Genome of a Novel Freshwater *Microcystis* Cyanophage Mwe-Yong1112-1

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Abstract

Freshwater *Microcystis* cyanophage Mwe-Yong1112-1 is the first virus isolated with *M. wesenbergii*. The complete genome of the cyanophage is 39,679 bp in length with a G+C content of 66.6%. BLASTx scanning results indicated “No significant similarity found”. Mwe-Yong1112-1 shared the highest pair-wise average nucleotide identity (ANI) value of 60.83% (below the ≥95% boundary to define a species) and the highest nucleotide sequence similarity of 17.48% (below the >50% boundary to define a genus) with the closest phage in pairwise sequence comparison (PASC). In the proteomic tree, Mwe-Yong1112-1 formed an independent branch in Siphoviridae family. Analysis indicated that cyanophage Mwe-Yong1112-1 should be considered as a novel phage presenting a novel genus in Siphoviridae family.

Main Text

Cyanobacterial blooms are increasing worldwide in frequency, magnitude, and duration in recent decades, causing toxin accumulation, disease, and even death of aquatic organisms, and risking to human drinking water and food safety [1]. One of the most pervasive and dominant bloom-forming cyanobacteria in freshwater ecosystems is *Microcystis* spp. [2]. Ubiquitous and microcystin producing *Microcystis wesenbergii* is one of the three most harmful and dominant freshwater Microcystis [3].

Viruses infecting cyanobacteria are referred to as cyanophages, which play a major role in the dynamics, genetic diversity and structure of cyanobacterial communities [4]. As compared to widely studied marine cyanophages, only little information on freshwater cyanophages was reported [5]. To date, only 10 freshwater *Microcystis* cyanophages have been reported. Only five *Microcystis* cyanophage genomes (MaMV-DC, Ma-LMM01, Mic1, vB_MelS-Me-ZS1 and PhiMa05) have been sequenced and characterized (Table 1) [6-10]. Formerly, no *M. wesenbergii* cyanophage was reported.

In this study, the complete genome of a *M. wesenbergii* cyanophage was sequenced and analyzed. The *Microcystis* cyanophage Mwe-Yong1112-1 was obtained by four serial single-plaque isolation using double-layer agar plate method [9] employing *M. wesenbergii* FACHB-1112 as host, from the stream in YongChengShiJia community (North latitude, 29.8; East longitude, 121.5) in Ningbo, Zhejiang, China. The infectivity of Mwe-Yong1112-1 to 38 cyanobacterial strains was studied according to the reference [9]. Whole genome sequencing of Mwe-Yong1112-1 was performed as descripted [9] using the Illumina MiSeq (San Diego, CA, USA) sequencing platform. Phage termini were analyzed as described previously [11]. Open reading frames (ORFs) were preliminarily predicted with RAST [12], and then identified with BLASTp [13] (E-values $<10^{-5}$), Hmmer [14] (E-value $\leq 10^{-5}$) and HHpred [15] (E-value $\leq 10^{-5}$, possibility >96%). The tRNAscan-SE program was used to search for regions encoding tRNAs [16]. Antibiotic resistance and virulence factor genes were searched in CARD database (http://arpcard.mcmaster.ca) and VFDB database (http://www.mgc.ac.Cn/VFs/main.htm), respectively. The pair-wise average nucleotide identity (ANI) values were confirmed using OrthoANI [17]. To estimate the nucleotide sequence similarity

between Mwe-Yong1112-1 and other phages in current (5 Jan. 2022) public databases, the Pairwise Sequence Comparison (PASC) classification tool was used [18].

