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Acquisition of intact polar lipids from the Prymnesiophyte *Phaeocystis globosa* by its lytic virus PgV-07T

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Abstract

Recent studies showed changes in phytoplankton lipid composition during viral infection and have indicated roles for specific lipids in the mechanisms of algal virus-host interaction. To investigate the generality of these findings and obtain a better understanding of the allocation of specific lipids to viruses, we studied the intact polar lipid (IPL) composition of virally infected and non-infected cultures of the Prymnesiophyte *Phaeocystis globosa* G(A) and its lytic virus PgV-07T. The *P. globosa* IPL composition was relatively stable over a diel cycle and not strongly affected by viral infection. Glycolipids, phospholipids and betaine lipids were present in both the host and virus, although specific groups such as the diacylglyceryl-hydroxymethyltrimethyl-β-alanines and the sulfoquinovosyldiacylglycerols, were present in a lower proportion or were not detected in the virus. Viral glycosphingolipids (vGSLs), which have been shown to play a role in the infection strategy of the virus EhV-86, infecting the Prymnesiophyte *Emiliania huxleyi* CCMP374, were not encountered. Our results show that the involvement of lipids in virus-algal host interactions can be very different amongst virus-algal host systems.

1 Introduction

Phytoplankton are the primary source of a large variety of organic compounds, including lipids. In the marine food web these lipids are not only an important nutritional source of carbon, but also contain substantial amounts of phosphorus and nitrogen. Several phytoplankton species belonging to the class Prymnesiophyceae are considered important players in organic carbon and sulfur cycling worldwide (Smith et al., 1991; Arrigo et al., 1999; Stefels and van Boekel, 1993; Moon-van der Staay et al., 2000). *Phaeocystis globosa* is an ecologically important species in temperate coastal regions (Schoemann et al., 2005), and can be a substantial source of intact polar lipids (IPLs) in particulate organic matter (Brandsma et al., 2012b). This species is found in

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a single celled flagellate stage as well as in colonies, and can develop dense blooms (see review by Schoemann et al., 2005). Blooms of *P. globosa* have been reported to be terminated or even prevented by viral infection (Brussaard and Martínez Martínez, 2008). Phytoplankton viruses play a fundamental role in the marine food web. Through the lysis of their algal hosts, viruses release nutritional compounds from the cells into new pools of dissolved and particulate organic matter, a process called the viral shunt (Wilhelm and Suttle, 1999). Viral infection may also affect the cellular composition of their host. Evans et al. (2009) have shown that viral infection of the Prymnesiophyte *Emiliania huxleyi* CCMP1516 can affect the host fatty acid composition. Viruses can thus potentially alter the composition of lipids in phytoplankton.

Additionally, it has been shown that lipids can play a role in the interaction between viruses and their algal hosts as elegantly shown for the phytoplankton-virus model system *E. huxleyi* CCMP374 and its virus EhV-86 (Vardi et al., 2009). Viral infection induced the production of specific viral glycosphingolipids (vGSLs) in cells of *E. huxleyi* that seem to play a role in a programmed cell death mechanism of the host and the timing of cell lysis. These compounds are found in the virus itself as well. In a natural coccolithophore population it was shown that these lipids may be used as biomarkers for viral infection of phytoplankton (Vardi et al., 2012). Members of the Group I *P. globosa* viruses (PgVs) belong to the nucleocytoplasmic large DNA viruses (NCLDVs) (Brussaard et al., 2004). Many members of the NCLDV's are known to possess a lipid membrane (Van Etten et al., 2010), that have been found to span the inner side (Yan et al., 2005) or the outer side of the capsid (Mackinder et al., 2009). However, questions remain about the acquisition and composition of the lipids in these membranes.

In this study we investigated the composition of IPLs in the Prymnesiophyte *P. globosa*, before and during viral infection. Our aims were to detect possible changes of the host IPLs induced by the virus and, since many members of the NCLDVs possess a membrane, clarify the IPL composition of the possible viral membrane as well. Additionally, we aimed to compare our findings to specific IPL dynamics in the

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phylogenetically related phytoplankton species *E. huxleyi*, in which vGSLs play an important role in the mechanisms of viral lysis.

