



First description of a cyanophage infecting the cyanobacterium *Arthrospira platensis* (Spirulina)

Stéphan Jacquet, Xu Zhong, Parvathi Ammini, Angia Sriram Pradeep Ram

► To cite this version:

Stéphan Jacquet, Xu Zhong, Parvathi Ammini, Angia Sriram Pradeep Ram. First description of a cyanophage infecting the cyanobacterium *Arthrospira platensis* (Spirulina). *Journal of Applied Phycology*, 2013, 25, pp.195-203. hal-00813900

HAL Id: hal-00813900

<https://hal.science/hal-00813900>

Submitted on 16 Apr 2013

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 First description of a cyanophage infecting the 2 cyanobacterium *Arthrospira platensis* (Spirulina)

3
4 Stéphan JACQUET¹, Xu ZHONG¹, Parvathi AMMINI^{1,2}, Angia Sriram PRADEEP
5 RAM³

- 6
7 1. INRA, UMR 042 CARRETEL, 75 Avenue de Corzent, 74203 Thonon-les-Bains cx, France
8 2. National Institute of Oceanography, Dr Salim Ali Road, PO Box 1913, Kochi, 682018 India
9 3. Laboratoire Microorganismes : Génome et Environnement, UMR CNRS 6023, Clermont Université,
10 Université Blaise Pascal, BP 80026 63171 Aubière Cedex, France

14 Abstract

15 Cyanobacteria constitute a versatile group of photosynthetic bacteria of immense commercial and
16 ecological importance. Some species of this group are cultivated and sold as food because of their
17 high nutritional value. This is typically the case for *Arthrospira platensis*. We describe for the first
18 time a virus infecting this economically important filamentous cyanobacterium isolated from
19 culture pools located in the South of France. This virus could be observed and discriminated easily
20 from other particles with flow cytometry. Based on morphology and molecular investigation, it was
21 proposed that the virus belongs to the cyanopodovirus group with a capsid and short tail of about
22 120 and 20 nm, respectively. Finally, the virus appeared to be highly specific (very narrow host
23 range) to *A. platensis*.

25 Keywords

26 Cyanobacteria; cyanophage; culture; characterisation; *Arthrospira platensis*

1 **Introduction**

2 The genus *Arthrospira* (ex *Spirulina*) is a non-heterocystous filamentous cyanobacterium,
3 characterised by multicellular, cylindrical and usually screwlike coiled trichomes, inhabiting
4 diverse environments including those of high salinity (Anagnostidis and Komarek 1988; Manen and
5 Falquet 2002). Several strains have been isolated worldwide, and made useful in a variety of
6 fundamental and applied research studies: commercial mass cultures have indeed been developed
7 for the food industry in local areas, but also for alternative biofuel feedstock, skin-care product
8 resources, etc (Ciferri and Tiboni 1985; Belay et al. 1996; Fox 1996). To the best of our knowledge,
9 nothing has been published yet on viruses associated to the dynamics of this species, despite its high
10 commercial value.

11 Bacteriophages infecting cyanobacteria, namely cyanophages, are tailed and contain
12 dsDNA. They belong to three families: the cyanomyoviruses (virus with a long contractile tail), the
13 siphoviruses (virus with a long non-contractile tail) and the podoviruses (virus with a short or non-
14 apparent tail) (Safferamn et al. 1983). Cyanophages were first studied in freshwater systems where
15 a virus infecting a filamentous cyanobacterium had been isolated about 50 years ago (Safferman
16 and Morris 1963). Following this discovery, the isolation and characterization of several freshwater
17 cyanophages were studied extensively between the 1960's and early 80's (Brown 1972; Padan and
18 Shilo 1973; Safferman 1973; Sherman and Brown 1978; Gromov 1983). Cyanophage description,
19 infecting both unicellular and filamentous marine cyanobacteria, occurred after 1980. It was not
20 until the early 1990's that cyanophages infecting the marine *Synechococcus* were isolated (Wilson
21 et al. 1993; Suttle 2000). The literature has become relatively rich over recent years with the
22 description of cyanophage structure and diversity, both for the ocean and for some lakes (Short and
23 Suttle 2005; Wilhelm et al. 2006; Chen et al. 2009; Matteson et al. 2011). On the other hand, studies
24 about the characterisation, ecological importance and dynamics of cyanophages infecting specific
25 cyanobacteria remain relatively scarce (Sandaa and Larsen 2008; Yoshida et al. 2008).

Being alerted during the summer of 2011 by an episode of high *Arthrospira platensis* mortality cultured in some pools located in the South of France, we suspected that such event mortality could be due to a mass lytic process involving specific viruses, i.e. cyanophages. We obtained water samples from different pools with or without mortality to test for the presence of viruses. We discovered a cyanophage, able to infect and lyse *A. platensis* and for which a basic description is provided.

Materials and methods

Site and sample description A dozen of samples were obtained from “le chant de l’eau”, an exploitation based in the south of France (Fuilla) and consisting of 8 pools of 70 -200 m². Growth conditions of the cyanobacterium have been described in Jourdan (2006). Briefly, *A. platensis* grew in outdoor and under glass pools inside which a soft agitation is provided with a temperature varying between 25°C and 35°C, natural light:dark cycles and nutrient concentrations as described thereafter (sodium bicarbonate = 8 g/l; potassium sulfate = 1 g/l ; sodium chloride = 5 g/l ; potassium nitrate = 2 g/l ; magnesium sulfate = 0.2 g/l ; calcium chloride = 0.1 g/l ; ammonium + ammoniac? = 0.2 g/l). Samples were taken from some of these different 15 cm depth pools where the cyanobacterium was observed to die (in a few hours to days) or not to die. The strain is referred to *paracas* from the species *A. platensis* owing to the original location where it was first isolated (i.e. Peru). The samples were subjected to flow cytometry and transmission electronic microscopy analysis, infection experiments and by various molecular techniques as described below. Alternatively, we also obtained samples from other farms of the South of France (referred to as Domaine algal, Carpio, Algosud) to test the infectivity of the virus and also to test for lysogenic induction.

Flow cytometry analysis Samples were pre-filtered through GF/F (Whatman) and polycarbonate 0.2 µm (Millipore) filters in order to remove all cellular materials. Viruses were observed and

1 counted using a FACS Calibur flow cytometer (Becton Dickinson) equipped with an air cooled
2 laser providing 15 mW at 488 nm. Samples were fixed with glutaraldehyde (0.5% final
3 concentration, grade I, Merck) for 30 min, then diluted in 0.02 µm filtered TE buffer (0.1 mM Tris-
4 HCL and 1 mM EDTA, pH 8), and incubated with SYBR Green I (at a final 10^{-4} dilution of the
5 commercial stock solution; Molecular Probes), for 5 min at ambient temperature in the dark,
6 followed by an incubation for 10 min at 75°C, and then another 5 min at room temperature, prior to
7 FCM analysis. Note that the viruses could also be observed without heating but the discrimination
8 was comparatively poor. Analysis was made on samples to which a suspension of 1-µm beads had
9 been added (Molecular probes). Flow cytometer listmode files obtained were then transferred and
10 analyzed on a PC using the custom-designed freeware CYTOWIN (Vaulot 1989).