Mwe-Yong1112-1 could lyse 23 of the 38 tested cyanobacterial strains.7 tested strains could be lysed fully within 3 days by Mwe-Yong1112-1 (Supplementary information Table S1). The 23 susceptible cyanobacterial strains respectively belong to 4 orders, Chroococcales, Nostocales, Oscillatoriales and Synechococcales (Table S1). Most reported phages have strict host specificity. Nevertheless, as more and more phages are isolated and studied, and the development of phage isolation techniques and host range experimental approaches, more and more broad-host phages were found [19]. For example, 8 of 12 tested cyanobacteria including genera *Anabaena* and *Nostoc* were found susceptible to cyanophage A-4L [20]. *Microcystic* cyanophage Me-ZS1 was found to be capable of infecting cyanobacterial strains across taxonomic orders (Chroococcales, Nostocales and Oscillatoriales) [9]. *Aquamicrobium* phage P14 was found to infect two *Aquamicrobium* strains of the Alphaproteobacteria class and three *Alcaligenaceae* strains of the Betaproteobacteria class [21].

The complete genome of cyanophage Mwe-Yong1112-1 (GenBank accession number MZ436628) is a double-stranded 39,679 bp DNA molecule with the G+C content of 66.6%. There are 53 predicted open reading frames (ORF), 29 exist in the positive strand and another 24 exist in the negative strand (Fig. 1a). The total length of the ORFs is 12,299 bp, viz., the coding rate is 93.13%. Analysis of the termini indicated that Mwe-Yong1112-1 had no fixed terminus. BLASTx scanning resulted “No significant similarity found”, indicating the very specific feature of genome sequence and unique phylogenetic status of Mwe-Yong1112-1.

No tRNA gene and ORF associated with virulence factors, toxins or antibiotic resistance gene was identified in the genome. Only 28 (52.83%) Mwe-Yong1112-1 ORFs can be assigned to encode putative functional proteins (E-value $<10^{-5}$). The predicted ORFs could be classified into six functional groups (Fig. 1a).

Lysogeny-associated proteins encoded by cyanophage Mwe-Yong1112-1 include the repressor protein CI (ORF 26), regulatory protein CII (ORF 27) and integrase (ORF 3). Mwe-Yong1112-1’s ORF 30 and ORF 31 were predicted to encode transposase and Mu B respectively. The transposition of transposing phage Mu requires the following three phage-encoded proteins: transposase, Mu A and, Mu B [22]. No ORF was found to encode Mu A in Mwe-Yong1112-1. The mechanisms that guide lysis-lysogeny decision are complex. Although harbouring lysogeny-associated genes, cyanophage Mwe-Yong1112-1 produced transparent circular plaques on *M. wesenbergii* lawns and caused *M. wesenbergii* cultures yellowing and clarifying in this study. One or more factors, such as high density of host cyanobacterial cells, might induce the cyanophage to enter a lytic cycle.

Mwe-Yong1112-1 harbours at least 3 ORFs predicted to encoding proteins playing a role in host cell lysis, including a peptidase (ORF 4), a lysozyme (ORF 44) and a holin (ORF 45). Mwe-Yong1112-1’s ORF 2 was predicted to encode regulatory phage protein Cox. This ORF may partly contribute to the

cyanobacteriolytic phenotype of the cyanophage Mwe-Yong1112-1. The phage Cox protein is a small multifunctional DNA-binding protein. It has three different functions: (i) control the immunity repressor and the expression of integrase, repression of the P2 Pc promoter (ii) activation of the P4 satellite phage and (iii) as an activator of excision [23]. Mwe-Yong1112-1's ORF 17 was predicted to encode a DNA adenine methylase (*dam*). To counteract bacterial defense systems (Restriction-modification systems, R-M), phages make extensive base modifications (substitutions) to block endonuclease restriction [24]. It is speculated that the products of phage *dam* genes may act to protect the phage DNA against restriction upon infection [25].

Dam was also reported to be a regulatory protein. It can regulated genes involved in diverse functions [26]. Besides, Mwe-Yong1112-1 genome harbours three ORFs predicted to encode three putative regulatory factors. GemA protein (ORF 39): early protein responsible for decreasing host DNA gyrase activity [27]. Middle operon regulator (ORF 43): activator of the Pm promoter, which allows the expression of viral endolysin and structural genes [28]. DksA-like zinc finger domain containing protein (ORF 46): critical for regulating transcription of ribosomal RNA [29].