2 Materials and methods

2.1 Culturing conditions

Axenic cultures of *Phaeocystis globosa* G(A) (Culture collection University of Groningen, the Netherlands) were grown in batch cultures at 15 °C in a 1 : 1 mixture of f/2 medium (Guillard, 1975) and modified artificial seawater (ESAW, Harrison 1980), i.e. enriched with Tris-HCl and Na_2SeO_3 (Cottrell and Suttle, 1991). Irradiance was supplied at 90 µmol quanta $m^{-2} s^{-1}$ in a light : dark cycle of 16 : 8 h. The lytic *P. globosa* virus, PgV-07T (Baudoux and Brussaard, 2005) was derived from the virus culture collection of the Royal Netherlands Institute for Sea Research. This dsDNA virus, belonging to PgV group I with a genome size of 470 kbp and a diameter of approximately 150 nm (Baudoux and Brussaard, 2005), was cultured under the same conditions on exponentially growing *P. globosa*. Both culture and lysate were regularly checked for axenity by epifluorescence microscopy, using the nucleic acid stain 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) (Life technologies Ltd. Paisley, UK). At all times during the experiments the cultures were axenic.

2.2 Detection of viral membrane

The presence of a lipid membrane in PgV-07T was confirmed by staining fresh viruses with the lipophilic dye N-(3-triethylammoniumpropyl)-4-[4-(dibutylamino)styryl] pyridinium dibromide (FM 1–43) (Life technologies Ltd. Paisley, UK) in TE buffer (pH = 8) at a final concentration of 10 µM for 10 min. Viral particles were detected using a benchtop BD FacsCalibur equipped with a 488 nm argon laser (BD Biosciences, San Jose, USA), after setting the trigger on green fluorescence. Additionally, to test whether

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this lipid membrane was required for the infectivity of PgV, freshly produced viral lysate was treated with the effective lipid solvents diethyl ether and chloroform (98 and 99 % respectively, J. T. Baker). Separate incubations with 10 or 50 % (v/v final concentration) of either of the two compounds were performed at room temperature for 60 min. After incubation, the solvent from the samples treated with diethyl ether, was removed from the virus suspension just by evaporation (overnight at 4 °C). The chloroform treatments were centrifuged (Eppendorf 5810R, Hamburg, Germany) at 4100 g for 5 min. The aqueous upper phase was recovered and remaining traces of chloroform were removed by evaporation (overnight at 4 °C). Positive controls, i.e. viruses not treated with solvent were taken along. For all treatments, virus infectivity was tested by most probable number (MPN) endpoint dilution assay (Suttle, 1993).

2.3 Experimental procedures of host lipid dynamics

For the viral infection experiment, 2 L cultures of exponentially growing *P. globosa* (8.6×10^4 cells mL^{-1}) were used. Duplicate cultures were inoculated with fresh 0.2 µm filtered (Minisart High-Flow syringe filter; Sartorius AG, Goettingen, Germany) PgV lysate, obtaining a virus to host ratio of 55 : 1. The duplicate non-infected control cultures were inoculated with the same volume of autoclaved seawater. Samples for algal abundance (3.5 mL), PgV enumeration (1 mL) abundances, and IPL composition (150 mL) were taken at regular intervals until the cultures were completely lysed. Algal samples were fixed with a 1 % final concentration of formaldehyde : hexamine solution (18 % *v*/*v* : 10 % *w*/*v*). Samples for virus enumeration were fixed with 25 % gluteraldehyde (EM-grade; Sigma–Aldrich, St. Louis, USA) to a final concentration of 0.5 %, and incubated for 30 min at 4 °C after which the samples were flash frozen in liquid nitrogen. IPL samples were filtered through 47 mm Whatman GF/F filters (Maidstone, UK), folded in aluminum foil and flash frozen in liquid nitrogen. All samples were stored at −80 °C until analysis. A second experiment was conducted, with similar set-up as described above, to allow isolation of PgV after complete host cell lysis (48 h).