11 ***Host-range experiment*** For this test, the samples with the virus of interest was filtered twice
12 through a 0.45 µm polycarbonate mesh syringe sterile (Fischer Scient.) filter to remove all particles
13 but not the viruses. Infection was processed classically by adding 500 µL of the cyanovirus isolate
14 to 2 to 5 mL of a variety of cyanobacterial strains, in duplicate, from the Thonon Culture Collection
15 (TCC) or other collections : 25 PE-rich *Synechococcus* spp (TCC 32, 185, 186, 789 to 808), 10 PC-
16 rich *Synechococcus* (PCC 6301, 6311, 6707, 6715, 7917, 7918, 7941, 7952, 9004 and 9005), 4 PC-
17 rich *Synechocystis* (PCC 6308, 6803, 6905, 7509), 1 colonial cyanobacterial form (*Microcystis*
18 *aeruginosa*, TCC 80) and 1 filamentous cyanobacterial form (*Planktothrix rubescens*, TCC 29), all
19 originated from freshwater ecosystems. Finally the virus was tested against *A. platensis* obtained
20 from the other farms mentioned above. The infectivity of the virus was not tested on marine species
21 or strains since *A. platensis* is a freshwater cyanobacterium that cannot be cultured in natural
22 seawater even if it accommodates a high salinity range up to 25 mg/L. Only when a clear lysate was
23 produced in the duplicates the infection was recorded as successful.

24 ***Infection of host cells*** The process of infection was studied by adding a suspension of <0.45 µm
25 suspension of virus particles to 4 different cultures of *A. plantensis* and changes within the algal

1 host was followed by fluorescence using a FluoScan (Metrastat). Note that we neither tested
2 different multiplicity of infection nor worked with many replicate treatments.

3 **Induction of lysogenic *A. platensis*** We addressed the prevalence of lysogeny within different
4 cultures of *A. platensis* following Dillon and Parry (2008). Briefly, a 1 mg/L stock solution of
5 mitomycin C (Sigma) was prepared in <0.02 µm filtered milliQ water and stored in the dark at 4°C.
6 10 ml aliquots of exponentially growing cultures of each *A. platensis* sample were incubated with
7 mitomycin C at final concentrations of 0 (control containing only water), 1, 5, and 20 µg/mL under
8 a 14:10 light: dark (L:D) cycle for 2 weeks in our temperature-controlled algal culture room. 1 mL
9 sub-samples were taken at time 0, 7 and 14 days to count viruses with FCM (the cyanophage and
10 the bacteriophages from the contaminating heterotrophic bacteria being easily discriminated by
11 FCM parameters due to different side scatter and green DNA-dye complex fluorescence as shown
12 on Figure 1).

13 **Transmission Electron Microscopy (TEM).** For visualization and characterisation of viral particles
14 by TEM, glutaraldehyde fixed samples (1% final concentration) stored at 4°C were then harvested
15 by ultracentrifugation onto 400 mesh NI electron microscope grids with carbon-coated Formvar
16 film, by using a Beckman Coulter SW40 Ti Swing-Out-Rotor run at 70,000 x g for 20 min at 4°C
17 (Weinbauer and Peduzzi 1994; Sime-Ngando et al. 1996). Each grid was stained at room
18 temperature (ca. 20°C) for 30 s with uranyl acetate (2% wt/wt), rinsed twice with 0.02 µm filtered
19 distilled water and dried on a filter paper. Grids were then examined using a JEOL 1200EX TEM
20 operated at 80 kV at a magnification of x 100,000. The photographic negatives were scanned with
21 Adobe Photoshop and cyanophage dimensions were determined using IMAGEJ software.

22 **Primers and Polymerase chain reaction (PCR) analysis** To identify the virus, different primers
23 (see Table 1) were used like the CPS1/CPS2, CPS4/CPS5, CPS1.1/CPS8.1, all targeting the portal-
24 vertex-capsid-protein encoded gene *g20* of Cyanomyovirus (Fuller et al. 1998; Wilson et al. 2000;
25 Sullivan et al. 2008), MZIA1bis/MZIA6 targeting the major-capsid-protein encoded gene *g23* of
26 Myoviridae (Filée et al. 2005), and *pol1* designed by Chen et al. (2009) and *pol2* designed in this

1 study (targeting the Cyanopodovirus specific DNA polymerase gene of cyanopodovirus). Briefly,
2 the *pol2* primers were designed based on the sequences of marine and estuarine podoviral *pol* gene
3 sequences available in GenBank (FJ872594 to FJ872816). The primers were designed using primer
4 3 software (<http://frodo.wi.mit.edu/primer3/>) and primer design software of NCBI (Primer-
5 BLAST). A total of 16 primers were obtained and tested for the efficiency to amplify the viral
6 concentrate. The primer pair initially called Jason 3Fa and 4R (hereafter referred to as *pol2*), was
7 designed to give a positive amplicon of 389 bp. The *pol2* primer was tested for its specificity on a
8 variety of viral concentrates (both cyanophage and algal virus isolates) supplied by different
9 colleagues (see acknowledgements) as both positive or negative controls. Other primers were also
10 tested such as MCP F/R and AVS1/2 targeting the major capsid protein of algal viruses (Chen and
11 Suttle 1995, Larsen et al. 2008) to test the identity of the virus. All these tests were performed both
12 on untreated sample but also on FCM sorted population after reconcentration using centrifugal filter
13 units (Amicon UltraCell 10 Kda, Millipore) if necessary for PCR analysis.

14 The PCRs were performed by using the DNA Thermal Cycler T-Professional (Biometra)
15 with the optimised conditions for each primer (Zhong et al. in preparation). Briefly, 25- μ l reaction
16 mix contained 1X PCR buffer, 2 mM MgCl₂, 200 μ M of each dNTP, 0.4 μ M of each primer, 0.5 U
17 of Platinum[®] *Taq* DNA polymerase (Invitrogen), and 1 μ l of sample. The virus concentrates
18 provided by colleagues (see acknowledgments) were also used either as positive or negative
19 controls for PCR. PCR products were subjected to electrophoresis on a 1.5 % (w/v) agarose gel in
20 0.5X TBE (89 mM Tris-base, 89 mM boric acid, 2 mM EDTA, pH 8.0) and visualized by ethidium
21 bromide staining on a UV transilluminator (Tex-35M, Bioblock Scientific) and photographed using
22 a gel documentation system (BioRad, Germany).

