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Genetic Diversity of Algal Viruses Which Lyse the Photosynthetic Picoflagellate *Micromonas pusilla* (Prasinophyceae)†

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The genetic similarity among eight clones of *Micromonas pusilla* virus (*Mp*V) isolated from five geographic locations was measured by DNA hybridization. Our objective was to explore the existence of genetically distinct populations of *Mp*V by comparing the similarity among *Mp*Vs isolated from a single water sample to the similarity among viruses isolated from geographically distant locations. The highest and lowest similarities we observed were 70% ± 1.1% (mean ± standard error [SE], $n = 3$) for virus strains SP1 and SP2 isolated from a California coastal water sample and 13% ± 1.9% for strains SP2 and PB6; the latter was isolated from New York estuarine water. However, the similarity between *Mp*V isolated from a single water sample was not always greater than the similarity between viruses isolated from different locations. Viruses PB7 and PB8 were isolated from a single New York estuarine sample but were only 16% ± 0.5% similar, whereas PB7 was quite similar (43% ± 2.9%) to PL1, a virus from Texas coastal water. Overall, the similarity among *Mp*Vs isolated from a single geographic location, 34% ± 12.6% (mean ± SE, $n = 4$), was not significantly different from the similarity among *Mp*Vs isolated from geographically distant locations, 26.6% ± 2.7% (mean ± SE, $n = 24$) ($P = 0.92$, Mann-Whitney U test). Clones of *Mp*V were more similar to each other than they were to the related algal virus PBCV-1, and three groups of *Mp*Vs consisting of (i) PL1, SG1, PB6, and PB7, (ii) PB8, and (iii) GM1, SP1, and SP2 were resolved. The genetic variation among *Mp*Vs isolated from a single water sample was as large as the variation between viruses isolated from different oceans. If *Mp*Vs within a geographic location share genetic characteristics not shared with *Mp*Vs from geographically distant locations, this was not reflected in the overall similarity of their genomes.

A diverse assemblage of viruses coexists with the microbes responsible for the bulk of primary production, nutrient regeneration, and respiration in marine food webs. Viruses in this assemblage infect each of the ecologically important groups of microbes, e.g., eukaryotic algae (3, 14, 25), cyanobacteria (23, 26), heterotrophic bacteria (15), and heterotrophic nanoflagellates (6). Consequently, viruses are an integral component of marine microbial food webs, causing a moderate amount of mortality of their microbial hosts (4, 16, 22, 24).

The phenotypic and genotypic diversity of virus populations is linked to their interaction with host populations. Consider the diverse host specificities of viruses which infect cyanobacteria, i.e., cyanophages (23, 26). Some strains of cyanophage have a very limited host range, giving them the potential to influence the composition of cyanobacterial populations without greatly influencing the overall abundance of cyanobacteria, whereas others infect many strains of cyanobacteria, allowing them to influence a larger portion of the population and thus potentially affect both the composition and overall abundance of cyanobacterial populations. An understanding of both the phenotypic diversity and the underlying genotypic diversity of virus populations should contribute to our understanding of the interaction between viruses and their host populations in nature.

Viruses known to lyse marine eukaryotic phytoplankton have narrow host ranges. *Chrysochromulina brevifilum* virus lysed only 2 (*C. brevifilum* and *C. strobilus*) of 10 *Chrysochromulina* species screened (25). The subject of this study, *Micromonas pusilla* virus (*Mp*V), appears to be specific for *M. pusilla*, as it lysed only this species among the 46 isolates of algae representing 35 species from five algal classes tested (13).

