Phylogenetic Diversity of Sequences of Cyanophage for *g*20 Gene from Napahai Wetland in China

C. Sun, J.K. Li, S. Li, Y.L. Wei, X.L. Ji*
College of Science and Technology, Kunming University of Science and Technology,
Kunming, 650500, Yunnan, China

***Abstract*—So far a large number of researches have shown that the cyanophages have a higher diversity in the marine and freshwater environment, but the report about the genetic diversity of cyanophages in wetland is little seen. In order to understand the genetic diversity of bacteriophage communities in Napahai wetland, the conserved structural gene (g20) of bacteriophage was amplified with the primers. The goal was to use this gene as a proxy to infer genetic richness in natural cyanophage communities and determine if sequences were more similar in Napahai wetland..In this study the distribution of g20 sequences were similar with paddy cyanophage sequences came from various regions through phylogenetic analysis. It indicated that the cyanophages in Napahai wetland had a rich diversity.**

Keywords-phylogenetic diversity; cyanophages g20 gene; Napahai wetland.

I. INTRODUCTION

As we all know viruses are abundant in various aquatic environment and play an important role in regulating the structure and diversity of microbial populations [1,2]. Because of greater amount and biodiversity of viruses, they are considered as the largest genomic reservoir and the vast majority of viruses are bacteriophages (phages) in the natural environments [1, 3]. So phage has important significance to microbial ecology food ring and influences on the genetic diversity of other microbiology.

Cyanophage is a class of bacteriophage capable of infecting cyanobacteria. Unicellular cyanobacteria in *Synechococcus* and *Prochlorococcus* are the most abundant picoplankton community in marine. Recently the study about the genetic diversity of cyanophages mainly based on the bacteriophage infecting *Synechococcus* and *Prochlorococcus* in ocean and lake [4-7].

Napahai is a unique seasonal plateau wetland with low latitude and high altitude in Yunnan province of China. It is an isolated and fragmented wetland ecosystem owning distinct rainy and dry seasons, and shows characteristics of terrestrial and aquatic environments. A large number of phages were one of the most important factors to balance wetland ecosystem. Undoubtedly, it is very important to study the phages systematic. The higher genetic diversity of *g*20 fragments were discovered in aquatic environments, including marines and lakes using the primers CPS1/CPS8 [8,9], while so far the research on genetic diverse of phage is rare in Napahai wetland. The use of primers CPS1/CPS8 promoted the further grouping of g20 genes in cyanophage in various water environments [1,10,11,12]. In this study, the amplified products of *g20* gene were similar with paddy cyanophage sequences came from various regions.

II. MATERIALS AND METHODS

A. Napahai Wetland Water Sampling and Ultrafiltration

The water sample was collected from the Napahai wetland of Shangri-La (E99°37'22", N27°53'32", 3266m, pH6.6) in 2013 and kept in the dark at 4℃[1].

Taken water samples from 4 ℃ refrigerator and centrifuged at 8000g for 30 min to remove soil particles and impurities etc. Then filtrated the samples with a 0.45-μm and 0.22-μm cellulose filter to completely remove bacteria. In finally concentrates of virus were stored in the dark at 4℃[1].

B. DNA Extraction and PCR Amplification

The treated water samples were frozen in liquid nitrogen or -80℃ frozen 8h, nozzle after laminating pierce holes. The lyophilized overnight freeze-dried into powder, DNA was extracted with the OMEGA virus genome extraction kit. The g20 gene fragment of cyanophage was amplified with the primers CPS1 (5′-GTAGWATTTTCTACATTGAYGTTGG-3′) and CPS8 (5′-AAATAYTTDCCAACAWATGGA-3′) [13]. Each 50μL PCR mixture contained 1μL forward and reverse primers, 1–2μL DNA template, 3μLdNTP (2.5 mM each), 0.5μL *Ex-Taq* polymerase (TaKaRa) and 5μL *Ex-Taq* buffer (TaKaRa); MilliQ water was added to yield the required volume per reaction. The amplification conditions were at 94℃ for 3min, followed by 35 cycles at 94℃ for 1min, 35℃for 1min and at 72℃ for 1min and a final extension at 72℃for 5min [1].

C. Clone Library Construction

The PCR amplification productions were attached into the pMD18-T vector, then transformed into DH5α competent cell according to the manufacturer's instructions. Picked positive clones randomly and transferred them into PCR tubes. Positive clones were numbered 1 to 80. And removed some of them to verify with colony PCR.

D. Phylogenetic Analysis

Homology comparison of respective g20 clones were examined by the BLAST Search engine at NCBI (http://www.ncbi.nlm.nih.gov/). Then constructed the phylogenetic tree using the MEGA4 software.

III. RESULTS

A. Primer Specificity and PCR Amplification

The primer was designed to detect the cyanophages in Napahai wetland. The goal was to amplify the family *Myoviridae*. Results show no cyanophages of *Podoviridae* or *Siphoviridae* or other phages are amplified with the primers CPS1 and CPS8. Strong bands about 700 bp were excised, purified and cloned (Figure 1). The result was conscience with those obtained with cyanophage isolates.

