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Isolation and Characterization of an *Aeromonas punctata* Bacteriophage

Cheng Kai[1,2], Zhang Denglan[1], Deng Jingxuan[2] and Zhao Yijun[1,*]

[1]*Key Laboratory of Ecological Remedlation of Lakes and Rivers and Algal Utilization of Hubei Province, College of Resources and Environmental Engineering, Hubei University of Technology, Wuhan, Hubei, 430068, P.R. China*

[2]*College of Life Science, Central China Normal University, Wuhan, Hubei, 430079, P.R. China*

Abstract: An *Aeromonas punctata* bacteriophage, named as DH1, was isolated from East Lake, Wuhan city, China. Morphologically, phage DH1 showed a typical *Myoviridae* structure consisting of an isometric head (50 nm in diameter) and a visible tail. The bacteriophage had a latent period of about 90 minutes and an average burst size of about 125 PFU•Cell^{-1}. Restriction enzyme pattern of the bacteriophage's genome showed that the genome is a double-stranded DNA and about 34kb in size. The sequenced genomic fragments showed highly similarities to gp04 and gp16 sequence of other *Myoviridae* bacteriophages at protein level.

Keywords: *Myoviridae*, bacteriophage, *Aeromonas punctata*.

1. INTRODUCTION

Aeromonas sp. has been well known to cause diseases in fishes, frogs, and even humans [1-4]. Till now, only one *Aeromonas hydrophila* bacteriophage has been isolated [5] and sequenced [6]. In this study, we reported the isolation and characterization of a novel *Aeromonas punctata* bacteriphage from a natural lake.

2. MATERIALS AND METHODS

Sampling: The sampling site was located in East Lake (30°31′37.66″N,114°22′09.18″E), Wuhan City, Hubei Province, China. Surface water samples (10-20cm depth) were collected and transported to laboratory immediately for bacteria and virus isolation during December 23, 2006 to January 8, 2007,

2.1. Isolation of Bacteria and Bacteriophage

A volume of 100μL water from lake was spread over solid LB media. After incubation at 37℃ for 24h, separated colony was picked and purified by standard methods. The isolation of bacteriophage was performed using phage plaque method. Briefly, 30mL fresh lake water was filtered through a 0.2μm filter (PN4612，PALL). A volume of 100μL filtered water was mixed with 200μL bacterial culture in LB media and incubated at 28℃ till the plaque was formed. Pure bacteriophage strain was obtained by five serial single-plaque isolations. Only one phage-host system was established from 60 purified bacterial colonies, the phage was named as DH1, and the host bacterium was designated MH1.

*Address correspondence to this author at the College of Resources and Environmental Engineering, Hubei University of Technology, Hubei, 430068, P.R. China; E-mail: 1829088302@qq.com

2.2. Characterization of MH1

The genomic DNA of bacteria MH1 was extracted with phenol-chloroform-isoamylol and precipitated with ethanol. A fragment of 16S rDNA was amplified by one pair of universal primer [7] and sequenced.

2.3. Phage Purification

Five hundred milliliters of cell lysates (the titer of the phage was about 10^9PFU•mL^{-1}) was centrifuged at 10000 g for 30 min in Jouan KR2.5i centrifuge to remove the cell debris. Then, the supernatant was centrifuged at 100,000 g for 2.5 h in Beckman J-30I (JS-24 rotor) to concentrate the phage stock to about 2mL. The phage stock was further purified on 20%-50% sucrose density-gradient centrifugation by Beckman optima^{Tm} MAX and MAX-E(MLS-50rotor). Finally, the phage stock was diluted in 3mL 20mmol/L $MgCl_2$ solution and stored at 4°C.

2.4. Transmission Electron Microscopy (TEM)

The purified phages were dropped onto a formvar carbon-coated copper grid (200 mesh) and negatively stained with 2% sodium phosphotungstate (pH7.0) and observed under TEM (Hitachi H-7000FA).

2.5. Phage DNA Extraction and Restriction Endonuclease Digestion

The nucleic acid of purified phage was extracted using protease K, followed by phenol-chloroform-isoamylol extraction and ethanol precipitation. The phage genomic DNA was digested with endonuclease *Acc* Ⅰ, *BamH* Ⅰ and *Sau3A* Ⅰ (Takara) and load onto a 0.8% agarose gel and analyzed using Ethidium Bromide staining.

2.6. Cloning and Sequencing

Two *BamH* I fragments, 1.4 kbp and 1.9 kbp were purified with a gel extraction kit (D2501-01, OMEGA), then cloned to pUC19 vector using standard protocol [8]. The recombined plasmids DNA were purified and sequenced with universal primers by Invitrogen Company.

2.7. Growth Cycle

Phage DH1 and exponential phase host cell MH1 were mixed at a MOI (multiplicity of infection) of 1:2000. The mixture was cultured at 28°C, and plaque assay was used to test the titer of DH1 every 30 min during 2.5 hours.