In contrast to their narrow host range, eight genetically distinct clones of *Mp*V possessing phenotypic variation in the molecular weights of their major capsid proteins were isolated from widely separated geographic locations, including the Gulf of Mexico and coasts of the Atlantic and Pacific Oceans (3). Furthermore, studies suggest that *M. pusilla* clones are phenotypically variable as well. Selenium enrichment enhanced the growth of an oceanic but not a coastal clone of a *Micromonas* sp. (20), and an immunofluorescence assay using antibodies against *M. pusilla* isolated from Woods Hole, Mass., had low cross-reactivity with *M. pusilla* from slope water of the Northwest Atlantic, the Gulf of Mexico, and the English Channel (19). The interaction between *Mp*V and *M. pusilla* in nature likely involves phenotypically and genotypically diverse virus and host populations.

In this study we explored the possibility that populations of *Mp*V which can be distinguished on the basis of their genotypic similarity exist. We measured the genetic similarity among *Mp*Vs isolated from five geographically distant locations to characterize the amount of genetic variation among *Mp*Vs and to determine if the similarity among *Mp*Vs isolated from a single geographic location is higher than the similarity among *Mp*Vs isolated from geographically distant locations.

MATERIALS AND METHODS

Virus clones. Virus clones PB6, PB7, and PB8 were isolated from Peconic Bay, New Suffolk, N.Y.; SP1 and SP2 were isolated from the coastal Pacific Ocean adjacent to Scripps Pier, La Jolla, Calif.; GM1 was isolated from the oligotrophic

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TABLE 1. Similarities among *Mp*V clones

Target	% Similarity with the following probe[a]:							
	PB6	PB7	PB8	SP1	SP2	GM1	PL1	SG1
PB6	100	36 ± 1.6	17 ± 1.5	19 ± 1.6	13 ± 1.9	17 ± 0.3	43 ± 1.6	42 ± 2.5
PB7	71 ± 4.9	100	16 ± 0.5	22 ± 2.9	16 ± 2.4	22 ± 0.6	43 ± 2.9	38 ± 2.6
PB8	57 ± 2.5	35 ± 1.4	100	26 ± 0.8	19 ± 1.9	27 ± 0.6	42 ± 0.5	43 ± 3.1
SP1	27 ± 1.2	16 ± 5.1	14 ± 0.1	100	70 ± 1.1	51 ± 0.8	24 ± 0.5	23 ± 0.7
SP2	28 ± 1.3	25 ± 0.9	18 ± 0.9	119 ± 4.0	100	53 ± 0.5	32 ± 1.3	30 ± 2.0
GM1	29 ± 1.1	30 ± 0.8	17 ± 0.5	63 ± 3.0	50 ± 1.7	100	30 ± 1.1	24 ± 1.4
PL1	73 ± 2.5	44 ± 1.9	22 ± 0.7	23 ± 0.2	17 ± 0.2	20 ± 0.2	100	54 ± 2.0
SG1	61 ± 4.2	41 ± 1.0	23 ± 0.8	27 ± 2.5	19 ± 0.8	19 ± 0.5	67 ± 2.2	100

[a] Values are means ± standard errors ($n = 3$).

Gulf of Mexico (26°19.5′N, 89°59.2′W); PL1 was isolated from the Marine Science Institute Pier, Port Aransas, Tex.; and SG1 was isolated from the Strait of Georgia, British Columbia, Canada. The protocol for virus isolation and general oceanographic characteristics of these environments were previously reported (3).

Virus DNA. *Mp*V was amplified by using *M. pusilla* (Butcher) (12) strain Plymouth 27 grown in ESAW (7) modified by the addition of Tris · HCl and selenium as described by Cottrell and Suttle (3). Cultures were grown at 18°C under a continuous quantum flux of 70 μmol of quanta m^{-2} s^{-1} photosynthetically active radiation. Preparation of 15-liter lysates was accomplished by sequential virus amplification using 40-ml, 1-liter, and 15-liter cultures. Cultures were initiated with 1×10^6 cells ml^{-1}, and virus addition was made ca. 2 days later when the cultures contained 4×10^6 to 6×10^6 cells ml^{-1} (ca. 17% of maximum culture yield, 3×10^7 cells ml^{-1}). The 40-ml cultures were infected with ca. 2 infective viruses per host cell by adding 5 ml of a virus stock (ca. 10^8 infective *Mp*V particles ml^{-1}); culture lysis was complete 2 to 3 days after virus addition. Two lysed 40-ml cultures were added to a 1-liter culture which also lysed after 2 to 3 days. Similarly, two of the lysed 1-liter cultures were added to the 15-liter culture, which lysed after 1 to 2 days, yielding a lysate containing ca. 10^8 infective *Mp*V particles ml^{-1}.