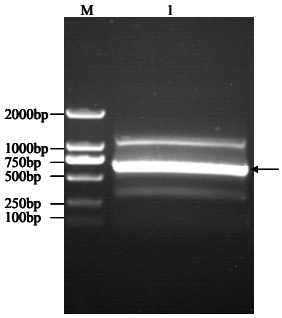

Figure 1. Temperature gradient amplified of concentrated phage of Napahai wetland with primers CPS1/CPS8
M: DNA markers, 1:G20 gene PCR amplification

B. Phylogenetic Diversity of Cyanophage Isolates

The sequence alignment of the *g*20 gene fragments from Napahai wetland indicated that the amplified region was highly conserved and suitable for inference of genetic relatedness among cyanophages by primers CPS1and CPS8[8]. Cyanophage isolates shared 73% to 92%. Clones 11 and 18 are similar with the floodwater of a Japanese paddy field, while the highest similar to clone in northeast China paddy field, and clone is 34% similar with Canadian Cultus Lake clone CUL02M-14 and other clones were distant from any isolates or clones, therefore, they were not grouped into any clusters [8](Figure 2).

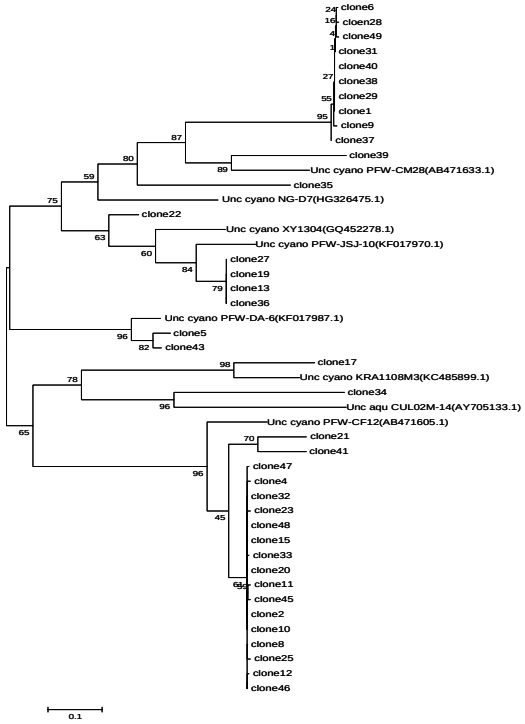

Figure 2. Phylogenetic relationships among the clones amplified of concentrated phage of Napahai wetland with primers CPS1/CPS8

IV. DISCUSSION

The primer CPS1/CPS8 is commonly used for PCR amplification of g20 gene of cyanophages in various aquatic environments, including lakes, marine and paddy. In this paper, we reported for the first time that the g20 genes in natural wetland water can also be amplified by this primer set. Though multiple PCR products were formed, most were g20 amplicons. It suggested that cyanophages also existed in Napahai wetland. These results showed that cyanophages in natural wetland were different from those in freshwater, marine water and even paddy floodwater. More reachers on the genetic diversity and populations of phage and host with changing environmental variables will provide new insights into the ecological function of wetland viruses.

V. ACKNOWLEDGMENTS

This research was supported by the National Natural Science Foundation of China (31160121), and State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences (SKLMR-20110606).

REFERENCES

[1] Jing R Y, Liu JJ, Yu Z H , Liu X B, Wang G H. Phylogenetic Distribution of the Capsid Assembly Protein Gene (g20) of Cyanophages in Paddy Floodwaters in Northeast China. *Plos One*,9: 1-11,2014.

[2] Hennes KP, Suttle CA, Chan AM. Fluorescently labeled virus probes show that natural virus populations can control the structure of marine

microbial communities. *Applied and Environmental Microbiology,* 61:3623–3627,1995.

[3] Kimura M, Jia Z, Nakayama N, Asakawa S. Viral ecology in soil: past,present, and future perspectives. *Soil Science and Plant Nutrition* ,54:1–32,2008.

[4] Waterbury JB, Watson SW, Valois FW, Franks DG. Biological and ecological characterization of the marine unicellular cyanobacterium Synecho-coccus. *Canadian Journal of Fisheries and Aquatic Sciences,* 214:71–120,1986.

[5] Partensky F, Hess WR, Vaulot D. Prochlorococcus, a marine photosynthetic prokaryote of global significance. *Microbiology and Molecular Biology Reviews* ,63:106–127,1999.

[6] Hambly E, Suttle CA. The viriosphere, diversity, and genetic exchange with phage communities. *Current Opinion In Microbiology,*8:444–450,2005.

[7] Wang K, Chen F. Prevalence of highly host-specific cyano-phages in the estuarine environment. *Environmental Microbiology* , 10:300–312, 2008.

[8] Zhong Y, Chen F, Wilhelm SW, Poorvin L, Hodson RE. Phylogenetic diversity of marine cyano-phage isolates and natural virus communities as revealed by sequences of viral capsid assembly protein gene g20. *Applied and Environmental Microbiology* ,68:1576–1584,2002.

[9] Wang K, Chen F. Genetic diversity and population dynamics of cyano-phage communities in the Chesapeake Bay. *Aquatic Microbial Ecology* ,34:105–116,2004.

[10] Short CM, Suttle CA. Nearly identical bacteriophage structural gene sequences are widely distributed in both marine and freshwater environments. *Applied and Environmental Microbiology,*71:480–486,2005.

[11] Jameson E, Mann NH, Joint I, Sambles C, Muehling M.The diversity of cyanomyovirus populations along a North-South Atlantic Ocean transect. *ISME Journal* ,5:1713–1721,2011.

[12] Dorigo U, Jacquet S, Humbert JF. Cyanophage diversity, inferred from g20 gene analyses, in the largest natural lake in France, Lake Bourget. *Applied and Environmental Microbiology* , 70:1017–1022, 2004.

[13] Zhong Y, Chen F, Wilhelm SW, Poorvin L, Hodson RE. Phylogenetic diversity of marine cyanophage isolates and natural virus communities as revealed by sequences of viral capsid assembly protein gene g20. *Applied and Environmental Microbiology* ,68:1576–1584,2002.