Significance of algal viruses and ecology of *Phaeocystis* host-virus interactions

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ABSTRACT

The ecological significance of algal viruses is suggested by the presence of algal cells containing virus like particles, the presence of viruses infecting specific algae and the succession of phytoplankton and virus in natural aquatic ecosystems. The interaction between *Phaeocystis pouchetii* and the lytic virus PpV01 was investigated in laboratory microcosms. The experiments show that *P. pouchetii* is susceptible to virus infection in all stages of growth, and that nitrate, phosphate and light limitation of algal growth do not prevent virus reproduction and cell lysis. Inferior growth conditions caused a decrease in burst size of > 50% compared to more copious growth conditions. The length of the lytic cycle and the infectivity of the viruses was apparently not affected by the host's growth conditions. Viral infection did not affect photosynthesis until near the onset of cell lysis. Release of dissolved organic carbon was low in non-infected cultures, while in infected cultures the entire algal biomass was converted to DOC. The DOC released during viral lysis was rapidly and efficiently utilized for bacterial growth.

Introduction

Little is known about algal viruses in general and it is only during the last decade that we have begun to consider their possible ecological roles. The ecological significance of algal viruses in natural aquatic ecosystems is suggested by three differed types of observations. The first is the presence of algal cells containing virus like particles (VLPs) in natural waters. VLPs have been found in cells from about 50 different algal species representing nearly all major algal classes [16, 21, 25]. Virus infection may thus be as common for microalgae as it is for any other group of organisms. Moreover, blooms of different marine phytoplankton species such as *Emiliania huxleyi* [1, 5], *Aureococcus anophagefferens* [11, 17] and *Heterosigma akashiwo* [13,14] have been found to have a significant fraction of VLP containing cells as the blooms were about to collapse.

Methods

The second type of observation is the presence of viruses in natural waters infecting specific algae. The two first microalgal viruses (*Micromonas pusilla* virus and Chlorella virus) were isolated in the 1970's [10, 22]. At present, less than 10 different microalga host virus systems have been reported to be available in culture, but four of these have been isolated during the last five years (Table 1). With an increasing awareness that algal viruses may be important, more effort has been invested in isolating algal host-virus systems for further

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Table 1. Microalgae host virus systems currently available in culture

Host algae	Reference
Chlorophyceae	
Chlorella sp.	22
Chrysophyceae	
Aureococcus anophagefferens	11
Prasiophyceae	
Micromonas pusilla	6
Pyramimonas orientalis	12
Prymnesiophyceae	
Chrysochromulina brevifilum	19
Phaeocystis pouchetii	9
Raphidophyceae	
Heterosigma akashiwo	15

investigation in the laboratory and the number of isolated viruses will presumably increase significantly in the next few years.

The third type of observations suggesting that algal viruses may be important in natural waters is case studies where the succession of phytoplankton and free virus like particles suggest a causative relationship [1,4].

A virus (PpV-01) infecting and lysing the marine prymnesiophyte *Phaeocystis pouchetii* has recently been isolated [9]. We have conducted a series experiments to elucidate how the virus production is affected by host cells growth and nutrition and how the virus infection affects primary production, release of dissolved organic carbon and bacterial secondary production [2,3].

Results

The experimental studies show that *Phaeocystis* was sensitive to virus infection in all phases of growth (exponential-, early stationary- and late stationary - senescence-phase) and that the population was decimated within 3 days after virus infection. Nutrient deficient cultures, i.e. batch cultures entering stationary phase due to phosphorus or nitrogen limitation, showed complete lysis within 3-4 days after virus infection as did control cultures growing in complete medium. Virus infected cultures incubated in the dark lysed more slowly than the control cultures incubated in the light. When comparing total virus counts and counts of infective viral particles (i.e. most probable number) we found no apparent relationship between host cell growth condition and infectivity of the viral particles produced. The fraction of infective particles in fresh algal lysates did however show a larger variation than has been reported for other systems (Table 2).