23

24 **Results and Discussion**

25 Use of cyanobacteria as a food supplement has a long history (Gantar and Svircev 2008) dating
26 back to Antiquity. Referred wrongly to as “Spirulina”, *Arthrospira* Stizenberger 1852, which is in

fact more related to *Planktothrix* and *Lyngbia* following Manen and Falquet (2002), have already been collected and used by Aztec populations (Ciferri 1983; Pulz and Gross 2004). Even today, malnutrition, especially due to a protein-poor diet is widespread in many parts of the world. The use of cyanobacteria as a non-conventional source of food and protein is a reality (Pulz and Gross 2004; Gantar and Svircev 2008; Rosenberg et al. 2008). At present, *Arthrospira* represents the second most important commercial microalga for the production of biomass as a health food and animal feed, after *Chlorella* (Voshak and Tomaselli 2000). Thus, in addition to purely fundamental aspects, it can be crucial to know the existence of factors such as viruses susceptible to impact severely culture systems and leading to economic losses.

The analysis with FCM (Fig 1) revealed a typical viral signature with a high FL1 (DNA-dye complex) level, different to what is usually observed in natural samples with the “classical” VLP1 to VLP4 (Larsen et al. 2004; Duhamel et al. 2006; Personnic et al. 2009). Such a FCM signature immediately suggested it could be a cyanophage, based on our own FCM cytogram experience and compared with different signatures of other cyanophage isolates (not shown). Obviously FCM signatures are not enough to characterise viruses (Brussaard et al. 2000) and other techniques were used to identify this particle unambiguously. The identity of the virus was further determined using both TEM and PCR. Such viruses can be observed easily with FCM that gives precise counts and thus the dynamics of such a virus in different culture conditions could be tested and followed.

TEM analysis revealed that there was a variety of phages in the different ponds with a majority of Podoviruses compared to the two other dsDNA Myo- and Siphovirus representatives. Once sorted using FCM and reconcentrated, TEM revealed that the virus infecting *A. platensis* was characterised by a short non contractile tail as it is the case for Cyanopodoviridae (Fig 5). The capsid and the tail were about 120 and 20 nm, respectively, which is expected for such a cyanophage (Suttle 2000).

As a latter matter of proof, the PCRs conducted with primers targeting various groups of viruses (i.e. Myovirus, Podovirus, algal viruses) were negative for all the primers except for *polI*

1 and *pol2* (Fig 3) from all the pools (Table 2). Here please change the titles inside the table as *pol1*
2 and *pol2* instead of *pol* chen and new *pol*. The pool without culture (i.e. with just the water medium
3 before to be cropped) was negative to all genes including *pol* gene PCRs performed in this study
4 (Fig 3). At last, only VLP2 was positive to *pol* primers compared to VLP1 and VLP3. All these
5 results thus supported that the virus was a cyanopodovirus. We refer to this cyanopodovirus
6 obtained in this study as SPIRUVIR hereafter. We sequenced the PCR product of *pol2* of
7 SPIRUVIR to confirm its identity. We obtained a sequence of 365 bp whose BLAST search
8 revealed 83 to 96% similarity to various DNA polymerase genes of cyanopodovirus sequences in
9 the GenBank. The evolutionary history was inferred using the Neighbor-Joining method [1]. The
10 percentage of replicate trees in which the associated taxa clustered together in the bootstrap test
11 (1000 replicates) are shown next to the branches [2]. The evolutionary distances were computed
12 using the Kimura 2-parameter method [3] and are in the units of the number of base substitutions
13 per site. The analysis involved 11 nucleotide sequences other than SPIRUVIR (Fig 4). Evolutionary
14 analyses were conducted in MEGA5 [4].

15 It is noteworthy that we could also expect a positive response to *psbA* gene investigation
16 since this photosynthesis gene has been found in many cyanophages except for the
17 cyanosiphoviridae which have been reported not to carry the photosynthesis genes, both *psbA* and
18 *psbD* (Huang et al. 2011).

19 The susceptibility of the various cultures was tested by the clarity of culture and
20 development of the virus compared to controls. The podovirus caused lysis of only *A. platensis*
21 cultures as against cyanobacterial and bacterial and eukaryotic hosts (not shown), demonstrating the
22 high host specificity of this phage. This result is not surprising since cyanopodoviruses are
23 generally reported as host specific, usually infecting only the host of isolation, compared to
24 myoviruses typically that display a broader host range (Chen and Lu 2002; Mann 2003; Sullivan et
25 al. 2003), even if Deng and Hayes (2007) also reported once that one podovirus could have a very
26 broad host range. It was also interesting to note that the lysis was clearly different between the

different cultures and that some resistance phenomena could be observed (Fig 6), even if we did not work on reasons and mechanisms behind such a resistance that can dress different aspects (Thomas et al. 2012). From the lab experiments, we were unable to confirm the short latent period as suggested by the rapid biomass decay observed *in situ* in the pools (in one night) by the producers and also generally reported for cyanopodoviruses compared to the cyanomyo- and cyanosiphoviruses (Sullivan et al. 2003; Wang and Chen 2008).

Techniques we employed revealed that the virus was a cyanophage belonging to the Podoviridae family, i.e. a cyanophage with an isometric capsid head and short tail, a virus responding only to (cyanopodovirus-derived) *pol* gene PCR primers and with typical characteristics of this family referred earlier to the LPP (for *Lyngbya*, *Plectonema*, *Phormidium*) viruses, i.e. a narrow host range acting as parasite unique to a specific host (only *Arthrospira* was indeed infected by this virus). This virus is thus of particular interest since it is well known that most algal (including cyanobacterial) viruses have been largely isolated from marine provinces (Nagasaki and Bratbak 2010). It is also noteworthy that most cyanophages isolated to date belong to the family Myoviridae (Mann 2003) which has been reported to be easier to isolate, has a greater proportion of lytic cycle and a host range broader than the two other families (Suttle 2005).

In contrast to Padan et al. (1972), Cannon et al. (1971), Ohku and Fujita (1996), Hewson et al. (2001) who documented lysogeny in a variety of filamentous cyanobacteria or again Dillon and Parry (2008) who reported a high level of induction of lysogenic cells and release of temperate cyanophages in the freshwater cyanobacterium *Synechococcus*, we did not obtain clear induction whatever the culture and the concentration of Mitomycin C used to induce cell lysis. As reported for other cyanobacterial genomes (Kettler et al. 2007; Dufresne et al. 2008), it is possible that there was intact prophages in *A. platensis*. The analysis of the genome of *Arthrospira platensis* using the Genbank database suggested however that a certain type of prophage may be present in the cyanobacterium, as in other cyanobacteria (Palenik et al. 2003; Sullivan et al. 2005), even if we failed to make the prophage element inducible if it is. If a cyanophage is integrated in the *A.*

1 *platensis* genome, the reason behind our incapacity to produce the virus and thus of this remarkable
2 resistance is not known. Other inducible agents should be tested such as high light and temperature
3 or UV radiation before any clear conclusion could be advanced. As the mitomycin treatment did not
4 lead to the production of new particles, it is unlikely however that the observed phage resistance
5 was due to lysogeny.