The 15-liter lysate was pressure filtered (5 to 10 lb/in^2) through a 142-mm-diameter borosilicate microfiber filter, grade GC50 (Micro Filtration Systems, Dublin, Calif.). The virus particles were then collected from the filtrate with a 30,000-molecular-weight-cutoff ultrafiltration cartridge, type S1Y30 (Amicon, Beverly, Mass.), and the retentate (200 ml) was centrifuged at 7,700 rpm (7,087 × *g*) for 10 min in an SS34 rotor (Sorvall) to pellet the small amount of bacteria and cell debris not retained by the prefilter. The viruses were pelleted from the supernatant by centrifugation at 17,000 rpm (38,400 × *g*) for 60 min in an AH-629 rotor (Sorvall). The pellets were resuspended in 200 μl of 50 mM Tris · HCl and then digested with DNase I (10 U μl^{-1}) and RNase A (1 U μl^{-1}) at room temperature for 3 h. The sample was layered on a linear sucrose step gradient (20, 30, 40, and 50%) made up with 50 mM Tris · HCl (pH 7.8) and centrifuged for 60 min at 20,000 rpm (50,445 × *g*) in an SW40 rotor (Beckman). The region of the gradient containing the virus particles (mostly within the 30% step) was pipetted from the gradient and diluted about 10-fold with 50 mM Tris · HCl (pH 7.8), and the virus particles were again collected by ultracentrifugation in the AH-629 rotor. The virus pellet was resuspended in 1 ml of 50 mM Tris · HCl (pH 7.8); incubated for 15 min at 68°C with 0.1% sodium dodecyl sulfate (SDS) and 5 mM EDTA (pH 8.0) to disrupt the viral proteins; and then extracted once with phenol, twice with phenol-chloroform (1:1), and once with chloroform. The final aqueous phase was adjusted to 1 M sodium chloride (5), and the DNA was precipitated with the addition of two volumes of 100% ethanol and overnight incubation at −20°C. The DNA precipitate was collected by centrifugation (12,000 × *g*, 30 min, 4°C), the supernatant was removed, and the remaining traces of sodium chloride were removed with two centrifuge tube volumes of 70% ethanol. The DNA was dried under a vacuum (<5 min) and dissolved in distilled water. The concentration of DNA was determined from the A_{260} (1 A_{260} unit = 50 μg of double-strand DNA ml^{-1}).

PBCV-1 DNA was provided by J. Van Etten, University of Nebraska, Lambda phage DNA was obtained from BRL Life Technologies, Inc., Gaithersburg, Md.

Probe DNA. Probe DNA was labeled with [α-^{35}S]dATP by using a nick translation reaction containing 20 μM unlabeled dCTP, dGTP, and dTTP; 1.75 μM unlabeled dATP; 10^8 dpm of [α-^{35}S]dATP (ca. 1,380 Ci/mmol) (NEN Products, Boston, Mass.); 1.0 μg of *Mp*V DNA; and 2 U of polymerase I-DNase I (no. 18162-016; BRL Life Technologies, Inc.). The 50-μl reaction mixture was incubated at 15°C for 90 min, and the reaction was terminated by adding 5 μl of 300 mM EDTA (pH 8.0). The labeled DNA was separated from the unincorporated nucleotides by using two iterations of ethanol precipitation (0.25 M sodium acetate, pH 7.0; 70% ethanol, 0°C, 10 min), centrifuged (12,000 × *g*, 30 min, 4°C), and dried under a vacuum (<5 min). The probe DNA was resuspended in 50 μl of distilled water and denatured by incubation at 95°C for 10 min.