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2.4 Isolation of PgV for lipid extraction

Viruses were isolated and purified according to Vardi et al. (2009) with the following adjustments to the original protocol. The 10 L of lysate was gently filtered through Whatman GF/C filters (47 mm diameter; Maidstone, UK) in 10 separate batches of 1 L to prevent clogging. After concentration by 30 kDa tangential flow filtration (Vivaflow 200; Sartorius Stedim Biotech GmbH, Goettingen, Germany), the remaining 50 mL of concentrated lysate was spun down into a 25 % OptiPrep™ (iodixanol) (Axis-Shield, Dundee, UK) solution. This was carried out by ultracentrifugation (Centrikon T-1080; Kontron Instruments, Watford, UK) in 12 mL ultraclear ultracentrifuge tubes (Beckman Coulter Inc., Brea, CA) in a swing-out rotor (SW41TI; Beckman Coulter, Palo Alto, USA) at approximately 100 000 × *g* for 2 h. Subsequently, the lower layer containing the 25 % Optiprep™ and viruses was pipetted on top of prepared density gradients containing 30, 35, 40 and 45 % Optiprep™. After ultracentrifugation as described above but at 200 000 × *g* for 4 h, the tubes were pierced with a sterile syringe needle to take out the clearly visible band containing PgV. The sample was gently filtered onto 0.02 µm Anodisc filters (25 mm diameter; Whatman, Maidstone, UK), and were stored in 20 mL glass scintillation vials (Packard bioscience, Meriden, USA) at −80 °C until analysis. During each step viral abundance samples were taken to confirm the recovery of viruses during filtration and centrifugation.

2.5 Algal and viral abundance

Samples for algal and viral enumeration were analyzed using a benchtop BD FacsCalibur flow cytometer with a 488 nm argon laser (BD Biosciences, San Jose, USA). Algal abundances were determined without dilution by chlorophyll red autofluorescence and side scatter (Marie et al., 1999). Virus samples were once thawed diluted 100 to 10 000-fold in sterile TE-buffer (pH = 8) according to Brussaard (2004). In short, PgVs were stained with the nucleic acid-specific dye SYBR green I to a final concentration of 0.5×10^{-4} of the commercial stock (Life technologies Ltd. Paisley, UK) for 10 min at

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80 °C and discriminated by their green fluorescence and side scatter signal. All flow cytometry data were analyzed using CYTOWIN 4.31 (Vaulot, 1989).

2.6 Intact polar lipid analysis

After freeze drying, the GF/F filters containing the infected *P. globosa* cells were cut into small pieces and extracted using a modified Bligh and Dyer (BD) extraction as described by Pitcher et al. (2011). All solvents used in the procedures described below were liquid chromatography/mass spectrometry (LC/MS) grade, with exception of the dichloromethane (DCM) which was high-performance liquid chromatography (HPLC) grade. A known volume of single-phase solvent mixture of methanol (MeOH) : DCM : phosphate buffer (2 : 1 : 0.8, *v* : *v* : *v*) was added to the sample in a glass centrifuge tube and placed in an ultrasonic bath for 10 min. The extract and residue were separated by centrifuging at 1000 × *g* for 5 min and the solvent mixture collected in a separate glass flask, this was repeated three times. DCM and phosphate buffer were added to the single-phase extract to give a new ratio of MeOH : DCM : phosphate buffer (1 : 1 : 0.9, *v* : *v* : *v*), and to induce phase separation. The extract was centrifuged at 1000 × *g* for 5 min. The DCM phase was collected in a round-bottom flask and the MeOH : phosphate buffer phase was washed two additional times with DCM. The combined DCM phases were reduced under a stream of N_2. The anodisc filters containing the viral isolate were extracted in the same way, but were ground directly in the BD solvent mixture in a glass tube, using a spatula. The polypropylene ring surrounding the filter was removed after the first sonication step. A blank filter extraction showed no background IPL contaminants.