6 We can only suggest here that such a virus could be indeed involved in the cyanobacterial
7 culture mass mortality following favourable conditions for the virus to become lytic. In forthcoming
8 studies, it will be important to be able to follow *in situ* population dynamics (both viruses and *A.*
9 *platensis*) but also many other parameters that are likely to be important in growth and mortality
10 processes of the cyanobacterium, such as light and nutrients, particularly nitrogen, both in terms of
11 quantity and quality, but also zooplanktonic predation or eukaryotic parasitism. Interactions
12 between cyanobacteria and cyanophages are complex and it will be also interesting to look in detail
13 what can be the effect of the virus on the morphology shape and helix architecture of the
14 cyanobacterium, known to be highly dependent on growth and environmental parameters (Vonshak
15 and Tomaselli 2000). Several viruses, belonging to several families, can infect one single species in
16 some cases. In other situations only one virus is virulent for one specific host. The environment is
17 likely to play an important regulating factor on lytic vs. lysogenic processes in these interactions.
18 We also know that cyanophages have probably played a key role in shaping some key metabolic
19 processes such as photosynthesis in cyanobacteria. Cyanobacteria can be responsible for important
20 resistance phenomena and associated costs. Differently said, cyanophages and cyanobacteria have a
21 long history of co-evolution, an arm-race where both entities win and loss tour a tour and for which
22 such interactions strongly depend on the environment.

25 **Conclusion**

26 We described for the first time a virus infecting one of the most commercially important species of
27 cyanobacteria, *Arthrospira*, which is used for health food and animal feed and also reported as a

source of food additives, fine chemicals or again for the production of biofuels. This cyanophage may be an important regulating factor of this cyanobacterium but we do not know what are the main factors intervening in such a regulation and if, for instance, some important resistance mechanism, have been developed by the cyanobacterium. Improving our knowledge of such a virus is obviously a key issue.

Acknowledgements

This study was supported by ANR AQUAPHAGE and région Rhone-Alpes (through a PhD grant for XZ). We are grateful to colleagues Martha Clockie, Ruth-Anne Sandaa, Rozenn Thomas, Steven Short, Steven Wilhlem, Marcia Osburne, and Feng Chen who provided different virus isolates and expertise. English was checked by an English-native speaker colleague. For PA, this is NIO contribution number 5170.

References

- Anagnostidis K, Komarek J (1988) Modern approach to the classification system of cyanophytes. 3. Oscillatoriales. Arch Hydrobiol Sppl 80, Algological studies 50/53:327-329.
- Belay A, Kato T, Ota Y (1996) *Spirulina* (*Arthrospira*): potential application as an animal feed supplement. J Appl Phycol 8:303-311.
- Brown RM Jr (1972) Algal viruses. Adv Vir Res 17:243-277.
- Brussaard CPD, Marie D, Bratbak G (2000) Flow cytometric detection of viruses. J Virol Methods 85:175-182.
- Cannon RE, Shane MS, Bush VN (1971) Lysogeny of a blue-green alga, *Plectonema boryanum*. Virol 45:149-153.
- Chen F, Lu JR (2002) Genomic sequence and evolution of marine cyanophage P60: new insight on lytic and lysogenic phages. Appl Environ Microbiol 68:2589-2594.
- Chen F, Suttle CA (1995) Amplification of DNA polymerase gene fragments from viruses infecting microalgae. Appl Environ Microbiol 61:1274-1278.
- Chen F, Wang K, Huang SJ, Cai HY, Zhao ML, Jiao NZ, Wommack E (2009) Diverse and dynamic populations of cyanobacterial podoviruses in the Chesapeake Bay unveiled through DNA polymerase gene sequences. Environ Microbiol 11:2884-2892.
- Ciferri O (1983) *Spirulina*, the edible micro-organism. Microbiol Rev 47:551-578.
- Ciferri O, Tiboni O (1985) The biochemistry and industrial potential of *Spirulina*. Annu Rev Microbiol 39:503-26.
- Clockie MRJ, Millard AD, Mehta JY, Mann NH (2006) Virus isolation studies suggest short-term variations in abundance in natural cyanophage populations of the Indian Ocean. J Mar Biol Ass UK 86:499-505.

1 Clockie MRJ, Shan J, Bailey S, Jia Y, Krisch HM, West S, Mann NH (2006) Transcription of a
2 photosynthetic T4 type phage that infects marine cyanobacteria. *Environ Microbiol* 5:827-835.

3 Deng L, Hayes PK (2008) Evidence for cyanophages active against bloom-forming freshwater
4 cyanobacteria. *Freshw Biol* 53:1240-1252.

5 Dufresne A, Ostrowski M, Scanlan DJ, Garczarek L, Mazard S et al. (2008) Unraveling the
6 genomic mosaic of a ubiquitous genus of marine cyanobacteria. *Genome Biol*
7 9:R90 doi:10.1186/gb-2008-9-5-r90.

8 Duhamel S, Domaizon I, Personnic S, Jacquet S (2006) Assessing the microbial community
9 dynamics and the role of viruses as bacterial mortality agents in Lake Geneva. *J Wat Sci* 19:115-
10 126.

11 Dillon A, Parry JD (2008) Characterization of temperate cyanophages active against freshwater
12 phycocyanin-rich *Synechococcus* species. *Freshw Biol* 43:1253–1261.

13 Filée J, Tétart F, Suttle CA, Krisch HM (2005) Marine T4-type bacteriophages, a ubiquitous
14 component of the dark matter of the biosphere. *PNAS* 102:12471-12476.

15 Fox RD (1996) *Spirulina* production and potential. Edisu, Aix-en-Provence, France.

16 Fuller NJ, Wilson WH, Joint I, Mann NH (1998) Occurrence of a sequence in marine cyanophages
17 similar to that of T4 *g20* and its application to PCR-based detection and quantification
18 techniques. *Appl Environ Microbiol* 64:2051-2060.

19 Gantar M, Svircev Z (2008) Microalgae and cyanobacteria: food for thought. *J Phycol* 44:260–268.

20 Gromov BV (1983) Cyanophages. *Ann Microbiol* 134:43-59.

21 Hewson I, O’Neil JM, Dennison WC (2001) Virus-like particles associated with *Lyngbia majuscula*
22 (Cyanophyta; Oscillatoriaceae) bloom decline in Moreton bay, Australia. *Aquat Microb Ecol*
23 25:207-213.

24 Huang S, Wang K, Jiao N, Chen F (2011) Genome sequences of siphoviruses infecting marine
25 *Synechococcus* unveil a diverse cyanophage group and extensive phage-host genetic exchanges.
26 *Environ Microbiol* 14:540-558.

27 Jenkins CA, Hayes PK (2006) Diversity of cyanophages infecting the heterocystous filamentous
28 cyanobacterium *Nodularia* isolated from the brackish Baltic Sea. *J Mar Biol Ass UK* 86:529-
29 536.