Target DNA. Triplicate target DNA samples (0.5 μg) were denatured by incubation in 0.4 M NaOH plus 0.5 M EDTA (pH 8.0) at 95°C for 10 min. The denatured DNA was collected by vacuum filtration with a slot blot filtration manifold onto a nylon filter (Zeta-Probe, catalog no. 162-0165; Bio-Rad) prewetted with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M trisodium citrate dihydrate, pH 7.0). After each sample was rinsed with 200 μl of 2× SSC the filter was air dried (80°C, 30 min). The DNA was bound to the filter by using UV light (120 mJ cm^{-2}) (UV cross-linker, FB-UVXL-1000; Fisher Biotech). The filter was divided into three pieces, each containing a single replicate sample of each target DNA. Each piece was wetted with distilled water and placed in a separate glass tube in preparation for hybridization.

Hybridization. Incubations were carried out in a hybridization incubator with a rotating rack (model 310; Robbins Scientific). The filters with bound DNA were incubated with prehybridization solution (6× SSC, 0.5% SDS, 5× Denhardt's reagent [50× Denhardt's reagent contains 5 g of Ficoll (type 400; Pharmacia), 5 g of polyvinylpyrrolidone, 5 g of bovine serum albumin (fraction V; Sigma), and H_2O to 500 ml], and 100 μg of denatured, fragmented salmon sperm DNA [Sigma type III sodium salt] ml^{-1}) at 65°C for 6 h. Probe DNA (125 pg, ca. 10^7 dpm) was added, and incubation was continued for 24 h at 65°C. After hybridization the filters were washed twice in each of the following: 2× SSC (5 min at room temperature), 2× SSC plus 0.5% SDS (30 min at 65°C), and 0.1× SSC (5 min at room temperature). The filters were dried overnight at room temperature, and rectangles (18 by 9 mm) containing each target DNA sample were cut from the filters. The radioactivity associated with each target DNA was measured by liquid scintillation counting with 3a70B cocktail (catalog no. 111154; Research Products International).

Calculation of similarities. The similarity between probe and target DNAs was calculated as follows: $S_{A \cdot B}\% = 100 \times [(dpm_{A \rightarrow B} - dpm_{A \rightarrow lambda})/(dpm_{A \rightarrow A} - DPM_{A \rightarrow lambda})]$, where $S_{A \cdot B}\%$ is the percent similarity between *Mp*V clones A and B, dpm is the radioactivity (disintegrations per minute) bound to the target DNAs after hybridization; and the subscripts A→B, A→A, and A→lambda denote hybridization between probe prepared from *Mp*V clone A and target prepared from *Mp*V clone B, *Mp*V clone A, and lambda phage, respectively. The similarity between probe and target composed of the same *Mp*V DNA is defined as 100%. When similarities from reciprocal hybridizations (probe and target switched) were unequal we used the lower value as the estimate of similarity between two *Mp*Vs, since the lower value is generally closer to the true similarity (10). Similarities were transformed to dissimilarities with the following equation: dissimilarity = 100 − similarity.

Data analysis. Statistical analyses were performed using SYSTAT, version 5.03 (SYSTAT, Inc., Evanston, Ill.). The neighbor-joining algorithm in MEGA, version 1.0 (8), was used to construct the phenogram from the dissimilarities. Starting with a starlike tree, the neighbor-joining algorithm performs an iterative clustering of operational taxonomic units chosen to minimize the total branch length of the phenogram (18).