An aliquot of the BD extracts was dissolved in an injection solvent composed of hexane/isopropanol/water (72 : 27 : 1; *v* : *v* : *v*) and filtered through a 0.45 µm regenerated cellulose filter (4 mm diameter; Grace, Deerfield, USA) prior to analysis by HPLC electrospray ionization MS (HPLC-ESI-MS^n) using methods modified from (Sturt et al., 2004). Briefly, HPLC separation was conducted on an Agilent 1200 series LC equipped with a thermostated autoinjector, coupled to a Thermo LTQ XL linear ion trap with Ion

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Max source with electrospray ionization (ESI) probe (Thermo Scientific, Waltham, MA). For details of gradient, column and ESI setting see Sinninghe Damsté et al. (2011). The IPLs were identified using a positive ion mode (m/z 400–2000). The four most abundant ions from each positive ion full scan were fragmented first to MS^2 (normalized collision energy (NCE) 25, isolation width (IW) 5.0, activation Qz 0.175) and then to MS^3 (NCE 25, IW 5.0, Qz 0.175). Structural identification of the IPLs was carried out by comparison with fragmentation patterns of authentic standards as described in Brandsma et al. (2012a). In addition to the characterization of routinely described glycolipids, phospholipids and betaine lipids, we surveyed extracts for the presence of viral glycosphingolipids (vGSL) using the mass spectral characteristics described by Vardi et al. (2009) and those of an authentic $C_{18:2}/C_{16:0}$ glucosylceramide standard (Avanti Polar Lipids, Inc., Alabaster, USA). In order to ascertain that our instrument sensitivity was sufficient to detect these compounds a positive control was carried out by analysis of an extract of *E. huxleyi* CCMP1516 (CCMP Culture collection, Bigelow) infected by EhV-86 (Wilson, 2013). For this host-virus system we were able to detect the described viral glycosphingolipid in MS^1 as its protonated molecule ($[M+H]^+$) at m/z 804, which gave rise to a diagnostic loss of 162 Da in MS^2 (data not shown), as in Vardi et al. (2009).

3 Results

3.1 Algal growth and viral dynamics

The non-infected *P. globosa* cultures grew at maximum growth rate (1.1 d^{-1}) throughout the experiment, while viral infection led to a rapid decline of cell numbers and full lysis 48 h post infection (p.i.; Fig. 1a). The one-step viral growth curve showed a release of newly produced viruses within 12 h p.i. (Fig. 1b) resulting in a burst size of 288 viruses $cell^{-1}$. PgVs stained well with the lipophilic dye FM 1–43 (Fig. 2a). The abundance of the FM 1–43 stained PgVs was slightly lower (8 %) than the SYBR green

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I-stained PgV abundance (Fig. 2b). The presence of a viral lipid membrane was, furthermore, confirmed by total loss of infectivity of PgV-07T upon treatment with 10 and 50 % (v/v) diethyl ether or chloroform.

3.2 Intact polar lipids in *P. globosa* and PgV

Ten classes of IPLs (see Fig. 3 for structures) were detected in the cultures at the start of the experiment (Fig. 4a), i.e. the glycolipids monogalactosyldiacylglycerols (MGDGs), digalactosyldiacylglycerols (DGDGs), glycosphingolipids (GSLs) and sulfoquinovosyldiacylglycerols (SQDGs); the phospholipids phosphatidylethanolamines (PEs), dimethylphosphatidylethanolamines (DMPEs), phosphatidylglycerols (PGs) and phosphatidylcholines (PCs) and the betaine lipids diacylglyceryl hydroxymethyltrimethyl-β-alanines (DGTAs) and diacylglyceryl carboxyhydroxymethylcholines (DGCCs). After 24 and 48 h the non-infected controls only showed minor changes in IPL composition, with a decrease of the relative contribution of the MGDGs and an increase of the PGs compared to the start (Table 1, Fig. 4b). Comparison between the control and virally infected cultures did not reveal any substantial differences (Fig. 4c, Table 1). The algal host DMPEs, which were detected in low levels in the control cultures at T48, were below the level of detection in the infected cultures at T48, most likely due to the decrease in cellular material over the course of the viral infection.

The IPL composition of PgV-07T was quite distinct from the host, with the PCs forming a much more substantial part of the IPLs than in the host. Of the other phospholipids (Fig. 4d, Table 1), PGs were also present, but PEs and DMPEs were below the limit of detection. Both betaine classes were detected in the viral isolate. The glycolipids were responsible for the largest difference in IPL composition between the virus and host. The contribution of the MGDGs and DGDGs to the sum of IPLs was reduced in the virus relative to the host and the SQDGs were not detected at all. Both *P. globosa* and PgV-07T display a series of glycosphingolipids in similar proportion. These were identified by the presence of a dominant MS^2 product ion arising from a loss of 180 Da from

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the $[M+H]^+$ ion, and a far less abundant product ion arising from loss of 162 Da. Viral glycosphingolipids (vGSLs), with a dominant loss of 162 Da, as described by Vardi and collaborators (2009), were, however, not detected in *P. globosa* or PgV.

