Viral Metagenomics and Phage Genomics of Korean Soil and Marine Environment

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Although viruses are known to be the most numerous biological entities in soil and seawater, little is known about their diversity in this environment. To investigate viruses in environment, we combined two kinds of approaches, the culture-independent and the culture-dependent method. We used the viral metagenomics and viral genomics to understand viral diversity and physiology. For the metagenomic approach, viruses were separated and concentrated with centrifugation and filtration from a soil and a marine environment. Viral DNA was extracted and amplified with the multiple displacement amplification (MDA) method, a whole genome amplification method which uses the phi29 DNA polymerase and random hexamer to amplify DNA isothermally. The metagenomes amplified by MDA were sequenced and/or compared with sequences from metagenomes amplified by the linker amplified shotgun library method (LASL) which amplified only double strand DNA. The analysis of sequences showed that the MDA method amplify single stranded DNA viral genomes more preferentially than other (mostly double stranded) viral DNA. Changes of circular DNA and linear DNA amount during MDA were observed with quantitative real time PCR to confirm the preferential amplification of circular DNA. As a result, we detected that various kinds of unknown single stranded DNA viruses exit in soil and marine environment. We also could assemble several circular genomic compounds of unknown putative single stranded DNA viruses from metagenomic sequences retrieved from soil and marine environment.

In addition to the metagenomic study, we isolated phages infecting *E. coli* and *Salmonella* and performed a genomic study of the phages. Among the isolates, a phage SP18 was selected to be sequenced its whole genome. We used the pyrosequencing method for whole genome sequencing and could assemble a contig of 170 kbp in length thought to be a genome of SP18. The analysis of whole genome of SP18 showed that it closely related to JS98, an enterophage isolated from human stool specimens. SP18 belongs to the T4-even phages and its genomic similarities and differences were compared to other related phages in this group.

References

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