RESULTS

Reciprocal hybridizations (probe and target switched) usually yielded different estimates of the similarity between *Mp*V clones. The higher estimate was on average 1.6 times the lower one, the largest inequality being a 3.4-fold difference between reciprocal hybridizations of PB6 and PB8 (Table 1). In addition, the higher similarity from the reciprocal hybridization of SP1 and SP2 exceeded 100% (119% ± 4%; similarities are means ± standard errors). The coefficient of variation [(standard deviation/mean) × 100] of the similarities estimated by using triplicate target DNA samples bound to three separate pieces of membrane incubated in separate containers was 8.8% (mean, $n = 56$).

The amount of radioactivity that was bound by using probes

TABLE 2. Rank order of similarities among *Mp*V clones calculated from the lower similarity obtained by reciprocal hybridizations

*Mp*V clones	% Similarity	Type of comparison[a]	Rank
PB6 and SP2	13	a	1
PB8 and SP1	14	a	2
PB7 and PB8	16	w	4
PB7 and SP1	16	a	4
PB7 and SP2	16	a	4
PB6 and PB8	17	w	7.5
PB6 and GM1	17	a	7.5
PB8 and GM1	17	a	7.5
SP2 and PL1	17	a	7.5
PB8 and SP2	18	a	10
PB6 and SP1	19	a	12
SP2 and SG1	19	a	12
GM1 and SG1	19	a	12
GM1 and PL1	20	a	14
PB7 and GM1	22	a	15.5
PB8 and PL1	22	a	15.5
PB8 and SG1	23	a	18
SP1 and PL1	23	a	18
SP1 and SG1	23	a	18
PB6 and PB7	36	w	20
PB7 and SG1	38	a	21
PB6 and SG1	42	a	22
PB6 and PL1	43	a	23.5
PB7 and PL1	43	a	23.5
SP2 and GM1	50	a	25
SP1 and GM1	51	a	26
PL1 and SG1	54	a	27
SP1 and SP2	70	w	28

[a] a, among-location comparison; w, within-location comparison.

and targets composed of DNA from different *Mp*V strains was ≥18% of the amount of radioactivity that was bound when the probe and target were composed of DNA from the same strain of *Mp*V (Table 1). In contrast, the amounts of *Mp*V probe radioactivity that bound to lambda phage DNA and an area of filter with no DNA addition were 7% ± 1.9% and 7% ± 2.2% ($n = 8$), respectively, and were not significantly different ($P = 0.65$, paired comparisons t test). Similarly, the amount of radioactivity that bound to PBCV-1 DNA was 6% ± 2.3% ($n = 8$) and was not significantly different from the amount that bound to the blank (lambda phage DNA) ($P = 0.47$, paired comparisons t test).

Given that the lower similarity obtained in a reciprocal hybridization is closer to the true similarity (10), the highest similarity observed was between *Mp*V clones SP1 and SP2 (70% ± 1.1%; $n = 3$) isolated from a California coastal water sample, whereas the lowest similarity (13% ± 1.9%) was between clones SP2 and PB6. PB6 was isolated from New York estuarine water. However, the similarity between viruses isolated from a single water sample was not always greater than the similarity between viruses isolated from different locations. Viruses PB7 and PB8 were isolated from a single New York estuarine sample but were only 16% ± 0.5% similar, whereas PB6 and PB7 were quite similar (43% ± 1.6% and 43% ± 2.9%, respectively) to PL1, a virus from Texas coastal water. The similarities among the *Mp*V clones isolated from a single geographic location ranked 4th 7.5th, 20th, and 28th among the 28 similarities measured (Table 2). The similarity among *Mp*V clones isolated from a single geographic location, 34% ± 12.6% ($n = 4$), was not significantly different from the similarity among *Mp*V clones isolated from geographically distant locations, 26.6% ± 2.7% ($n = 24$) ($P = 0.92$, Mann-Whitney U test).

