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Letter

Two Virus-like Particles that Cause Lytic Infections in Freshwater Cyanobacteria

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Dear Editor,

We report the results of the preliminary isolation of two virus-like particles (VLPs) that are infectious to freshwater cyanobacteria from Lake Donghu, the largest urban lake in China, located in Wuhan City, Hubei Province. By light and transmission electron microscopy, we observed VLPs causing lytic infections in freshwater bloom-forming cyanobacteria, and we detected their infections by exposing the VLPs to 12 cyanobacterial strains, including *Microcystis aeruginosa* HAB1801 and *Anabaena spiroides* HAB1211. They were termed '*Anabaena spiroides* geminivirus-like (AsGV-L)' particles and '*Microcystis aeruginosa* corticovirus-like (MaCV-L)' particles, based on their ultrastructural morphological characteristics and host specificities.

The viruses and virus-like particles in aquatic ecosystems specific to cyanobacteria (cyanophages) and eukaryotic algae (phycoviruses) are recognized as having a worldwide distribution (Ackermann H W, 2007; Middelboe M, et al., 2008). They are the most abundant and likely the most diverse biological entities in aquatic environments; increasingly they are being recognized as important players in global geochemical cycles (Danovaro R, et al., 2011; Suttle C A, et al. 2007). Cyanophages are now known to play a critical role in cyanobacterial ecology, diversity, mortality and evolution (Avrani S, et al., 2011; Lindell D, et al., 2007). This has been an increasing cause for concern; the distribution, population diversity, morphology and genome of cyanophages have been widely reported in recent years (Gao E B, et al., 2012; Sullivan M B, et al., 2006).

Some cyanobacterial species are harmful to humans and animals because they produce cyanobacterial toxins (Dietrich D, et al., 2005). *Microcystis aeruginosa* and *Anabaena spiroides* are common toxic cyanobacteria in freshwaters and may form dense blooms in eutrophic waters (Rueckert A, et al., 2007; Wood S A, et al., 2012). Although viruses are most often studied as pathogens, some viruses are beneficial to humans and their own hosts (Roossinck M J, 2011). It has been suggested that cyanophages are environmentally effective both in controlling certain types of bloom-forming cyanobacteria and in assisting gene transfer between microorganisms in aquatic ecosystems (Partensky F, et al., 2011; Rohwer F, et al., 2009).

Many marine cyanophages have been isolated, thus allowing characterization of the cyanophages and detection of host–cyanophage interactions (Bryan M J, et al., 2008). Certain cyanophage strains that infect freshwater cyanobacteria have also been detected (Baker A C, et al., 2006). For example, studies on the diversity and distribution of virioplankton have been reported in a shallow freshwater lake, Lake Donghu (Liu Y M, et al., 2006; Zhang Q Y, et al., 2012). However, only a few freshwater cyanophage strains were reported to be isolated (Gao E B, et al., 2009). In order to gain a better understanding of the VLPs that lead to cell lysis of bloom-forming toxic cyanobacteria in freshwater, we need more information about host ranges and infection mechanisms from the culturable cyanophages. The description of a cyanophage's morphology (size, shape, etc.) is beneficial to elucidate the possible infection mechanism. In this study, we have described the results of light microscopy and electron microscopy observations which revealed the host ranges and morphological features of the VLPs.

Table 1 lists the 12 strains of cyanobacteria used to study the infection and isolation of VLPs, and the susceptibility

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Table 1. The cyanobacterial strains used in our studies and their susceptibilities to AsGV-L and MaCV-L

Cyanobacterial strain	AsGV-L	MaCV-L
Microcystis aeruginosa HAB1801	−	+
Microcystis aeruginosa HAB0334	−	−
Microcystis aeruginosa FACHB-526	−	−
Microcystis aeruginosa FACHB-905	−	−
Microcystis aeruginosa FACHB-915	−	−
Anabaena spiroides HAB1211	+	−
Anabaena spiroides HAB0502	−	−
Anabaena spiroides HAB0508	−	−
Anabaena spiroides PCC7120	−	−
Anabaena oumiana HAB0984	−	−
Synechococcus sp. FACHB-1061	−	−
Botryococcus sp. FACHB-1108	−	−

AsGV-L: *Anabaena spiroides* geminivirus-like particle
MaCV-L: *Microcystis aeruginosa* corticovirus-like particle
+ sensitive; − not sensitive

of the cyanobacteria to the VLPs. We obtained the specimens from the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Sciences. Each cyanobacterial strain was clonally grown in BG-11 medium at 25 ℃ under a 14 h light to 10 h dark cycle of 35 μmol photons $m^{-2}·s^{-1}$ with cool white fluorescent illumination. To isolate and cultivate freshwater VLPs from the aquatic ecosystems, many water samples were collected from eutrophicated areas of Lake Donghu in September 2010. The water samples were centrifuged at 8000 ×g for 10 min at 4 ℃ and filtered through a 0.45 μm pore size filter (Millipore, Bedford, MA, USA) to remove zooplankton, phytoplankton, bacteria and cellular debris from the samples. Aliquots (200 μL) of the samples were added to 1 mL of cyanobacterial cultures per well in 24-well plates and fresh BG-11 medium was used for negative control. They were incubated under the same conditions as stated above. The cultures were visually inspected and examined by light microscopy daily to check for visible changes in cyanobacterial morphology.

