

Isolation and Molecular Phylogenetic Analysis of Thermophilic Halotolerant Virus from Coastal Geothermal Area

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Summary

Bacteriophage (phage), viruses attacking bacteria, are considered to be the most abundant and diverse biological entities on earth, and more than hundred million phage species are estimated on earth. Phage are continuously regulating microbial ecology and activity, including carbon and nutrient fluxes, food web dynamics, and microbial diversity and diversification. The interest in the applied use of bacteriophages in a diverse range of fields. In sea hydrothermal vent communities, viruses play very important roles. However vent thermophilic phages remain largely unexplored.

Six phages were isolated from sediment of Obama hot spring, Nagasaki, Japan. ϕ OH2 and ϕ OH3 which were isolated phages formed turbid plaques on the double layer agar plates. The phages ϕ OH2 and ϕ OH3 were classified by transmission electron microscopy as a Family *Siphoviridae* and a Family *Inoviridae*, respectively. The thermostability assay showed that ϕ OH2 and ϕ OH3 were most stable at 60°C. ϕ OH2 and ϕ OH3 were most stable at pH 7.0 and pH value didn't markedly affect their survival. The phages also exhibited salt-tolerance at from 0.1 to 0.5 M NaCl.

Nucleotide sequences of the phage genomes were determined. ϕ OH2 contains a double-stranded linear DNA of 38,099 bp, which encodes 60 putative open reading frames (ORFs). The 5,688-nucleotide genome of ϕ OH3 was a circular single-stranded DNA and had a replication form. Six putative ORFs were found in the ϕ OH3 genome, and all predicted proteins showed no similarity to proteins in databases. These results indicated that there are novel phages in sea hydrothermal vent and the phages play roles on horizontal gene transfer.

For further characterization of phage ecology, cloning, expression and purification of the endolysin and holing genes of the ϕ OH2 were done. Based on homology searching result, the endolysin gene showed that it consists of an N-terminal catalytic domain (PGRP domain) and the C-terminal putative lysin motif (LysM) repeat regions. ϕ OH2 endolysin exhibited activity against *G. kaustophilus*, *T. thermophiles*, *L. johnsonii* and *E. coli* strains. Optimal conditions for ϕ OH2 endolysin reactivity were pH 7.0 and 70°C. ϕ OH2 endolysin showed high thermostability, with 40% of initial activity remaining following 20 min of treatment at 100°C. ϕ OH2 holin gene were expressed in *E. coli* with pET21 expression vector as a fusion protein. Although its activity against *E. coli* was detected after 15 min of induction with IPTG, mass production has been achieved. Therefore, the gene was inserted into pColdTF expression vector and was expressed. The purified ϕ OH2 holin also showed high thermostability.