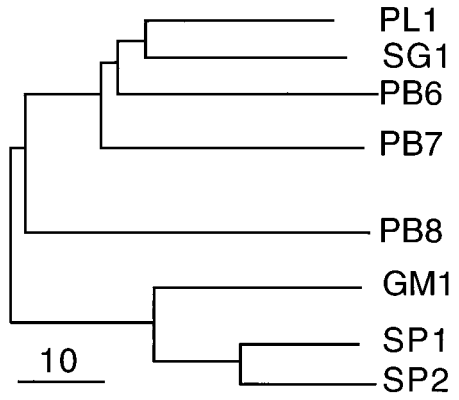

FIG. 1. Phenogram of MPV clones. The scale bar represents a dissimilarity of 10 (dissimilarity = 100 − similarity).

A two-dimensional representation of the affinities among the *Mp*V clones revealed three groups: (i) PL1, SG1, PB6, and PB7; (ii) PB8; and (iii) GM1, SP1, and SP2 (Fig. 1).

DISCUSSION

Inequality between reciprocal hybridizations was not unique to this study (9–11). In a study of the species composition of natural bacterioplankton assemblages, Lee and Fuhrman (10) also observed inequality between reciprocal hybridizations while examining the ability of DNA hybridization to accurately quantify DNA similarity. DNAs from single bacterial species were combined in various proportions to create mixtures with a range of similarities, and the observed similarity measured by hybridization between mixtures was compared with the known similarity between mixtures. DNA hybridization usually produced estimates close to the known similarities (i.e., within 15% or 1 standard error), and reciprocal hybridizations generally agreed unless the species in common between two mixtures was a large fraction of one mixture and a small fraction of the other mixture. In this case reciprocal similarities were unequal and the lower value was closer to the true similarity. The higher value of a reciprocal hybridization results from the species in common being more abundant in the target than in the probe.

Inequality of reciprocal hybridizations between *Mp*V DNAs suggests that the sequences in common between *Mp*V clones were more abundant in the genomes of some clones than in others. This suggests that *Mp*V genomes likely contain repeated sequences which are very similar among clones and that the number of times the sequences are repeated varies among clones. Although direct evidence of repeated genes is not available for *Mp*V, this does occur in related algal viruses. The genome of a large double-strand DNA virus (PBCV-1) which infects a *Chlorella*-like alga and is likely related to *Mp*V (30) contains 13 tandem repeats of 61 to 65 amino acids in the gene coding for Vp260, a glycoprotein located on the surface of PBCV-1 (17). A modified form of the Vp260 gene in a spontaneously derived PBCV-1 antiserum-resistant mutant contained both deletions and duplications of repeat sequences, causing the number of repeats to differ between the wild-type and mutant viruses. In addition, PBCV-1 has 2.2-kb inverted terminal repeat sequences (21) and repeated sequences in the form of DNA methylation and possibly DNA restriction enzyme pseudogenes (28). The genomes of *Mp*V clones contain

methylated bases, and their abundance varies among clones from undetectable levels to 8.6% 5-methylcytosine and 3.9% *N*-6-methyladenine (29). It is very likely that *Mp*V genomes contain genes and pseudogenes for DNA methylation and DNA restriction enzymes as well, and these may contribute to the pool of repeated sequences in *Mp*V genomes.

The wide range of similarities we measured among *Mp*V clones did not fall into groups of high within-location similarities and low among-location similarities. Some isolates of *Mp*V from widely separated geographical locations (e.g., PL1 and SG1, 54% similar) were more similar than isolates from a single location (e.g., PB7 and PB8, 16% similar). Examination of the rank order of the similarities revealed within-location similarities distributed uniformly across the among-location similarities (Table 2), and the phenogram revealed some closer affinities among isolates from distant locations than among isolates from the same location (e.g., PL1 and SG1 versus PB8 and PB6). If isolates from within a geographic location share genetic characteristics that they do not share with *Mp*V from distant locations, this was not reflected in the overall similarities of their genomes. One might anticipate that nucleotide sequence analysis of the highly variable region of a gene would distinguish within- from among-location similarities, yet preliminary analysis of 400 bases of a variable domain of the DNA polymerase gene of these *Mp*V clones also failed to reveal higher within-location similarities (2).