Table 2. Infectivity of algal viruses

Host virus system	% Infectivity	Reference
Phaeocystis pouchetii - PpV01	7 - 100	3
Chlorella - PBCV-1	25 - 50	21
Micromonas pusilla - MpV-SP1	12 - 34	7

Discussion

The conclusions that can be drawn from these experiments are that *P. pouchetii* was susceptible to virus infection in all stages of growth, and that nutrient (nitrate or phosphate) or light limitation of algal growth did not prevent viral reproduction and cell lysis. The main effect of host cell growth condition on virus production appeared to be on the number of viruses produced per infected host cell. A high burst size (maximum 510) was found in exponentially growing cultures, while low burst size (minimum 15) was found in stationary phase cultures, in nutrient depleted cultures and in light limited cultures. This is qualitatively comparable to what has been found for other algal host-virus systems (Table 3) but P-deficiency does not seem to be as important as has been found for some other systems.

Table 3. Virus proliferation as a function of host cell growth condition

Host	Effect of host growth condition on virus proliferation	Reference
*Phaeocystis pou*chetii	Burst size decreased by 50-90% at inferior growth conditions.	3
Chlorella	Poor replication in stationary-phase cells.	20,21
Emiliania huxleyi	No virus production and cell lysis when phosphate was depleted.	1
Synechococcus	Length of lytic cycle increased and the burst size decreased by 80% when cell growth was phosphate limited.	24

Virus infection in *Phaeocystis pouchetii* does not seem to affect the cells photosynthetic apparatus and it allowed primary production in the cells to continue throughout most of the lytic cycle which lasts for 12-18 h [9]. In this respect, the response of *Phaeocystis* to virus infection resembled virus infection in *Synechococcus*, but it was apparently different from that of other eucaryotic algae where photosynthesis is shut down immediately or shortly after the infection (Table 4).

Accumulation of dissolved organic carbon (DOC) and bacterial biomass in cultures of *Phaeocystis* indicate that excretion amounts to 20-30% of the algae's particulate production (bacterial growth efficiency assumed to be 50%). Viral lysis of the algal cells resulted in a massive release of DOC which in terms of carbon approximated that of the entire algal biomass. Growth of bacteria succeeding cell lysis demonstrated that the DOC released was readily available for bacterial secondary production and the net effect of the virus infection was an efficient conversion of algal biomass into bacterial biomass. For viral lysis of *Aureococcus anophagefferens*, Gobler et al [8] estimated that bacterial carbon production was 22% of the total algal carbon while 15% was released to the dissolved phase. Compared to our results obtained with *Phaeocystis pouchetii* this suggests that both the amount and bioavailability of organic material released from algae due to viral lysis, and the bacterial secondary production succeeding such lysis, may vary significantly.

Table 4. Effect of virus infection on photosynthesis in different algal host virus systems

Host-virus system	Observed effect of virus infection on photosynthesis	Reference
Phaeocystis pouchetii - PpV01	Photosynthesis is active during most of the lytic cycle.	2
Micromonas pusilla - MpV	Significantly reduced within 2h after infection.	23
Chlorella - PBCV-1	CO_2 fixation inhibited almost immediately.	20
Synechococcus (BCC1) - S-BBS1	Photosynthetic rates in infected cultures were similar to those in non-infected control cultures until near the onset of cell lysis.	18

