unsuitable hosts for this virus.

Transmission electron microscopy. Samples fixed with 2.5% glutaraldehyde were harvested and prepared for total counts of virus for transmission electron microscopy as described in Bratbak and Heldal (1993). Viral particles were counted in 20–50 fields at 100,000× magnification in a JEOL 100CX transmission electron microscope.

Samples for thin sectioning of cells were prefixed with 2.5% glutataldehyde, concentrated by centrifugation, postfixed with 2% OsO_4 in Na-cacodylate buffer (pH 7.3) for 1–2 h, dehydrated in a graded ethanol series, and embedded in LR White acrylic resin (Agar Scientific). Thin sections were poststained with 2% uranyl acetate.

TABLE 2. *Literature data on growth and reproduction of algal viruses.*

Host algae	Latent period (h)	Burst size	Reference
Chlorella	3–4	200–350	van Etten et al. 1991
Micromonas pusilla	7–14	70	Waters and Chan 1982
Chrysochromulina brevifilum	nd[a]	>320	Suttle and Chan 1995
Emiliania huxleyi	nd	350–700	Bratbak et al. 1996
Phaeocystis pouchetii	12–18	350–600	This paper

[a] nd = not determined.

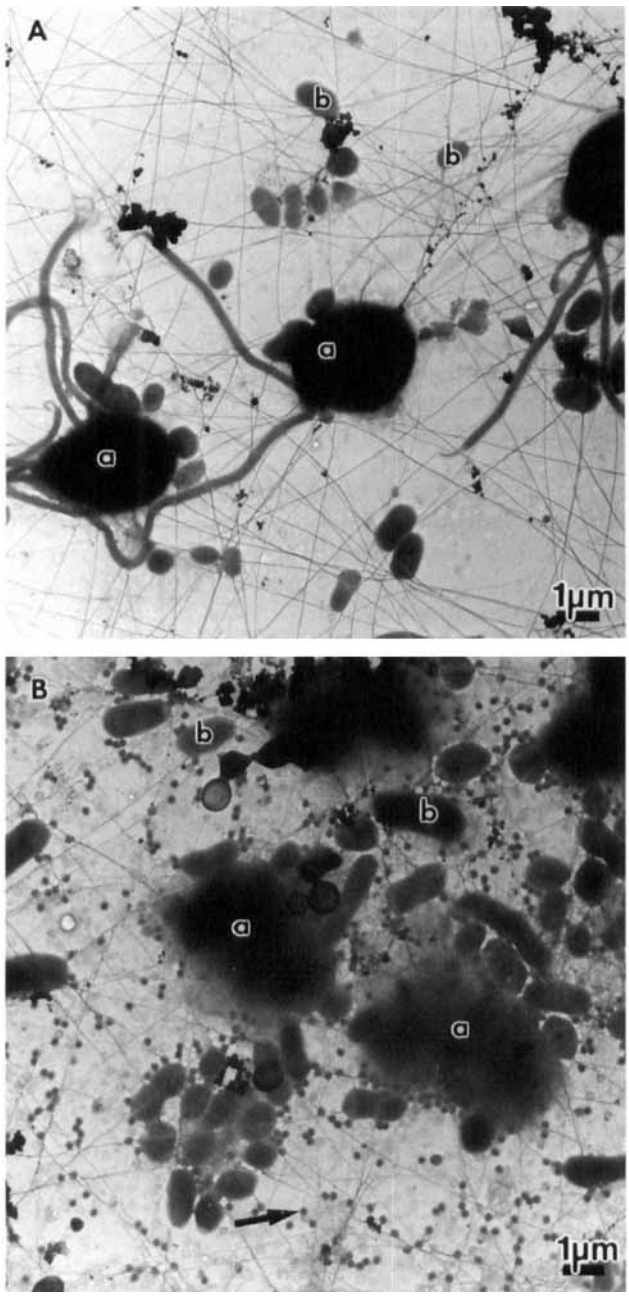

FIG. 2. Transmission electron micrographs of a *Phaeocystis pouchetii* culture infected with PpV01 viruses. A) Time zero. Healthy cells (a), no viruses and few bacteria (b). B) 48 h after adding ca. 10 viral particles per cell. The cells (a) are embedded in organic material. Viral particles (arrow) are abundant and bacteria (b) flourish.