4 Discussion

The present study showed that the IPL composition of the Prymnesiophyte *P. globosa* G(A) was not affected by viral infection. However, the IPL composition of the virus PgV-07T was different from the host's lipid composition, suggesting a selective acquisition of these compounds. We have not encountered any virus-specific lipids that were not detected in the non-infected host. This is in contrast to the finding of viral glycosphingolipids in another Prymnesiophyte virus-host model system, i.e. EhV-86 and *Emiliania huxleyi* CCMP374. Phylogenetically related virus-phytoplankton systems may thus have very different lipid related features during viral propagation.

4.1 Impact of viral infection on host lipid composition

The IPL classes detected in *P. globosa* originate from all cellular components, including the cell membrane as well as intracellular membranes from the chloroplasts and other organelles. In previous studies, environmental factors such as irradiance (Guschina and Harwood, 2009), but also viral infection (Vardi et al., 2009) were shown to affect the composition of these host IPLs. It is for instance known that viral infection typically induces a membranous rearrangement in the host to compartmentalize viral replication (den Boon et al., 2010). In our study we did not observe a strong impact by viral infection on the composition of IPL classes of *P. globosa*, nor did the diel cycle have an effect. However, within these IPL classes specific compounds may still be affected by viral infection. Evans et al. (2009) showed that viral infection of *E. huxleyi* CCMP1516 led to a restructuring of the host fatty acid composition in terms of saturation state. The main classes of IPLs may not necessarily be affected in such case. To date no data on

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the main IPL classes of infected *E. huxleyi* or fatty acids of infected *P. globosa* have been reported.

4.2 Acquisition of intact polar lipids from the host

By staining the viruses with a lipophylic dye, we have shown that PgV-07T possesses a lipid membrane. The complete loss of infectivity of PgV-07T after treatment with diethyl ether and chloroform indicates that this membrane plays a crucial role in the infection of *P. globosa*. Based on transmission electron microscopy thin section images, the Group-I PgVs, i.e. 150 nm large NCLDVs with a thin outer layer surrounding a layered inner core, to which PgV-07T belongs, are assembled in the cytoplasm and leave the host after disruption of the cell membranes (Baudoux and Brussaard, 2005). Thus, in contrast with EhV-86 (Mackinder et al., 2009), PgV seems to obtain its lipid membrane in the host cytoplasm and not from the cell membrane. The lipids of PgV are then either allocated, newly synthesized or obtained from other membranes, such as the endoplasmatic reticulum (E.R.) or the Golgi system (den Boon et al., 2010; Heaton and Randall, 2011). Meints et al. (1986) showed the appearance of membranous structures at the sites of virus assembly in *Chlorella* sp. This virus, called PBCV-1, belongs to the Phycodnaviridae and is thought to obtain these lipid membranes from the host endoplasmatic reticulum (Wilson et al., 2009). The origin and patterns of PgV replication in the host cell, i.e. formation of virions in the cytoplasm and virus release in a single burst, (Baudoux and Brussaard, 2005) show a strong resemblance with PBCV-1 (Meints et al., 1986). Using electron cryomicroscopy, Yan et al. (2000, 2005) confirmed that not only PBCV-1 but also the *Phaeocystis pouchetii* virus PpV01, contains a membrane covering the inside of the capsid. Regarding the similarity in patterns of infection between these viruses and PgV-07T, it is likely that PgV-07T also possesses an intracapsid membrane.