30 Jourdan JP (2006) Cultivez votre spiruline. Manuel de culture artisanale pour la production de
31 spiruline, 146 p (<http://www.antenna.ch/documents>).

32 Kettler GC, Martiny AC, Huang K, Zucker J, Coleman ML et al. (2007) Patterns and implications
33 of gene gain and loss in the evolution of *Prochlorococcus*. *PLoS Genet* 3: e231.
34 doi:10.1371/journal.pgen.0030231.

35 Larsen A, Flaten GAF, Sandaa RA, Castberg T, Thyrrhaug R, Erga SR, Jacquet S, Bratbak G (2004)
36 Spring phytoplankton bloom in Norwegian coastal waters: Microbial community dynamics,
37 succession and diversity. *Limnol Oceanogr* 49:180-190.

38 Larsen JB, Larsen A, Bratbak G, Sandaa RA (2008) Phylogenetic Analysis of Members of the
39 *Phycodnaviridae* Virus Family, Using Amplified Fragments of the Major Capsid Protein Gene.
40 *Appl Environ Microbiol* 74: 3048–3057.

41 Manen JF, Falquet J (2002) The *cpcB-cpcA* locus as a tool for the genetic characterization of the
42 genus *Arthrospira* (Cyanobacteria): Evidence for horizontal transfer. *Int J Syst Evol Microbiol*
43 52:861–867.

44 Mann NH (2003) Phages of the marine cyanobacterial picophytoplankton. *FEMS Microb Rev*
45 27:17-34.

46 Mann NH, Cook A, Millard A, Bailey S, Clockie MR (2003) Marine ecosystems: bacterial
47 photosynthesis genes in a virus. *Nature* 424:741.

48 Matteson AR, Loar SN, Bourbonniere RA, Wilhelm SW (2011) Molecular enumeration of a
49 cyanophage in a Laurentian Great Lake: quantitative evidence for ecological importance. *Appl*
50 *Environ Microbiol* 77:6772-6779.

51 McDaniel LD, delaRosa M, Paul JH (2006) Temperate and lytic cyanophages from the Gulf of
52 Mexico. *J Mar Biol Ass UK* 86:517-527.

- 1 Millard A, Clockie MRJ, Shub DA, Mann NH (2004) Genetic organization of the *psbAD* region in
2 phages infecting marine *Synechococcus* strains. PNAS 101:11007-11012.
- 3 Nagasaki K, Bratbak G (2010) Isolation of viruses infecting photosynthetic and non-photosynthetic
4 protists. Limnology and Oceanography: Methods, Manual on Aquatic Viral Ecology, p 92–101.
- 5 Ohki K, Fujita Y (1996) Occurrence of a temperate cyanophage lysogenizing the marine
6 cyanophyte *Phormidium persicinum*. J Phycol 32:365-370.
- 7 Padan E, Shilo M, Oppenheim B (1972) Lysogeny of the blue-green alga *Plectonema boryanum* by
8 LPP2-SPI cyanophage. Virol 47:525-526.
- 9 Padan E, Shilo M (1973) Cyanophage-viruses attacking blue-green algae. Bacteriol Rev 37:343-
10 370.
- 11 Palenik B (2003) The genome of a motile marine *Synechococcus*. Nature 424:1037-1042.
- 12 Personnic S, Domaizon I, Sime-Ngando T, Jacquet S (2009) Seasonal variations of microbial
13 abundances and of virus- vs. flagellate-induced mortality of picoplankton in some peri-alpine
14 lakes. J Plank Res 31:1161-1177.
- 15 Pulz O, Gross W (2004) Valuable products from biotechnology of microalgae. Appl Microbiol
16 Biotechnol 65:635–648.
- 17 Rosenberg JN, Oyler GA, Wikinson L, Betenbaugh MJ (2008) A green light for engineered algae:
18 redirecting metabolism to fuel a biotechnology revolution. Curr Opin Biotechnol 19:430–436.
- 19 Safferman RS (1973) Phycovirus. In The biology of blue-green algae (Eds NG Carr and BA
20 Whitton), Blackwell Scient Pub, Oxford, pp 214-237.
- 21 Safferman RS, Cannon RE, Desjardins PR, Gromov BV, Haselkorn R, Sherman LA, Shilo M
22 (1983) Classification and nomenclature of viruses of cyanobacteria. Interviol 19:61-66.
- 23 Sherman LA, Brown RM Jr (1978) Cyanophages and viruses of eukaryotic algae. In
24 Comprehensive virology (Eds H Fraenkel and RR Wagner), Plenum Press, New York, pp145-
25 234.
- 26 Short S, Suttle CA (2005) Temporal dynamics of natural communities of marine algal viruses and
27 eukaryotes. Aquat Microb Ecol 32:107-119.
- 28 Sime-Ngando T, Mignot JP, Amblard C, Bourdier G, Desvillettes C, Quiblier-Lloberas C (1996)
29 Characterization of planktonic virus-like particles in a French mountain lake: methodological
30 aspects and preliminary results. Intern J Limnol 32: 259-263.
- 31 Sullivan MB, Waterbury JB, Chisholm SW (2003) Cyanophages infecting the marine
32 cyanobacterium *Prochlorococcus*. Nature 424:1047-1051.
- 33 Sullivan MB, Coleman ML, Weigle P, Rohwer F, Chisholm SW (2005) Three *Prochlorococcus*
34 cyanophage genomes: signature features and ecological interpretations. Plos Biol 3:0790-0806.
- 35 Sullivan MB, Coleman MC, Quinlivan V, Rosenkrantz JE, DeFrancesco AS, Tan G, Fu R, Lee JA,
36 Waterbury JB, Bielawski JP, Chisholm SW (2008) Portal protein diversity and phage ecology.
37 Environ Microbiol 10:2810-23.
- 38 Suttle CA (2000) Cyanophages and their role in the ecology of cyanobacteria. In The Ecology of
39 cyanobacteria, (Eds BA Whitton and M Potts), Kluwer Acad Press, pp563-589.
- 40 Tamura K, Peterson D, Peterson N, Stecher G, Nei M, and Kumar S (2011) MEGA5: Molecular
41 Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and
42 Maximum Parsimony Methods. Molecul Biol Evol 28:2731-2739.
- 43 Thomas R, Jacquet S, Grimsley N, Moreau H (accepted) Strategies and mechanisms of viral
44 resistance in phytoplankton. Adv Oceanogr Limnol
- 45 Vault D (1989) CytoPC: processing software for flow cytometric data. Signal Noise 2:8.
- 46 Vonshak A, Tomaselli L (2000) *Arthrospira (Spirulina)*. In The ecology of cyanobacteria (eds BA
47 Whitton and M Potts). Kluwer Acad Pub, pp 505-522.
- 48 Wang K, Chen F (2008) Prevalence of highly host-specific cyanophages in the estuarine
49 environment. Environ Microbiol 10:300-312.
- 50 Waterbury JB, Valois FW (1993) Resistance to co-occurring phages enables marine *Synechococcus*
51 communities to coexist with cyanophages abundant in seawater. Appl Environ Microb 59:3736-
52 3743.