The inferred amino acid sequence of the entire DNA polymerase gene of *Mp*V SP1 contains strings of 11 and 9 amino acids which are conserved among *Micromonas* and *Chlorella* viruses but were not found in any other sequences in GenBank, which suggests that these two groups of viruses have a common ancestor (30). These viruses are probably only distantly related, since no specific hybridization was detected between DNAs from *Mp*V and PBCV-1; clones of *Mp*V are clearly more closely related to each other than they are to PBCV-1. However, the genetic differences among *Mp*V clones are as large as the differences among genera of bacteria. The range of similarities among clones of *Mp*V (13 to 70%) overlaps the similarities among strains of bacterial species (60 to 70%) and the similarities among genera of bacteria (<20%) (1).

It is not understood how genetically distinct clones of *Mp*V coexist in a single geographic location (e.g., PB6, PB7, and PB8 in Peconic Bay). Coexistence of *Mp*V clones may indicate that there is no competition among these genotypes, that the different genotypes are phenotypically equivalent, and/or that no genotype is competitively superior to another. However, the potential for competition among *Mp*V clones is high, since growth of *Mp*V populations in nature is likely limited by the abundance of *M. pusilla*. A study of the dynamics of *Mp*V and its effect on *M. pusilla* populations implied an *Mp*V production rate of 0.79 day^{-1} (4). This rate is much lower than the maximum production rate of *Mp*V during a one-step growth experiment (7.0 day^{-1}) calculated by using Figure 2 of reference 27. Any clone in a population of *Mp*V having the ability to increase its successful encounter with and adsorption to *M. pusilla* would grow faster and should dominate. The high diversity of *Mp*V suggests that competition among clones of *Mp*V is restricted.