Of the viral IPL classes the phospholipids appear to be acquired in higher proportions than the glyco- and betaine lipids. The PCs, considered to be the most dominant phospholipid in phytoplankton (Van Mooy et al., 2009) were well represented in *P. globosa*

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and, probably due to its ubiquity in all cellular membranes (Thompson, 1996), in the virus as well. The second most important group of phospholipids in *P. globosa*, the PGs, are known to be abundant in chloroplasts and other organelles, such as the endoplasmatic reticulum (Schwertner and Biale, 1973). The source of the viral lipid membrane could be associated with such organelles. The PEs and DMPEs were not detected in the virus, but even in the infected cultures they were only present in trace amounts. These compounds have been shown to be precursors in the methylation pathway of PC in bacteria (Aktas and Narberhaus, 2009) and yeast (Kanipes and Henry, 1997), while this is the case for PEs in the phytoplankton species *Chlamydomonas reinhardtii* (Yang et al., 2004). PEs and DMPEs have furthermore been shown to be associated to small organelles such as mitochondria (Schwertner and Biale, 1973). For the infected lysing *P. globosa* cultures, such organelles might have been lost by passing through the GF/F filter during sampling of the infected cultures.

The betaine lipids in the PgV membrane were relatively low in comparison to their contribution to the total lipids in the algal host. Interestingly, DGTAs have been found in algal thylakoid membranes (Murata, 1998). TEM thin sections and epifluorescence microscopy of infected *P. globosa* cells (data not shown) illustrate that the integrity of the chloroplast of *P. globosa* is largely unaffected far into the viral growth cycle, leaving the chloroplast membranes intact. The DGTAs in *P. globosa* might also be mainly associated to the membranes of the unaffected chloroplast and therefore present in lower relative amounts in the membrane of PgV-07T.

The relative loss of glycolipids, including the apparently complete loss of SQDGs in the virus, is probably due to the same process. The four classes of glycolipids detected *in P. globosa* are generally associated with the chloroplast in algae and higher plants (Guschina and Harwood, 2009; Sato, 2009). With roughly a 50 % contribution, the MGDGs often comprise the majority of the chloroplast lipids, while the other half is composed of DGDGs, SQDGs and phospholipids. GSLs have not been found in chloroplasts, but their synthesis is directly related to chloroplast metabolism and vice versa the disruption of sphingolipid biosynthesis was found to lead to the disruption

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of the chloroplast membranes in the vascular plant *Datura stramonium* (Abbas et al., 1992; Chen et al., 2009). Additionally, GSLs are also found in plant cell membranes, where they can account for up to 27 mol% of lipids (Chen et al., 2009). In this case both the association to the chloroplast and the cell membranes could account for the relative loss of GSLs in PgV-07T.

In summary, our results suggest that PgV-07T acquired its lipid membrane mainly in the host cytoplasm and that the chloroplast membranes as well as the cell membrane were not utilized. Since we did not find any IPL-group in the infected cultures or the virus that were not also detected in the non-infected culture, we hypothesize that PgV-07T selectively recruits its lipids from existing membranes, such as the E.R. or the Golgi-apparatus. Examination of the composition of these membranes could clarify this issue.

4.3 Glycosphingolipids as markers for viral infection

Although we detected a group of GSLs in both the non-infected and infected *P. globosa* cells, we did not observe the specific vGSLs that Vardi et al. (2009) described as being synthesized in infected *E. huxleyi* cells and being part of the viral membrane. Indeed, the four viral genes, involved in the synthesis of this vGSL and transcribed during infection (Wilson et al., 2005) were found to be absent in the genome of PgV-016T (Santini et al., 2013), a virus from the same Group I PgVs as PgV-07T (Baudoux and Brussaard, 2005). This is especially interesting since *P. globosa* and *E. huxleyi* both belong to the same class of algae, namely the Prymnesiophyceae, and both PgV-07T and EhV-86 are NCLDVs (Baudoux and Brussaard, 2005; Wilson et al., 2005). *E. huxleyi* has been extensively used as a model system to study specific features of algal virus-host interaction such as caspase activity (Bidle et al., 2007), host entry, departure and viral envelope acquisition (Mackinder et al., 2009) and now the involvement of vGSLs (Vardi et al., 2009). Comparison of these and additional virological aspects with those of PgV-07T (Group I) and other known members of phytoplankton infecting NCLDVs, i.e. PgVs from Group II, PpV-01, PBCV-1, *Chrysocromulina ericina* virus (CeV-01B) and

