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Letter

Host Re-identification of Cyanophage PP and Its Implications for Host Range and Specificity

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Dear Editor,

In this study, we re-identified the hosts of cyanophage PP. Twenty-three candidate algal strains were tested, and the results indicated that seven strains belonging to *Plectonema* and *Phormidium* are the hosts of cyanophage PP, including two previously reported filamentous cyanobacteria, *Plectonema boryanum* and *Phormidium foveolarum* (Zhao et al. 2002). However, several species or strains within the two genera were found not to be hosts of cyanophage PP, implying that the host range is relatively specific.

Cyanophages are virus-infecting cyanobacteria. The first to be isolated was cyanophage LPP, which infects *Lyngbya*, *Plectonema*, and *Phormidium* species (Safferman and Morris, 1963). Cyanophage PP, a lytic virus with short-tailed, icosahedral-shaped, double-stranded DNA, was the first cyanophage to be isolated and identified in China, and its hosts were reported to be *Plectonema* (*Plectonema boryanum*IU594) and *Phormidium* (*Phormidium foveolarum* IU427) (Zhao et al. 2002).

In the present study, cyanophage PP (isolated by Prof. Yijun Zhao, Central China Normal University) provided by Dr. Fei Deng (Wuhan Institute of Virology, Chinese Academy of Sciences) was used. The plasmid was stored in 10% chloroform at 4°C and prepared as described previously (Cheng et al. 2007). The 23 algal strains (Table 1) used for the host re-identification of cyanophage PP were originally obtained from the Freshwater Algae Culture Collection (Institute of Hydrobiology, Chinese Academy of Sciences; FACHB collection) and were all cultured in BG11 medium (Rippka et al. 1979) at 25±1 °C, 30 $\mu E \cdot m^{-2} \cdot s^{-1}$. White light was provided by a 36-W white fluorescent tube (FSL YZ36 T8/880, Foshan, China) under a 12-h light/12-h dark photoperiod.

For the preliminary screening, 0.5 mL 10^8 plaque-forming units (PFU)·mL^{-1} cyanophage PP were added to each of 5.0 mL algal culture (23 strains) in the logarithmic phase cultured in a buret. The effect was assessed by observing the color change of the culture; 15 strains faded to various degrees of yellow. These 15 strains were selected for the second round of screening. In this round, 0.2 mL 10^8 PFU·mL^{-1} cyanophage PP were added to each 3.0 mL algal culture (15 strains; Table 1) at the logarithmic phase as an aliquot in 12-well cell culture plates (Corning, New York, NY, USA), and the effect of the algae on the virus was assessed every day under an inverted microscope. In the two tests, both control groups were free of cyanophage PP. Continuous light was provided for cyanophage infection during the test period, and each treatment was tested in duplicate.

We found that four strains of *P. boryanum* (IU594, FACHB-402, FACHB-246, and FACHB-240) and three species of *Phormidium* (FACHB-238, FACHB-239, and FACHB-161) faded to yellow and lysed in less than 12 h (Fig. 1 shows microscopic images of *P. boryanum* IU 594 without and with cyanophage PP infection), indicating that these seven cyanobacteria are the hosts of cyanophage PP (Table 1). In contrast, the remaining eight strains (FACHB-1136, FACHB-1129, FACHB-1099, FACHB-857, FACHB-723, FACHB-890, FACHB-722, and FACHB-388) were not lysed, suggesting that these strains are not hosts for cyanophage PP.

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Table 1. Host range of cyanophage PP