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REFERENCES

1. **Brock, T. D., and M. T. Madigan.** 1991. Biology of microorganisms, 6th ed. Prentice-Hall, Englewood Cliffs, N.J.
2. **Chen, F., and C. A. Suttle.** Unpublished data.
3. **Cottrell, M. T., and C. A. Suttle.** 1991. Wide-spread occurrence and clonal variation in viruses which cause lysis of a cosmopolitan, eukaryotic marine phytoplankter, *Micromonas pusilla*. Mar. Ecol. Prog. Ser. **78:**1–9.
4. **Cottrell, M. T., and C. A. Suttle.** 1995. Dynamics of lytic viruses: the effect of *Micromonas pusilla* virus on natural populations of the photosynthetic picoflagellate *Micromonas pusilla*. Limnol. Oceanogr. **40:**730–739.
5. **Fang, G., S. Hammar, and R. Grumet.** 1992. A quick and inexpensive method for removing polysaccharides from plant genomic DNA. BioTechniques **13:**52–65.
6. **Garza, D. R., and C. A. Suttle.** 1994. Isolation of lytic viruses which infect a marine heterotrophic nanoflagellate, p. a-29. *In* Abstracts of the American Society of Limnology and Oceanography and the Phycological Society of America Joint Meeting 1994. American Society of Limnology and Oceanography, Inc., Lawrence, Kans.
7. **Harrison, P. J., R. E. Waters, and F. J. R. Taylor.** 1980. A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. J. Phycol. **16:**28–35.
8. **Kumar, S., T. Koichiero, and M. Nei.** 1993. MEGA: molecular evolutionary genetics analysis, version 1.0. Pennsylvania State University, University Park.
9. **Lambert, D. L., P. N. Taylor, and R. Goulder.** 1993. Between-site comparisons of freshwater bacterioplankton by DNA hybridization. Microb. Ecol. **26:**189–200.
10. **Lee, S., and J. A. Fuhrman.** 1990. DNA hybridization to compare species compositions of natural bacterioplankton assemblages. Appl. Environ. Microbiol. **56:**739–746.
11. **Lee, S., and J. A. Fuhrman.** 1991. Spatial and temporal variation of natural bacterioplankton assemblages studied by total genomic DNA cross-hybridization. Limnol. Oceanogr. **36:**1277–1287.
12. **Manton, I., and M. Parke.** 1960. Further observations on small green flagellates with special reference to possible relatives of *Chromulina pusilla* Butcher. J. Mar. Biol. Assoc. U.K. **39:**275–298.
13. **Mayer, J. A.** 1978. Ph.D. thesis. University of British Columbia, Vancouver, Canada.
14. **Mayer, J. A., and F. J. R. Taylor.** 1979. A virus which lyses the marine nanoflagellate *Micromonas pusilla*. Nature (London) **281:**299–301.
15. **Moebus, K.** 1991. Preliminary observations on the concentration of marine bacteriophages in the water around Helgoländ. Helgol. Meeresunters. **45:** 411–422.
16. **Proctor, L. M., and J. A. Fuhrman.** 1990. Viral mortality of marine bacteria and cyanobacteria. Nature (London) **343:**60–62.
17. **Que, Q., Y. Li, I.-N. Wang, L. C. Lane, W. G. Chaney, and J. L. Van Etten.** 1994. Protein glycosylation and myristylation in *Chlorella* virus PBCV-1 and its antigenic variants. Virology **203:**320–327.
18. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
19. **Shapiro, L. P., L. Campbell, and E. M. Haugen.** 1989. Immunochemical recognition of phytoplankton species. Mar. Ecol. Progr. Ser. **57:**219–224.
20. **Shapiro, L. P., and R. R. L. Guillard.** 1986. Physiological ecology of the marine eukaryotic ultraplankton. Can. Bull. Fish. Aquat. Sci. **214:**371–389.
21. **Strasser, P., Y. Zhang, J. Rohozinski, and J. L. Van Etten.** 1991. The termini of the *Chlorella* virus PBCV-1 genome are identical 2.2-kbp inverted repeats. Virology **180:**763–769.
22. **Suttle, C. A.** 1995. The significance of viruses to mortality in aquatic microbial communities. Microb. Ecol. **28:**237–244.
23. **Suttle, C. A., and A. M. Chan.** 1993. Marine cyanophages infecting oceanic and coastal strains of Synechococcus: abundance, morphology, cross-infectivity and growth characteristics. Mar. Ecol. Prog. Ser. **92:**99–109.
24. **Suttle, C. A., and A. M. Chan.** 1994. Dynamics and distribution of cyanophages and their effects on marine *Synechococcus* spp. Appl. Environ. Microbiol. **60:**3167–3174.
25. **Suttle, C. A., and A. M. Chan.** 1995. Viruses infecting the marine Prymnesiophyte *Chrysochromulina* spp.: isolation, preliminary characterization and natural abundance. Mar. Ecol. Prog. Ser. **118:**275–282.
26. **Waterbury, J. B., and F. W. Valois.** 1993. Resistance to co-occurring phages enables marine *Synechococcus* communities to coexist with cyanophages abundant in seawater. Appl. Environ. Microbiol. **59:**3393–3399.
27. **Waters, R. E., and A. T. Chan.** 1982. Micromonas pusilla virus: the virus growth cycle and associated physiological events within the host cells; host range mutation. J. Gen. Virol. **63:**199–206.
28. **Zhang, Y.** 1991. Ph.D. thesis, University of Nebraska, Lincoln.
29. **Zhang, Y.** Unpublished data.
30. **Zhang, Y., and C. A. Suttle.** 1994. Design and use of PCR primers for B-family DNA polymerase genes to detect and identify viruses and microbes, p. a-85. *In* Abstracts of the American Society of Limnology and Oceanography and the Phycological Society of America Joint Meeting 1994. American Society of Limnology and Oceanography, Inc., Lawrence, Kans.