1 Weinbauer MG, Peduzzi P (1994) Frequency, size and distribution of bacteriophages in different
2 marine bacterial morphotypes. *Mar Ecol Progr Ser* 108:11-20.

3 Wilhelm SW, Carberry MJ, Eldridge ML, Poorvin L, Saxton MA, Doblin MA (2006) Marine and
4 freshwater cyanophages in a Laurentian Great Lake: evidence from infectivity assays and
5 molecular analyses of *g20* genes. *Appl Environ Microbiol* 72:4957-4963.

6 Wilson WH, Joint IR, Carr NG, Mann NH (1993) Isolation and molecular characterisation of five
7 marine cyanophages propagated on *Synechococcus* sp. WH 7803. *Appl Environm Microbiol*
8 59:3736-3743.

9 Wilson WH, Nicholas JF, Joint IR, Mann NH (2000) Analysis of cyanophage diversity in the
10 marine environment using denaturing gradient gel electrophoresis. In: Bell CR, Brylinsky M,
11 Johnson-Green P (eds) *Microbial biosystems: New Frontier. Proc 8th Int Symp Microb Ecol.*
12 Halifax, Canada. Atlantic Canada Society for Microbial Ecology, Kentville, p 565–570.

13 Yoshida T, Nagasaki K, Takashima Y, Shirai Y, Tomaru Y, Takao Y et al. (2008) Ma-LMM01
14 infecting toxic *Microcystis aeruginosa* illuminates diverse cyanophage genome strategies. *J*
15 *Bact* 190:1762–1772.

16
17
18

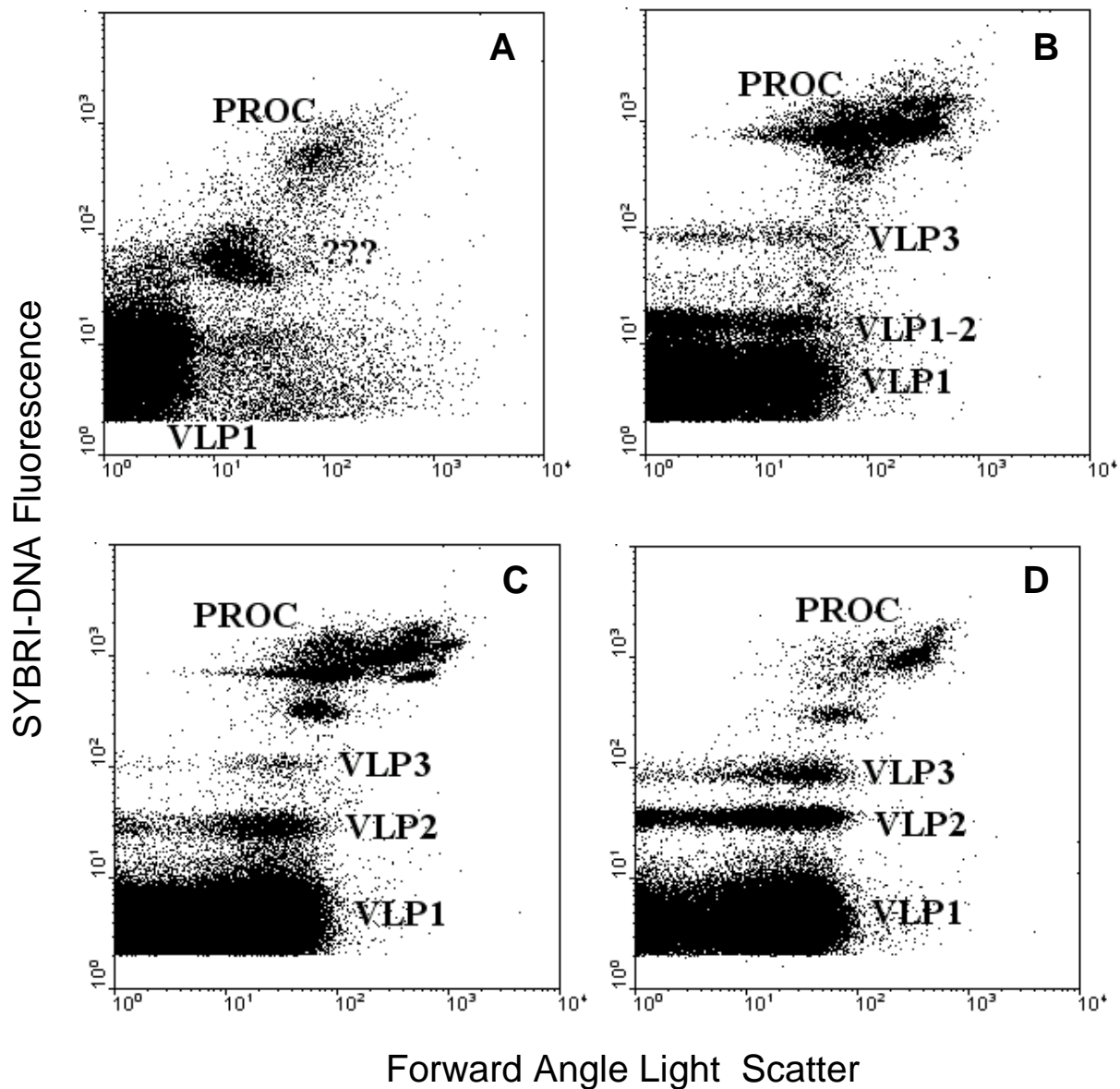
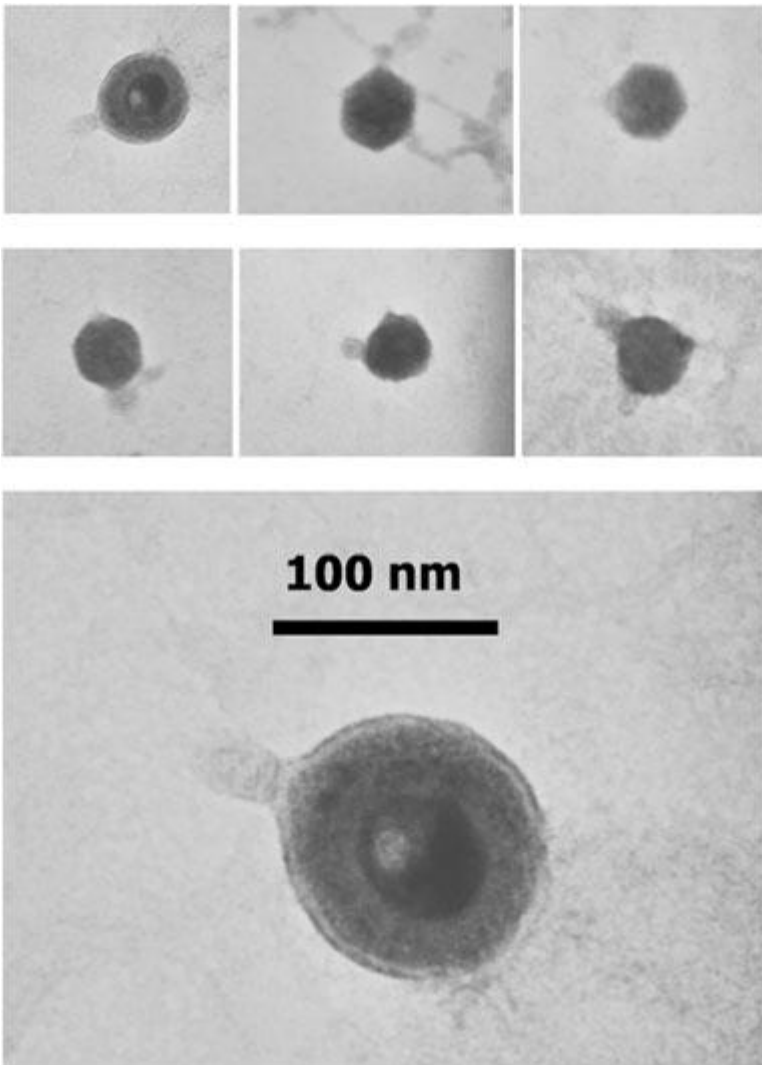