Strains	Latin name	Host	Strains	Latin name	Host
IU 594*	*Plectonema boryanum*	Yes	FACHB-723*	*Phormidium mucicola*	No
FACHB-402*	*Plectonema boryanum*	Yes	FACHB-401	*Phormidium autumnale*	No
FACHB-246*	*Plectonema boryanum*	Yes	FACHB-239*	*Phoridium foveolarum*	Yes
FACHB-240*	*Plectonema boryanum*	Yes	FACHB-238*	*Phormidium lucidum*	Yes
FACHB-200	*Plectonema phormidiodes*	No	FACHB-161*	*Phormidium ambiguum*	Yes
FACHB-1137	*Phormidium* sp.	No	FACHB-890*	*Lyngbya cryptovaginatus*	No
FACHB-1136*	*Phormidium* sp.	No	FACHB-722*	*Lyngbya* sp.	No
FACHB-1129*	*Phormidium* sp.	No	FACHB-388*	*Lyngbya kuetzingii*	No
FACHB-1128	*Phormidium* sp.	No	FACHB-258	*Lyngbya spiralis*	No
FACHB-1099*	*Phormidium* sp.	No	FACHB-197	*Lyngbya attenuata*	No
FACHB-886	*Phormidium tenue*	No	FACHB-905	*Microcystis aeruginosa*	No
FACHB-857*	*Phormidium innudatun*	No			

*Strain was tested both in burets and 12-well cell culture plates

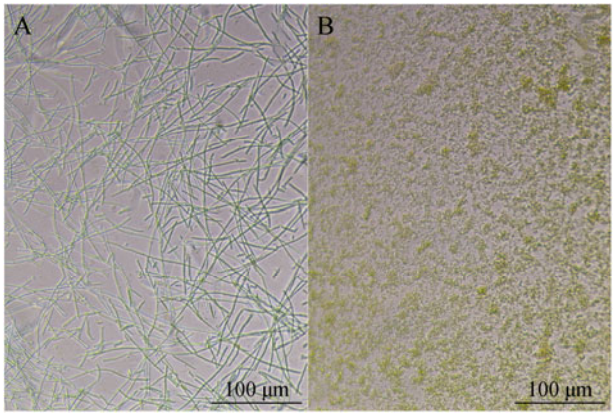

Fig. 1 Microscopic images of *Plectonema boryanum* IU 594 (A) without and (B) with cyanophage PP infection (Scale bar= 100 μm).

The designation of cyanophages is based on the initial letter of the host's Latin name (Safferman et al. 1983; Zhao et al. 1999). In the present study, all the strains defined as cyanophage PP hosts belonged to the two genera *Plectonema* and *Phormidium*. Although *Lyngbya* also belongs to the same order, Oscillatoriales, and its DNA has a similar mol% G+C content (46%) and buoyant density (1.705 g·cm^{-3}) as *Plectonema* and *Phormidium* (Edelman M, et al., 1967), all five *Lyngbya* species tested in this study were found not to be hosts of this cyanophage.

In addition, some species or strains in the same genera as *P. phormidiodes* and *Phormidium* sp. are also not hosts of cyanophage PP. This paradox was reported in a previous study that sought to determine the host range of cyanophage LPP (Johnson and Potts 1985). The likely reason for this discrepancy lies in the disarray of cyanobacterial taxonomy and species designations in the past (Safferman et al. 1983, Suttle 2002). It was reported that different species or strains assigned to the same genera by traditional taxonomy are genetically distinct based on techniques such as 16S rRNA sequence amplification, random amplified polymorphic DNA, and pigment assay (Wilmotte 2004, Palinska et al. 2011), and this may partly explain the difference in host specificity of cyanophage PP, even within in the same genera. In addition, it should be noted that cyanophage PP hosts are not limited to the species found in the present study.

It has been gradually recognized that virioplankton, including cyanophages, play a key role in the aquatic ecosystem (Suttle 2002, 2005). They can regulate aquatic microbial community structure and diversity and have a potential effect in controlling harmful algal blooms (e.g., cyanophage and phycovirus). The presence of distinct taxonomic cyanobacteria groups in raw water is a universal phenomenon, implying that it is important to investigate cyanophage host range and specificity in order to apply cyanophages based on ecological function. For example, if the dominant cyanobacteria groups during bloom are cyanophage's hosts, we could use the cyanophage to reduce the biomass of dominant cyanobacteria groups selectively. In addition, it may be necessary to combine morphological and molecular methods for more accurate classification of host cyanobacteria.

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Author contributions

Q Zhou, N Wei, L Zheng and L Song, designed the research. Q Zhou and N Wei conducted the experiments. Q Zhou and L Song wrote the paper. All authors discussed the results and commented on the final manuscript.

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