Density centrifugation and protein analysis. Viruses were harvested from the lysate of a 1-L *Phaeocystis pouchetii* culture by polyethyleneglycol 6000 (PEG) precipitation. The salt concentration of the lysate was increased to 1 M by the addition of NaCl, and the lysate was then centrifuged at 1800 × *g* for 5 min to remove cellular debris. PEG (100 g·L^{-1}) was dissolved by slow stirring at room temperature for 1 h, and the suspension was left overnight at 4° C. The PEG was then pelleted by centrifugation at 10,000 × *g* for 20 min and resuspended in a few milliliters of f/2 medium. CsCl was added to give a final density of 1.5, and the suspension

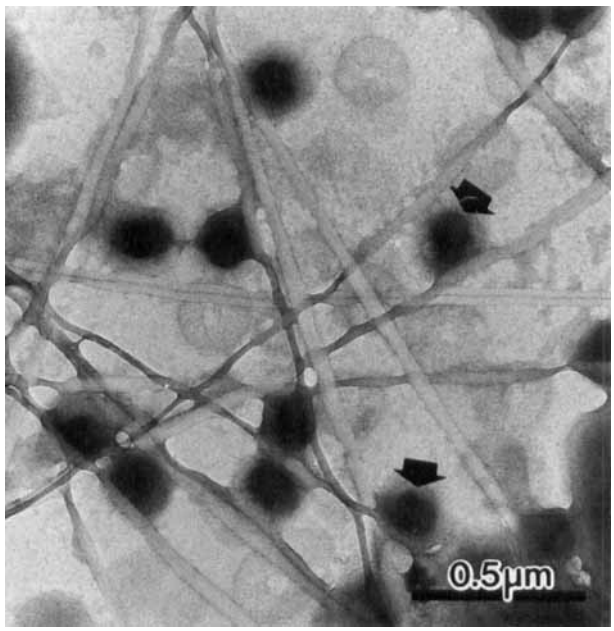

FIG. 3. Transmission electron micrographs of free viral particles (arrows) and scales entrapped in mucilage.

was centrifuged at 120,000 × *g* for 40 h. The virus band was removed with a syringe, and the density was determined with a refractometer. The viruses were concentrated by centrifugation in an Amicon microconcentrator (MW cut off 30,000) and washed twice with f/2 medium to remove the CsCl.

Viruses were disintegrated by boiling for 5 min in sample buffer, and the proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (12% polyacrylamide, 200 V) using a Mini Protean II Dual Slab Cell (Bio-Rad). The gel was silver-stained (Bio-Rad Silver Stain kit), and SDS-PAGE standards (Bio-Rad, 10,000–100,000 MW) were used for molecular weight calibration.

RESULTS AND DISCUSSION

Isolation of the virus. A virus infecting and lysing *Phaeocystis pouchetii* (PpV01) was propagated from the plankton concentrates exposed to UV light for 15 and 30 s. The same approach was used to isolate a virus infecting *Emiliania huxleyi* (Bratbak et al. 1996). Although this method obviously is useful for isolating algal viruses, the mechanism by which it works is uncertain. Concentrating the host population may increase the infection rate of lytic viruses and thus cause the number of viruses to increase to a level that may be detected by inoculation of a pure host culture. Exposure to UV light is intended to cause induction of virus production in algae carrying proviruses, but we had no evidence that the algae indeed were carrying any virus. The third possibility is, of course, that the virus concentration in the sample was unaffected by these treatments and that a virus could have been isolated by adding untreated seawater to a pure algal culture.

Our approach for isolating viruses has been to concentrate the host population while other authors have concentrated the virus population (Suttle et al.

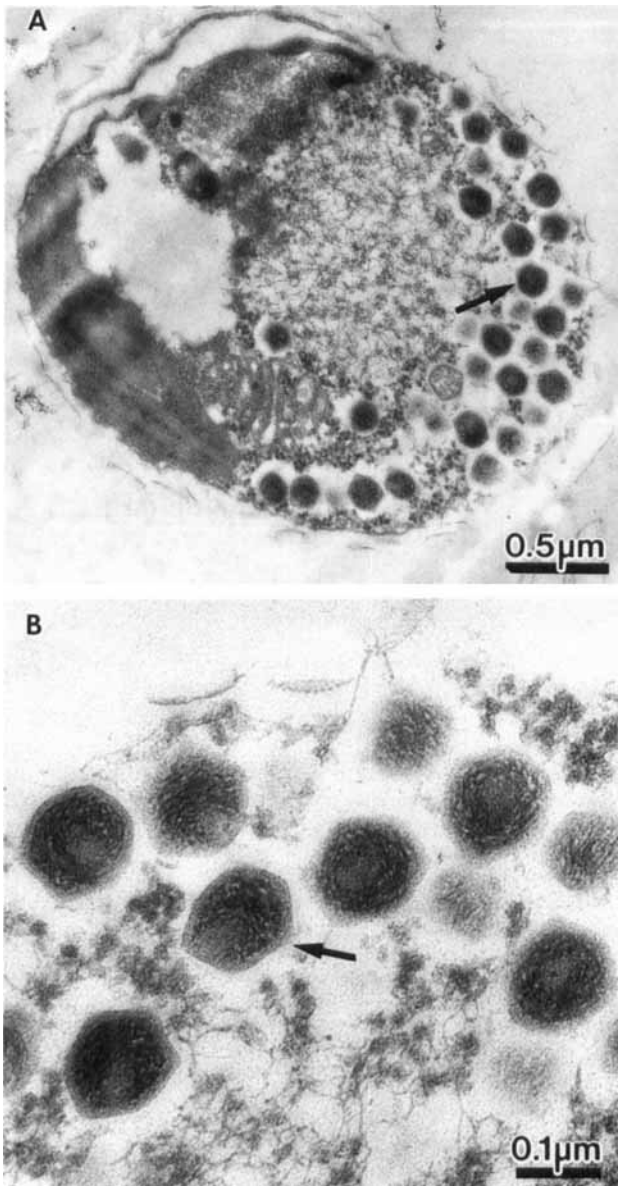

FIG. 4. Thin section of a *P. pouchetii*. A) A cell 48 h after adding PpV01 viruses to the culture showing the localization of the viral particles (arrow) in the cytoplasm. B) Closeup of intracellular viral particles (arrow).