Among these water samples, only two of them could induce obvious host cell lysis within one week. Host cell lysis by one sample was observed in *Anabaena spiroides* HAB1211 cultures, and the other in *Microcystis aeruginosa* HAB1801 cultures. In the infected *Anabaena spiroides* cultures, host filaments settled to the bottom of the cultured wells with a series of pathological changes, including random lysis of the cyanobacterial vegetative cells and shortening of the filament lengths. Lysis of almost all the host cells then occurred within 4–5 days of the inoculation (Fig. 1A and 1B). In the infected *Microcystis aeruginosa* cultures, most of the cyanobacterial cells were destroyed and the initial green color visibly changed to light yellow. The presence of these freshwater samples in Lake Donghu

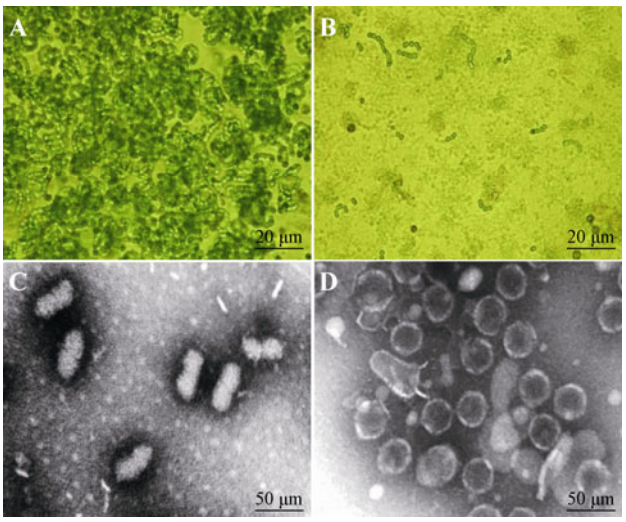

Fig. 1. Micrographs of cyanobacterial cultures and virus-like particles. A: Normal *Anabaena spiroides* HAB1211 cultures; B: *Anabaena spiroides* HAB1211 cultures infected with *Anabaena spiroides* geminivirus-like (AsGV-L) particles. Scale bar = 20 μm. C: Negative staining electron microscope image of AsGV-L particles; D: Negative staining electron microscope image of *Microcystis aeruginosa* corticovirus-like (MaCV-L) particles. Scale bar = 50 nm.

indicated the presence of unreported cyanophages in this lake.

We determined the host ranges of the VLPs by adding 0.2 mL aliquots of the above cyanobacterial lysates to triplicate 0.8 mL cultures of the exponentially growing cyanobacterial species listed in Table 1. Growth of cyanobacterial cultures without the suspension served as a negative control. The occurrence of host cell lysis was monitored daily by light microscopy. Cyanobacterial strains in which lysis did not occur after 14 days of incubation were considered not to be susceptible hosts for the VLPs. In the four tested cyanobacterial species (12 strains), the VLPs only lysed *Anabaena spiroides* HAB1211 and *Microcystis aeruginosa* HAB1801, corresponding to AsGV-L particles and MaCV-L particles, respectively. The results showed a very narrow host range for both AsGV-L and MaCV-L particles, but all VLPs together could have a wider host range.

We used transmission electron microscopy to obtain morphological information for the classification of the viruses. AsGV-L and MaCV-L particles were propagated in *Anabaena spiroides* HAB1211 and *Microcystis aeruginosa* HAB1801, respectively, and then the VLPs were purified by sucrose density gradient centrifugation as described previously (Gao E B, et al., 2009). We examined the negatively stained VLP samples. AsGV-L particles displayed geminivirus-like morphology, noticeably consisting of two incomplete icosahedra joined together, 79 ± 5 nm

(mean ± SD) in length and 28 ± 3 nm in diameter (Fig. 1C). MaCV-L particles appeared round in shape with icosahedral symmetry and a non-enveloped capsid, 50 ± 3 nm in diameter (Fig. 1D). To our knowledge, this is the first time that geminivirus-like particles and corticovirus-like particles have been reported to infect cyanobacteria. We are unclear as to why the corticovirus-like particles shown in Fig. 1D seem to have a low electron density and do not contain DNA in the nucleocapsids.