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Pyramimonas orientalis virus (PoV-01B), suggests that the infection strategy of EhV-86 is rather uncommon amongst the known phytoplankton viruses (Yan et al., 2000; Sandaa et al., 2001; Baudoux and Brussaard, 2005; Van Etten et al., 2010). The absence of vGSLs (and the possible selective acquisition of IPLs) as shown for PgV-07T, might be a more general feature amongst phytoplankton-infecting NCLDVs. This would also imply that the use of lipid biomarkers for viral infection of phytoplankton (Vardi et al., 2012) might only be successful for specific virus-host systems such as *E. huxleyi* and EhV-86 and not be generally applicable.

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Table 1. The relative distribution of the intact polar lipids (IPLs) in the control and infected *P. globosa* cultures at three time points and in the virus PgV-07T. Relative ionization of the compounds per treatment is depicted by "plusses" (+), or by "trace" (Tr.) when below 5 % of the total ionization. Minus (–) shows complete absence. Due to their low abundance, detection of the PEs and DMPEs was not consistent; hence they were not included in the IPL sum.

		MGDGs	DGDGs	SQDGs	PC	PG	DGTA	DGCC	GSL
T0	Control	++	+	Tr.	+ + +	Tr.	+	++	Tr.
	Infected	++	+	Tr.	+ + +	Tr.	+	++	Tr.
T24	Control	+	+	Tr.	+ + +	+	+	++	Tr.
	Infected	+	+	Tr.	+ + +	+	+	++	Tr.
T48	Control	+	+	Tr.	+ + +	+	+	++	Tr.
	Infected	+	+	Tr.	+ + +	+	+	++	Tr.
	PgV	Tr.	Tr.	–	+ + ++	+	+	++	Tr.

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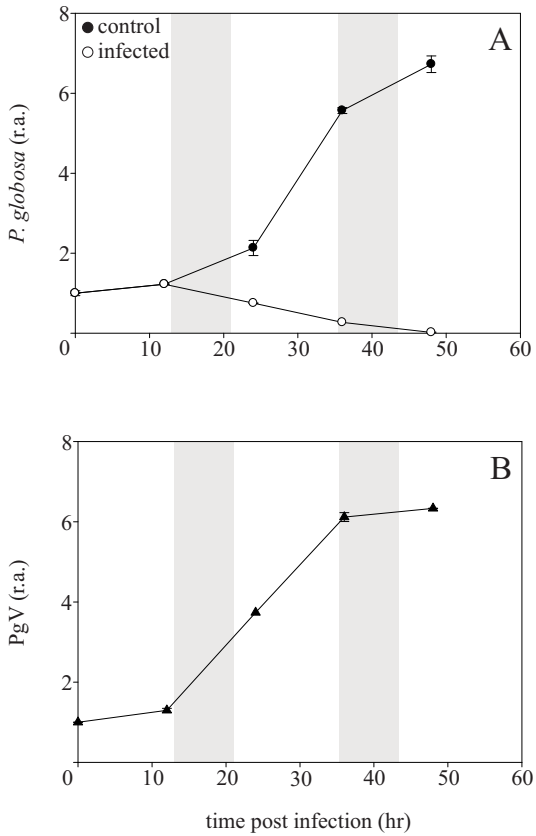

Fig. 1. Abundances (normalized to T0) of algal host *Phaeocystis globosa* **(A)**, and of the virus PgV-07T **(B)**. Closed symbols represent the non-infected cultures, the open symbols the virally infected cultures. If standard deviation bar is not visible, it falls within the symbol. Shaded areas depict dark (night) period and r.a. stands for relative abundance.

