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].

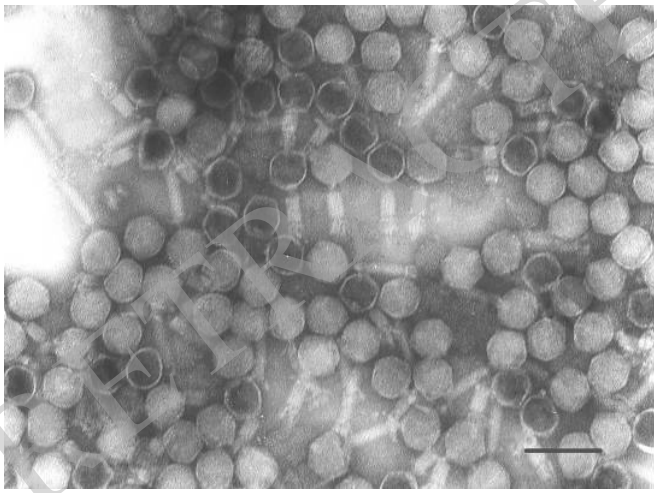

Fig. (1). Electron micrograph of negatively stained phage DH1.

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].

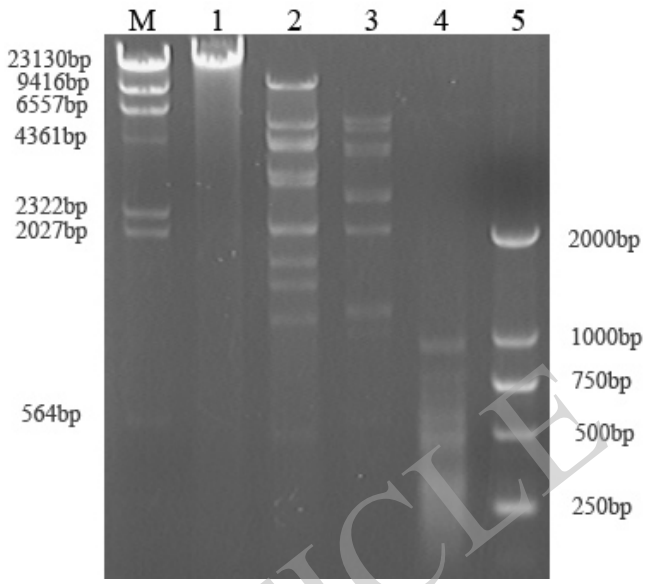

Fig. (2). Restriction enzyme digested patterns of DH1 genome DNA in 0.8% agarose gel electrophoresis.

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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