Multiplication of such particles in cyanobacterial cell cultures allowed us to prepare purified VLPs so that we could investigate their characteristics. However, the VLPs gradually lost infectivity over three generations. The same phenomenon was reported in earlier studies. Nine cyanophages of the A(L) series were isolated, but most of them had been lost over several generations (Hu N T, et al., 1981). Although multiple methods, such as drying, autoclaving and UV radiation, can inactivate phages, the mechanisms of cyanophage inactivation over generations have been rarely studied. Resistance to co-occurring cyanophages acquired by more and more host cyanobacterial cells has been considered as a possible reason for loss of cyanophage infectivity over generations. In general, cultivating cyanophages is very difficult and pure cyanobacterial culture is an essential precondition for effectively purifying cyanophages. In order to further identify the VLPs, we need to improve cyanophage cultivation techniques to prevent phage inactivation and obtain a high phage yield.

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Author contributions

Conceived and designed the experiments: QY Zhang; Performed the experiments: SH Li and T Ou; Analyzed the data: SH Li and QY Zhang; Contributed reagents/materials: SH Li and QY Zhang; Wrote the paper: T Ou and QY Zhang.

References

Ackermann H W. 2007. **5500 phages examined in the electron microscope**. Arch Virol, 152: 227-243.

Avrani S, Wurtzel O, Sharon I, Sorek R, and Lindell D. 2011. **Genomic island variability facilitates prochlorococcus-virus coexistence**. Nature, 474: 604-608.

Baker A C, Goddard V J, Davy J, Schroeder D C, Adams D G, and Wilson W H. 2006. **Identification of a diagnostic marker to detect freshwater cyanophages of filamentous cyanobacteria**. Appl Environ Microbiol, 72: 5713-5719.

Bryan M J, Burroughs N J, Spence E M, Clokie M R J, Mann N H, and Bryan S J. 2008. **Evidence for the intense exchange of MazG in marine cyanophages by horizontal gene transfer**. PLoS One, 3: e2048.

Danovaro R, Corinaldesi C, Dell'Anno A, Fuhrman J A, Middelburg J J, Noble R T, and Suttle C A. 2011. **Marine viruses and global climate change**. FEMS Microbiol Rev, 35: 993-1034.

Dietrich D, and Hoeger S. 2005. **Guidance values for microcystins in water and cyanobacterial supplement products (blue-green algal supplements): A reasonable or misguided approach?.** Toxicol Appl Pharm, 203: 273-289.

Gao E B, Gui J F, and Zhang Q Y. 2012. **A novel cyanophage with a cyanobacterial nonbleaching protein a gene in the genome**. J Virol, 86: 236-245.

Gao E B, Yuan X P, Li R, and Zhang Q Y. 2009. **Isolation of a novel cyanophage infectious to the filamentous cyanobacterium planktothrix agardhii(cyanophyceae) from lake donghu, china**. Aquat Microb Ecol, 54: 163-170.

Hu N T, Thiel T, Giddings Jr T H, and Wolk C P. 1981. **New *Anabaena* and *Nostoc* cyanophages from sewage settling ponds**. Virology, 114:236-246.

Lindell D, Jaffe J D, Coleman M L, Futschik M E and others. 2007. **Genome-wide expression dynamics of a marine virus and host reveal features of co-evolution**. Nature, 449: 83-86.

Liu Y M, Yuan X P, and Zhang Q Y. 2006. **Spatial distribution and morphologic diversity of virioplankton in lake donghu, china**. Acta Oecol, 29: 328-334.

Middelboe M, Jacquet S, and Weinbauer M. 2008. **Viruses in freshwater ecosystems: An introduction to the exploration of viruses in new aquatic habitats**. Freshwater Biol, 53: 1069-1075.

Partensky F, and Garczarek L. 2011. **Microbiology: Arms race in a drop of sea water**. Nature, 474: 582-583.

Rohwer F, and Thurber R V. 2009. **Viruses manipulate the marine environment**. Nature, 459: 207-212.

Roossinck M J. 2011. **The good viruses: Viral mutualistic symbioses**. Nat Rev Microbiol, 9: 99-108.

Rueckert A, Wood S A, and Cary S C. 2007. **Development and field assessment of a quantitative pcr for the detection and enumeration of the noxious bloom-former anabaena planktonica**. Limmol Oceanogr-Meth, 5: 474-483.

Sullivan M B, Lindell D, Lee J A, Thompson L R, Bielawski J P, and Chisholm S W. 2006. **Prevalence and evolution of core photosystem ii genes in marine cyanobacterial viruses and their hosts**. PLoS Biol, 4: e234.

Suttle C A. 2007. **Marine viruses—major players in the global ecosystem**. Nat Rev Microbiol, 5: 801-812.

Wood S A, Kuhajek J M, Winton M, and Phillips N R. 2012. **Species composition and cyanotoxin production in periphyton mats from three lakes of varying trophic status**. FEMS Microbiol Ecol, 79: 312-326.

Zhang Q Y, and Gui J F. 2012. **Freshwater viroplankton**. In: Zhang Q Y, Gui J F. Eds. Atlas of Aquatic Viruses and Viral Diseases, Beijing, Science China Press. pp 404-460.