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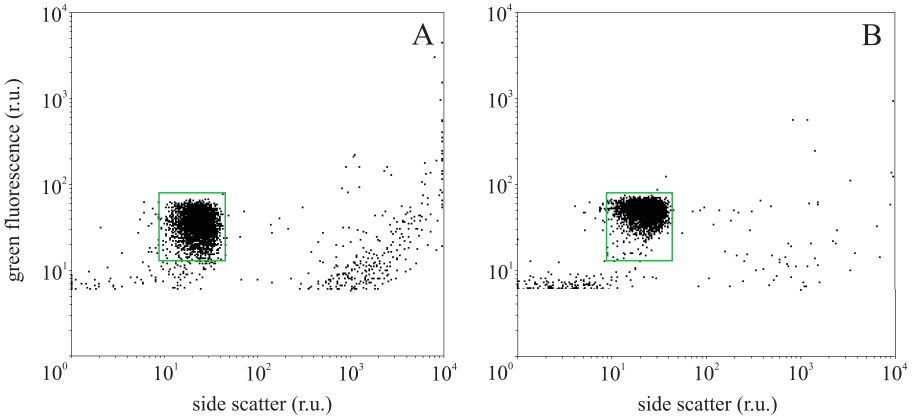

Fig. 2. Flow cytometric signatures of PgV after staining with the green fluorescent lipid stain FM 1–43 **(A)** and the nucleic acid-specific dye SYBR Green I **(B)**. r.u. stands for relative units.

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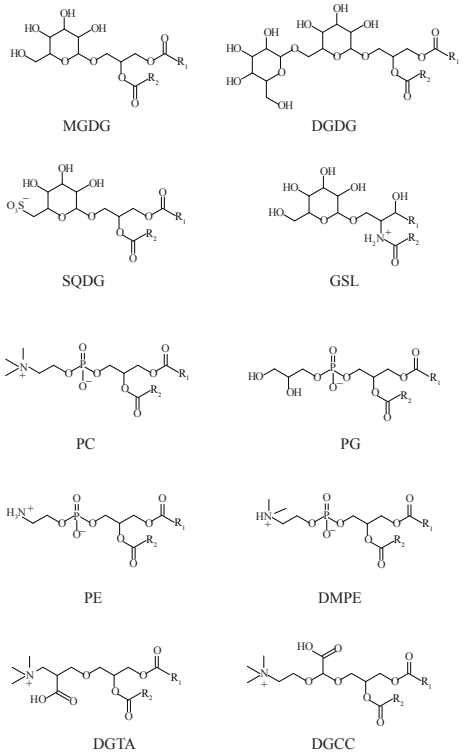

Fig. 3. Structural formulas of the main IPL classes belonging to the glycolipids monogalactosyldiacylglycerols (MGDGs), digalactosyldiacylglycerols (DGDGs), glycosphingolipids (GSLs) and sulfoquinovosyldiacylglycerols (SQDGs); the phospholipids phosphatidylethanolamines (PEs), dimethylphosphatidylethanolamines (DMPEs), phosphatidylglycerols (PGs) and phosphatidylcholines (PCs) and the betaine lipids diacylglyceryl hydroxymethyltrimethyl-β-alanines (DGTAs) and diacylglyceryl carboxyhydroxymethylcholines (DGCCs). The acyl groups are represented by R1 and R2.

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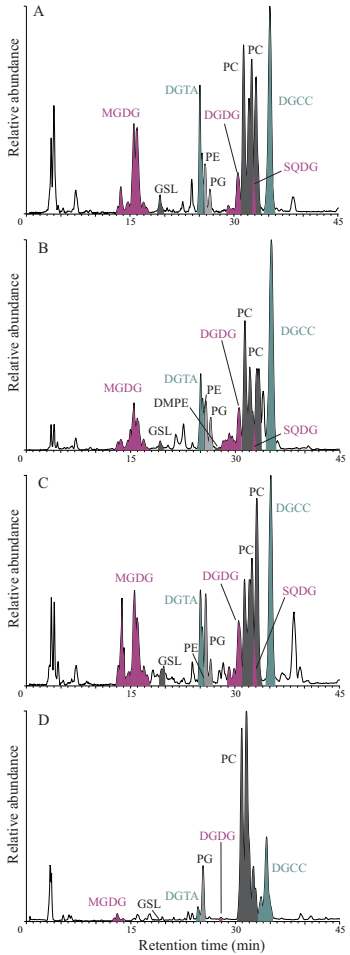

Fig. 4. Partial base peak chromatograms (MS1, m/z 400–2000) of non-infected *P. globosa* at T0 and T48 h **(A, B)**, virally infected *P. globosa* at T48 **(C)**, and the virus PgV-07T **(D)**.

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