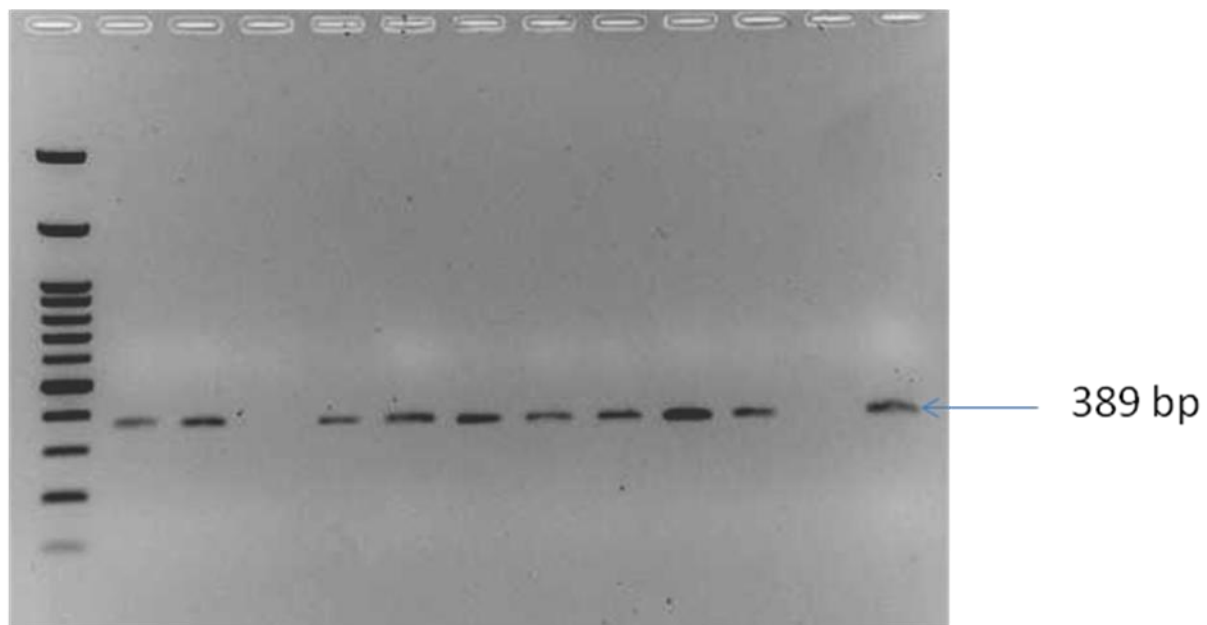
Fig1 FL1 (Sybr Green I – DNA complex fluorescence) vs. FSC (Forward Angle Light Scatter) cytograms showing typical viral signatures from the original samples received from *le chant de l'eau*. Samples were issued from a pool where there was no cyanobacterial mortality and recently fed with new medium (A), an important cyanobacterial mortality (B), recently inoculated with a new cyanobacterial culture but on a former contaminated site (C) and the beginning of symptoms of cyanobacterial mortality (D). Different signatures of viruses were referred to as VLP1, VLP1-2, VLP2 and VLP3. The prokaryotic (bacteria, cyanobacteria and archaea) community was referred to as PROC. VLP2 was the signature of the virus infecting *A. platensis*.

1

2



1
2
3 Fig. 2 TEM micrographs of the cyanopodivirus infecting *A. platensis*
4



Lanes:

M: 100 bp DNA ladder

1: Sample 1

2: Sample 2

3: Sample 3

4: Sample 4

5: Sample I

6: Sample II

7: Sample III

8: Sample IV

9: Sample V

10: Sample VI

11: Negative control

12: Positive control (D23)

Fig3 PCR amplification using *pol2* primers for the cyanopodovirus resulting in an amplicon of 389 bp. All the samples received from different pools of the farms were subjected to PCR with *pol2* primers. Lanes 1: S1, 2: S2, 3: S3, 4: S4, 5: SI, 5: SII, 6: SIII, 7: SIV, 8: S V, 9: S VI, N: Negative control 10: Positive control (D23), M: 100 bp ladder. Band absence is only observed in the negative control and for the pool in which only medium water was added.

1

2

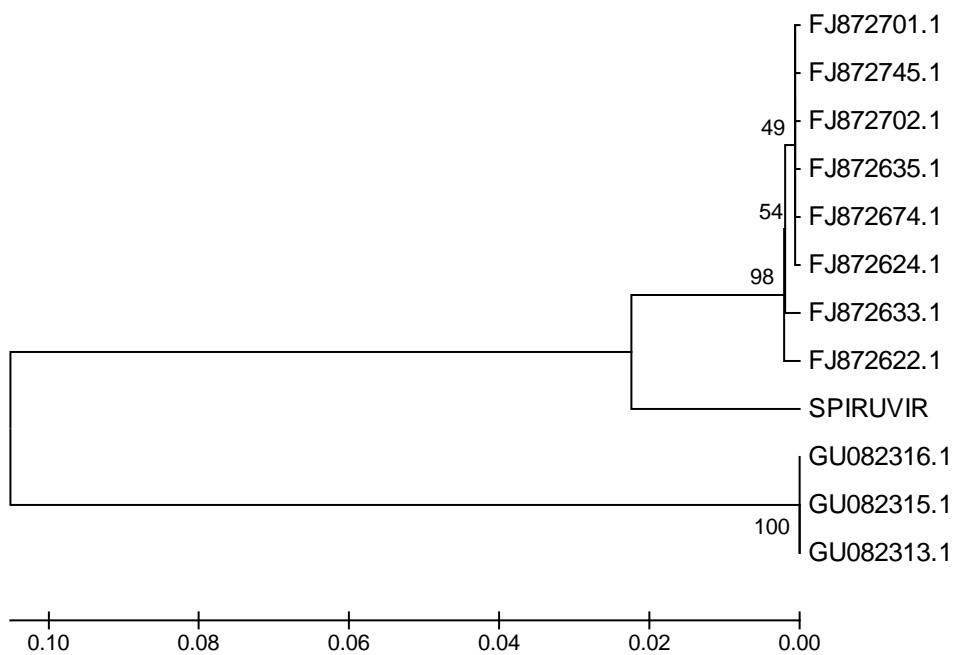


Fig4 Phylogenetic (Neighbour joining tree) tree showing the similarity of Spiruvir DNA polymerase gene to the podoviral DNA polymerase sequences in NCBI. The bootstrap value was given as 1000 using Kimura 2 parameter model for constructing the tree.