1990, 1991, Milligan and Cosper 1994). The advantage of our approach is that algal cells may be more easily concentrated with high efficiency than viral particles and that we may have a better chance of including temperate viruses in addition to lytic viruses.

Infection cycle. When exponentially growing cultures of *P. pouchetii* were inoculated with ca. 10 virus particles per algal cell, viral abundance first increased after 12–18 h, and lysis was almost complete after 48 h (Fig. 1). The initial decrease in cell number was not accompanied by any increase in viral abundance, so it is possible that some cells lysed shortly after being infected without producing mature viruses. The viral abundance in our experiments was estimated as total counts. Cottrell and Suttle (1995) found that the infectivity of the *Mp*V-SP1 virus of *Micromonas pusilla* was about 20%, and van Etten et al. (1991) estimated the infectivity of the PBCV-1 virus of *Chlorella* to be 25–50%. Thus, if the infectivity of the *P. pouchetii* virus PpV01 was comparable to that of other algal viruses, the multiplicity of infection in our experiments was estimated to be 2–5.

The length of the latent period and the time required for complete lysis of infected cultures for this virus is comparable to that of *Micromonas* viruses. It is longer than that of *Chlorella* viruses and shorter than that reported for other algal viruses (Table 2). These parameters, as well as the burst size of the cells (Table 2), may, however, be expected to vary with the growth and physiological conditions of the cells, so differences should be interpreted with care until more data on growth and reproduction of algal viruses are available.

As the virus infection was completed bacteria flourished on the organic material released from the lysed cells (Fig. 2B). This, in addition to the possibility that viral particles might have been trapped and hidden in organic material surrounding lysed cells (Figs. 2B, 3), may have caused the viral counts to be low. The burst size estimated from decrease in cell counts and increase in viral counts (Table 2) may therefore have been underestimated.

Thin-sectioned cells revealed that the fraction of cells containing visible virus particles was 10–20% from 12 to 36 h postinfection (Fig. 1). At 42 h, when almost no cells remained in the culture, the fraction was as high as 60%. The fraction of cells observed to contain virus particles throughout the lytic cycle was low even though all cells were infected and produced virus. This may to some extent be explained by the probability of observing intracellular viral particles in thin sections of cells. It is, however, also possible that the time required for biosynthesis and maturation of virus varies in the infected population, depending on the physiological condition and cell cycle stage of the individual cells.

Description of the PpV01. Free viral particles were 130–170 nm in diameter, and they had no distinct appendages or tail structure (Figs. 3, 4). In thin sections, they were 130–160 nm in diameter and had a hexagonal outline suggesting icosahedral symmetry (Fig. 4). The particles had a distinct capsid surrounding a heavily stained internal region, which appeared to be open and less stained in the center. This lighter stained center was characteristic and could also be observed on free viral particles. The viral particles were located in the cytoplasm of infected cells and, as viral production progressed, disruption of organelles and breakdown of the cell wall were evident (Fig. 4A).

Staining culture lysates with the DNA-specific stain 4′,6-diamidino-2-phenylindole, or DAPI (Porter and

Feig 1980), revealed that the genome of the virus is double-stranded DNA. The virus band formed after CsCl centrifugation had a density of 1.4. It was in some cases diffuse, indicating that the viruses could be associated with more or less organic material of similar density such as polysaccharides. We found one major polypeptide with a molecular weight of 59 kDa and at least five minor polypeptides with molecular weights between 30 and 50 kDa. The virus appears to be quite specific for *Phaeocystis pouchetii*, as none of the *P. globosa*, *Phaeocystis* sp., and *P. antarctica* strains tested lysed (Table 1).

The ecological significance of viruses infecting and lysing *Phaeocystis* is unknown, but the virus was isolated from the end of a bloom so it is tempting to suggest a causal relation. Cell lysis releases proteins and other polymers that may cause foaming, and *Phaeocystis* is notoriously known for its foam formation at the European continental coast (Lancelot et al. 1987, 1994). The possible connection between collapse of blooms, virus infection, and foam formation should thus be investigated. The cells we used as hosts for isolation and propagation of virus in cultures were free-living flagellates. In natural waters *Phaeocystis* often forms gelatinous colonies, and we do not know whether or not the cells in these colonies are susceptible to viral infection. It may be that the gelatinous material serves as a protection against viral infection.

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