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References

1. Bratbak G, Egge JK, Heldal M (1993) Viral mortality of the marine alga *Emiliania huxleyi* (Haptophyceae) and termination of algal blooms. Mar Ecol Prog Ser 93:39-48
2. Bratbak G, Jacobsen A, Heldal M (1998a) Viral lysis of *Phaeocystis pouchetii* and bacterial secondary production. Aquat Microb Ecol 16:1-9
3. Bratbak G, Jacobsen A, Heldal M, Nagasaki K, Thingstad F (1998b) Virus production in *Phaeocystis pouchetii* and its relation to host cell growth and nutrition. Aquat Microb Ecol 16:11-16
4. Bratbak G, Levasseur, M, Michaud, S, Cantin, G, Fernández, E, Heimdal, BR, Heldal M (1995) Viral activity in relation to *Emiliania huxleyi* blooms: a possible mechanism of DMSP release? Mar Ecol Prog Ser 128:133-42
5. Brussaard C P D, Kempers, R S, Kop, A J, Riegman, R, Heldal, M (1996) Virus like particles in a summer bloom of *Emiliania huxleyi* in the North Sea. Aquatic Microb Ecol 10:105-113
6. Cottrell MT, Suttle CA (1991) Wide-spread occurrence and clonal variation in viruses which cause lysis of a cosmopolitan eucaryotic marine phytoplankter, *Micromonas pusilla*. Mar Ecol Prog Ser 78:1-9
7. Cottrell MT, Suttle, CA (1995) Dynamics of a lytic virus infecting the photosynthetic marine picoflagellate *Micromonas pusilla*. Limnol Oceanogr 40:730-739
8. Gobler CJ, Hutchins, DA, Fisher, NS, Cosper, EM, Sañudo-Wilhewlmy SA (1997) Release and bioavailability of C,N,P, Se and Fe following viral lysis of a marine chrysophyte. Limnol Oceanogr 42:1492-1504
9. Jacobsen A, Bratbak G and Heldal M (1996) Isolation and characterisation of a virus infecting *Phaeocystis pouchetii* (Prymnesiophyceae). J Phycol 32:923-927

10. Mayer JA, Taylor FJR (1979) A virus which lyses the marine nanoflagellate *Micromonas pusilla*. Nature (London) 281:299-301
11. Milligan KLD, Cosper EM (1994) Isolation of a virus capable of lysing the brown tide microalga, *Aureococcus anophagefferens*. Science 266:805-807
12. Mortimer E, Patching J W (1998) Characterisation of a microalgal virus isolated from Irish waters and initial genome studies. Algal Virus Workshop. Abstract. Bergen, Norway. June 14 - 18, 1998
13. Nagasaki K, Ando M, Imai I, Itakura S, Ishida Y (1994a) Virus-like particles in *Heterosigma akashiwo* (Raphidophyceae): a possible red tide disintegration mechanism. Mar Biol 119:307-312
14. Nagasaki K, Ando M, Itakura S, Imai I, Ishida Y (1994b) Viral mortality in the final stage of *Heterosigma akashiwo* (Raphidophyceae) red tide. J Plankton Res 16:1595-1599
15. Nagasaki K, Yamaguchi M (1997) Isolation of a virus infectious to the harmful bloom causing microalga *Heterosigma akashiwo* (Raphidophyceae). Aquat Microb Ecol 13:135-140
16. Reisser W (1993) Viruses and virus-like particles of freshwaterand marine eucaryotic algae - a review. Arch Protistenkd 143:257-265
17. Sieburth JMcN, Johnson PW, Hargraves PE (1988) Ultrastructure and ecology of *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyceae): The dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, summer 1985. J Phycol 24:416-425
18. Suttle CA, Chan AM (1993) Marine cyanophages infecting oceanic and coastal strains of *Synechococcus*: abundance, morphology, cross-infectivity and growth characteristics. Mar Ecol Prog Ser 92:99-109
19. Suttle CA, Chan AM (1995) Viruses infecting the marine Prymnesiophyte *Chrysochromulina* spp: isolation preliminary characterization and natural abundance. Mar Ecol Prog Ser 118:275-282
20. van Etten JL, Burbank DE, Xia Y, Meints RH (1983) Growth cycle of a virus, PBCV-1, that infects *Chlorella*-like algae. Virology 126:117-25
21. van Etten JL, Lane LC, Meints RH (1991) Viruses and virus-like particles of eucaryotic algae. Microbiol Rev 55:586-620
22. van Etten JL, Meints RH, Burbank DE, Kuczmarski D, Cuppels DA, Lane LC (1981) Isolation and characterisation of a virus from the intracellular green algae symbiotic with *Hydra viridis*. Virology 113: 704-711
23. Waters RE, Chan AT (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
24. Wilson WH, Carr NG, Mann NH (1996) The effect of phosphate status on the kinetics of cyanophage infection in the oceanic cyanobacterium *Synechococcus* sp WH7803. J Phycol 32:506-516
25. Zingone A (1995) The role of viruses in the dynamics of phytoplankton blooms. Giornale Botanico Italiano 129:415-423