Mwe-Yong1112-1' ORF 7 was predicted to encode a putative outer membrane lipoprotein. λ and lambda-like pahgeswere reported to encode a widely conserved outer-membrane lipoprotein [30]. A phylogenetic tree (Fig. 1b) was constructed using the Maximum Likelihood method employing the JTT matrix-based model in MEGA 10.0.5 [31] based on the outer membrane lipoprotein sequences of 5 phages of *Lambdavirus* genus and Mwe-Yong1112-1. Results indicated the far evolutionary distance between Mwe-Yong1112-1 and the *Lambdavirus*.

The proteomic tree was generated using ViPTree online [32] based on genome-wide similarities determined by tBLASTx. 56 type species of the 14 families of order Caudovirales, the five *Microcystis* cyanophages with reported genome sequences and *Microcystis* cyanophage Mwe-Yong1112-1 were included in the analysis. In the proteomic tree (Fig. 1c), although cyanophage Mwe-Yong1112-1 and Siphoviridae phages, except for the phages of *Lambdavirus* genus, clustered together, there were long evolutionary distance between Mwe-Yong1112-1 and other Siphoviridae phages. Mwe-Yong1112-1 was most closely related to *Rhodobacter* phage RC1 and *Rhodovulum* phage RS1. In the PASC scanning, Mwe-Yong1112-1 shared the highest nucleotide sequence similarity, as low as 17.48% (below the ≥50% boundary to define a genus), with the most closely related phage. Mwe-Yong1112-1 shared the highest ANI value of 60.83% with *Rhodobacter* phage RC1. Genome comparison of Mwe-Yong1112-1 and RC1 by Easyfig (version 2.2.3) [33] demostrated very low similarity (Fig. 1d). Therefore, we propose a new phage genus with *Microcystis* cyanophage Mwe-Yong1112-1 as the representative specie. In addition, although *Lambdavirus* genus belongs to Siphoviridae family, there was a far evolutionary distance between the phages of *Lambdavirus* genus and other Siphoviridae phages in the proteomic tree. Hence, we propose that the creation of a new subfamily emboding the *Lambdavirus* phages in Siphoviridae family in the next versions of ICTV taxonomic update may be better.

Declarations

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Conflicts of interest:

The authors declare no conflict of interest.

Ethical approval:

This article does not contain any studies with human participants or animals performed by any of the authors.

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Table

Table 1. A list of *Microcystic* cyanophages whose genomes have been sequenced

Phage name	Accession number	Genome length (bp)	Host (reference)
MaMV-DC	NC_029002.1	169,223	*M. aeruginosa* FACHB-524[6]
Ma-LMM01	NC_008562.1	162,109	*M. Aeruginosa* NIES-298[7]
Mic1	MN013189.1	92,627	*M. aeruginosa* FACHB-1339[8]
vB_MelS-Me-ZS1	MK069556.2	49,665	*M. elabens* FACHB-916[9]
PhiMa05	MW495066.1	273,876	*Microcystis* sp.[10]
Mwe-Yong1112-1	MZ436628	39, 526	*M. Wesenbergii* FACHB-1112

Figures

Figure 1

(a) Genome map of cyanophage Mwe-Yong1112-1. The outermost circle represents 53 ORFs encoded in the genome, with different colors representing different functions. **(b)** Maximum likelihood phylogenetic tree of the outer membrane lipoproteins of 5 phages of *Lambdavirus* genus and Mwe-Yong1112-1. **(c)** Viral proteomic tree of the 6 *Microcystis* cyanophages and 56 type species of the 14 families of order Caudovirales. Red stars indicate *Microcystis* cyanophage Mwe-Yong1112-1. Blue stars indicate the other 5 *Microcystis* cyanophages. Triangles indicate the 5 phages of *Lambdavirus* genus harbouring ORF predicted to encoding outer membrane lipoproteins. **(d)** Genome comparison of *Microcystis* cyanophage Mwe-Yong1112-1 and the most closely related phage *Rhodobacter* phage RC1.

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