1

2

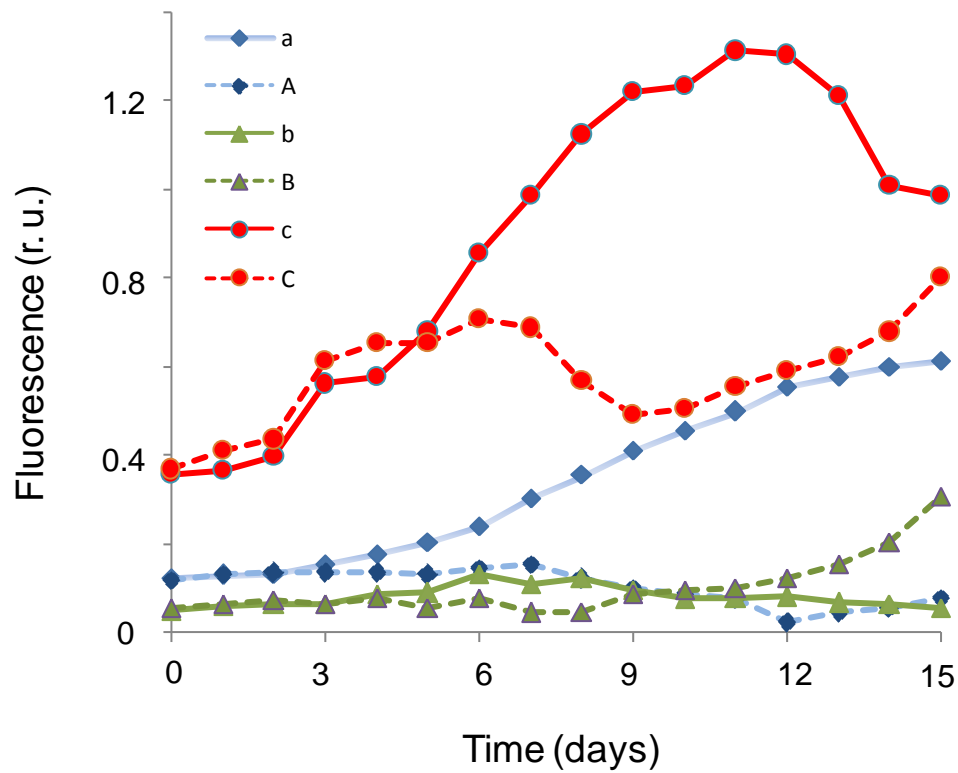


Fig5 Different cultures of *A. platensis* were inoculated with the virus at day 1 with a MOI of 1. It was clear from these infection experiments that infectivity and mortality processes differed in the cultures and that resistance could also be detected. A, B; C: Infected; a, b, c : not infected

Tab1 Table showing the details of the various primers used in this study.

Sl. No	Primer name	Primer sequence (5'-3') (F/R)	Target	Reference
1	CPS1/CPS2	GTAGWATTTTCTACATTGAYGTTG G/ GGTARCCAGAAATCYTCMAGCAT,	Portal-vertex-capsid-protein encoded gene <i>g20</i> of Cyanomyovirus	Fuller et al. 1998
2	CPS4/CPS5	GTAGAATTTTCTACATTGATGTTGG / GGTAACCAGAAATCTTCAAGCAT	Portal-vertex-capsid-protein encoded gene <i>g20</i> of Cyanomyovirus	Wilson et al. 2000
3	CPS1.1/CPS8.1	GTAGWATWTTYTAYATTGAYGTW GG/ ARTAYTTDCCDAYRWAUWGGWTC	Portal-vertex-capsid-protein encoded gene <i>g20</i> of Cyanomyovirus	Sullivan et al. 2008
4	MZIA1bis/MZIA6	GATATTTGIGGIGTTCAGCCATGA/ CGCGGTTGATTTCCAGCATGATTT C	Major-capsid-protein encoded gene <i>g23</i> of Myoviridae	Filée et al. 2005
5	<i>pol1</i> (CP-DNAP-349F/533Ra/533 Rb)	CCAAAYCTYGCMCARGT/ CTCGTCRTGSACRAASGC/ CTCGTCRTGDATRAASGC	DNA polymerase gene of cyanopodovirus	Chen et al. 2009
6	<i>pol2</i> (New pol)	ACTGCAACGCCTGGGATGGTG/ AGCAATGCGGCGACCGTCAA	DNA polymerase gene of cyanopodovirus	This study
7	MCP F/R	GGYGGYCARCGYATT / TGIARYTGYTCRAYIAGGTA	Major capsid protein of algal viruses	Chen and Suttle 1995
8	AVS1/2	(GARGGIGCIACIGTIYTIGAYGC / GCIGCRTAICKYTTYTTISWRTA)	Major capsid protein of algal viruses	Larsen et al. 2008

Tab2 Table showing PCR results for different primers targetting different viral groups, for all the samples received from different pools. The samples were positive only for the *pol* primers (referred in the table as pol-Chen and New pol, *i.e.* *pol1* and *pol2* in the text) targeting the cyanopodovirus. The absence of positive result is only observed for S3 that corresponded to a pool just filled with the culture medium and in which no cyanobacteria (and viruses) were added.

Samples	Primer Sets								
	mcp R/F	avs 1/2	psbA F/R	pol-Chen	g23	CPS1,1/8,1	CPS1/2	CPS4/5	New pol
S1	-	-	-	+	-	-	-	-	+
S2	-	-	-	+	-	-	-	-	+
S3	-	-	-	-	-	-	-	-	-
S4	-	-	-	+	-	-	-	-	+
SI	-	-	-	+	-	-	-	-	+
SII	-	-	-	+	-	-	-	-	+
SIII	-	-	-	+	-	-	-	-	+
SIV	-	-	-	+	-	-	-	-	+
SV	-	-	-	+	-	-	-	-	+
SVI	-	-	-	+	-	-	-	-	+