3. RESULTS AND DISCUSSION

3.1. Identification of the Host

A fragment of 196 bp of 16S rDNA was amplified and sequenced from bacteria MH1, and the sequence showed 100% identity to *Aeromonas punctata* and was submitted to GenBank (access number: EU515214).

3.2. Identification of the Phage

TEM analysis showed that phage DH1 had an icosahedral head (50 nm in diameter) and a visible tail (less than 100 nm in length) (Fig. **1**), which is morphologically similar to but much smaller than *Aeromonas hydrophila* phages Aehl and Aeh2 [5].

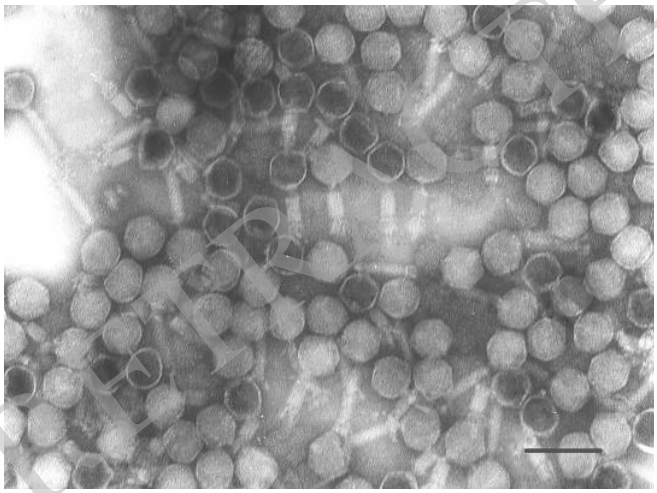

Fig. (1). Electron micrograph of negatively stained phage DH1.

Bar: 100nm

3.3. Genome Analysis of DH1

The result of enzyme digestion showed that the genome size is approximately 34±3kb (Fig. **2**) and much lesser than Aeh1(230kb) [9]. The sequence of two fragments, 1.4 and 1.9kb, were submitted to GenBank (access number: EU515215 and EU515216) and showed 42% and 50% identities to gp04 and gp16 of a *Myoviridae* bacteriophage Becp43, respectively [10].

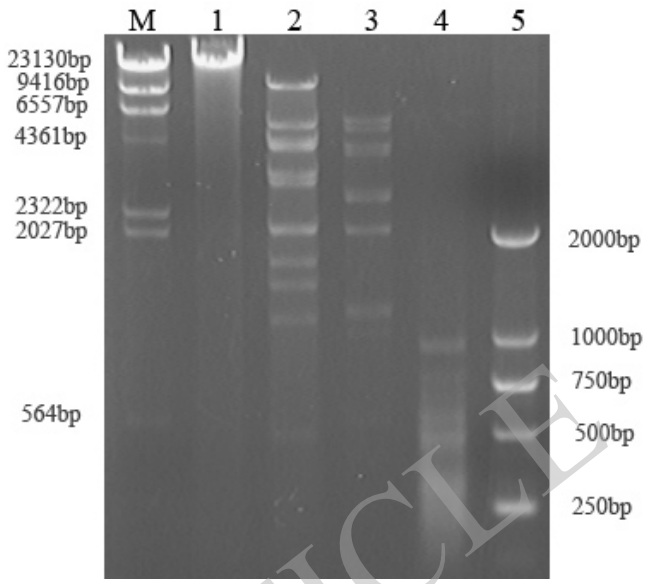

Fig. (2). Restriction enzyme digested patterns of DH1 genome DNA in 0.8% agarose gel electrophoresis.

M: λ phage DNA digested by HindIII, 1: phage genome DNA without digestion, 2: Acc I, 3: BamH I, 4: Sau3A I, 5:DL2000 marker.

3.4. Growth Cycle

The one-step growth curves of DH1 indicated that the latent periods of phage DH1 were 90 min, which was much longer than *Aeromonas hydrophila* phages Aehl and Aeh2 [5]. The average burst sizes of phage DH1 were about 125 PFU•Cell^{-1}, which was also bigger than Aehl and Aeh2 [5].

The majority of *Aeromonas sp.* described to date are from soil and water and known to be pathogenic to cold-blooded animals. Some studies have demonstrated that the presence of *A. sp.* in drinking water is a potential risk, since some strains can produce a wide range of virulence factors [2, 4]. Thus, by studying the interaction of the phage-host system, *A. sp.* phage may be significant for controlling the risk [11].

CONCLUSION

A. punctata bacteriophage DH1 was a typical Myoviridae bacteriophage, and its genome is a 34kb double-stranded DNA. The sequenced genomic fragments showed highly similarities to other Myoviridae bacteriophages.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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