

An introduction to algal viruses

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Algae are very diverse aquatic organisms that are photosynthetic, oxygenic autotrophs, typically smaller and less structurally complex than land plants. They have neither root nor leafy shoot, and are lack vascular tissues. Algae significantly influence aquatic environments, both as primary producers in the food chain and as pollutant when growth becomes uncontrolled.

Viruses that infect algae are widely distributed in nature, and have been isolated from freshwater and seawater sources throughout the world. Their concentrations typically range from 10^5 to 10^8 /ml. As the viruses of land plants, viruses infecting algae can affect their host and can cause significant mortality. Viruses or virus-like particles have been observed intracellularly in many different genera and classes of eukaryotic algae, varying from unicellular to multicellular, swimming to nonswimming, bloom-forming to non-bloom-forming, free-living to symbionts. Recent studies showing that these viruses are involved in the disappearance of algal blooms suggest that they play important roles in aquatic environments. They could also be used for the control of toxic red algae.

Viruses or virus-like particles have been reported in at least 44 taxa of eukaryotic algae since the 1970s. However, most of these viruses are not well characterized, because they are difficult to obtain in large quantities. *Paramecium bursaria* *Chlorella virus 1* (PBCV-1) is the most often studied virus of those found in algae, because it can be produced in large quantities by using exosymbiotic *Chlorella*-like green algae that are derived from *P. bursaria*. PBCV-1 is the prototype of the *Chlorovirus* genus in the Phycodnaviridae family, which encompasses large, polyhedral, plaque-forming dsDNA algal viruses. In addition to the *Chlorella* virus genus, *Coccolithovirus* genus (type species: *Emiliana huxleyi* virus 86), *Prasinovirus* genus (type species: *Micromonas pusilla* virus SP1), *Prymnesiovirus* genus (type species: *Chryochromulina brevifilum* virus PW1) and *Phaeovirus* genus (type species: *Ectocarpus siliculosus* virus 1) belong to this family.

A DNA sequence analysis of the 330,742bp PBCV-1 genome revealed over 700 open-reading frames (ORFs). Of these, 375 were protein-encoding genes. The viral-encoded proteins include transcriptional and translational factors, restriction/modification enzymes, topoisomerase, chitinase, and hyaluronan synthase. In addition, the PBCV-1 genome contains 11 tRNA genes, which indicates that some components of the host-protein

synthesis machinery might be replaced by virus-encoded tRNAs. Recent completion of the DNA sequence analysis of *Ectocarpus siliculosus* virus (EsV) *Emiliana huxleyi* virus (EhV) showed their phylogenetic relationship with PBCV-1.

In addition to viruses with dsDNA genome, several small algal viruses distinct from the family Phycodnaviridae have been reported: *Heterosigma akashiwo* nuclear inclusion virus (HaNIV), *H. akashiwo* RNA virus (HaRNAV), *Heterocapsa circularisquama* RNA virus (HcRNAV), *Schizochytrium* sp. RNA virus (SssRNAV). Also, algal virus with dsRNA genome, MpRNAV, has been reported recently from photosynthetic protist *Micromonas pusilla*.

In addition to many useful genes encoded by the large size genomes, algal viruses represent a novel source of promoters for expressing genes in foreign hosts. For example, the upstream region of the PBCV-1 adenine methyltransferase gene functions extremely well in several higher plants and in many bacteria.

Despite their potential importance, research on the algal viruses in Korea is very limited. We have isolated over 40 chlorella viruses from Korea since 1999. Analysis of their genomic DNA with restriction enzymes and Southern blot indicated their relationship with PBCV-1. However, analysis of the tRNA gene cluster revealed the presence of 14-16 tRNA genes contrast to 11 genes in PBCV-1.

Transformation of chlorella protoplast with vectors containing the green fluorescence protein (GFP) gene fused to putative early gene promoters from chlorella viruses isolated from Korea showed higher fluorescence intensity compared to that from chlorella cells transformed with CaMV 35S-GFP fusion. These results shows that algal virus including chlorella virus can be useful sources of useful genes and promoters.

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