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Analysis of Algal Virus Genomes and Possible Application

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Abstract

The genomic sequence of Chlorella virus SS-2 isolated in Korea and Feldmannia species Virus (FsV) have been determined. The genome of SS-2 was estimated to be 310kbp, and over 300kbp nucleotide sequence was analyzed. Among the 372 open reading frames identified from PBCV-1, the prototype of chlorella virus, 331 were identified from SS-2, which suggest that SS-2 is very similar to PBCV-1. The genome of FsV is composed of 153,259 bp and encodes about 128 major open reading frames on both strands. About 90% genes revealed the amino acids sequence identities to other genes of algae viruses including EsV-1, FirrV-1 and PBCV-1. Five putative early gene promoter regions from the open reading frames A67R, A312L, A342L A548L and A158L that encode the procyclin precursor, aspartyl-tRNA synthetase, regulatory protein, helicase and an unknown protein were used to make chlorella transformation vectors containing the green fluorescence protein (GFP) gene fused to these promoters. Compared to GFP intensity from chlorella cells transformed with CaMV 35S-GFP fusion (100%), cells transformed with the A67R, A158L, A312L, A342L and A548L promoter-GFP fusion construct showed 131.5%, 135.19%, 116.79%, 110.29%, 105.18% intensity, respectively. A DNA adenine methylatarnsferase and an exonuclease cloned from FsV showed specific activity when they expressed in *E. coli*. These results suggested that the promoters and genes derived from algal viruses could be used in other research systems and industry.

Introduction

Algae include diverse aquatic organisms that are photosynthetic, oxygenic authotrophs, typically smaller and less structurally complex then land plats. 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.

As their counterpart on land, algae also are infected many viruses. 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 reported in at least 44 taxa of eukaryotic algae since the 1970s. Including the most studied *Paramecium bursaria Chlorella virus* 1 (PBCV-1), genomic sequences of several algal viruses have been recently determined. DNA sequence analysis of the viral genomes revealed that their large genomes encodes many useful genomes including restriction/modification enzymes, topoisomerase, chitinase, and hyaluronan synthase. In addition, their promoters have been proved to be active in plants and bacterial cells. In this presentation, sequence analysis of the genome a chlorella virus isolated from Korea and a brown algae infecting virus, and application of the information will be discussed.

Genome Analysis of *Chlorella* Virus SS-2

The *Chlorella* virus SS-2 was isolated from fresh water of Seosan, Chungnam in 2000, and amplified using *Chlorella* strain NC64A that were cultured in a modified Bold's basal medium (MBBM). Virus was purified from cell lysate and DNA was extracted using 40-60% (w/w) CsCl gradient. Genomic DNA library was constructed and sequence of over 300kb was obtained from about 310kb genome. Among

Table 1. Genes encoded by the genome of Chlorella virus SS-2

Biochemical Pathways	Proteins Encoded
Protein Synthesis, Modification and Degradation	Translation elongation factor-3, Prolyl 4-hydroxylase α-subunit, Thiol oxidoreductase, Protein disulfide isomerase, Ubiquitin C-terminal hydrolase, Ubiquitination of cell cycle protein, Zn metallopeptidase
Nucleotide Metabolism	Aspartate transcarbamylase, Ribonucleotide reductase, Thioredoxin, Glutaredoxin, dUTP pyrophosphatase, dCMP deaminase, dG/dA kinase, Nucleotide triphophatase, cytidine deaminase, Thymidylate synthase complement, ATPase
Lipid Manipulation	Glycerophosphoryl diesterase, 2-hydroxyacid dehydrogenase, Lysophospholipase, N-acetyltransferase
Signaling	Ser/Thr protein kinase, Tyr protein kinase, Tyr phosphatase, K+channel protein, Ligand-gated channel
Nerve Synapse-like Proteins	Potassium ion channel, NMDA glutamate receptor channel, AMPA glutamate receptor channel, Ornithine decarboxylase, Homospermidine synthase, Histidine decarboxylase, Tyrosine phosphatase
Glycosyltransferase	Glycosyltransferase, Fucosyltransferase, Glucosyltransferase
Sugar Manipulations	Hyaluronan synthase, Glucosamine synthase, UDP-glucose dehydrogenase, GDP-mannose dehydratase, Fucose synthase
Cell Wall Degrading Enzymes	β-1,3 glucanase, Chitinase, Alginate lyase, Endochitinase, Chitosanase
DNA Replication, Recombination & Repair	δ DNA polymerase, ATP dependent DNA ligase, DNA topoisomerase II, PCNA, Replication factor C, Rnase H, Superfamily III helicase, DNA binding protein, Exonuclease, Pyrimidine dimer-specific glycosylase
Miscellaneous	Ornithine decarboxylase, Homospermidine synthase, Monomine oxidase, O-alanine synthase, Fibronectin binding protein, Cu/Zn superoxidase dismutase, Amidase, BCS1 protein, Histidine decarboxylase
Transcription	Transcription factor TF II B, Transcription factor TF II S, Transcription factor TF II D, VLTF2-type transcription factor, RNA guanylyltransferase, RNA triphosphatase, Superfamily II helicase, SWI/SNF helicase, Ski1 helicase, RNase III

the 372 open reading frames identified from PBCV-1, the prototype of chlorella virus, 331 were identified from SS-2, which suggest that SS-2 is very similar to PBCV-1.

Based on the analyzed sequence, five putative early gene promoter regions were cloned from the open reading frames A67R, A312L, A342L A548L and A158L that encode the procyclin precursor, aspartyl-tRNA synthetase, regulatory protein, helicase and an unknown protein, respectively. Sequence analysis of these promoter regions indicated the presence of many cis-acting elements for transcription factors including TATA box, CAAT box, GAGA box and CCAAT. Chlorella transformation vectors containing the green fluorescence protein (GFP) gene fused to these promoters were constructed and introduced in chlorella protoplasts. Compared to GFP intensity from chlorella cells transformed with CaMV 35S-GFP fusion (100%), cells transformed with the A67R, A158L, A312L, A342L and A548L promoter-GFP fusion construct showed 131.5%, 135.19%, 116.79%, 110.29%, 105.18% intensity, respectively.

Table 2. Relative activity of promoters from Chlorella virus SS-2 in transformed Chlorella

Origin of the promoters	GFP intensity (%)
CaMV 35S promoter	100
Procyclin precursor of SS-2	131.5
Aspartyl-tRNA synthetase of SS-2	135.19
Regulatory protein of SS-2	116.79
Helicase of SS-2	110.29
Unknown protein of SS-2	105.18

Sequence analysis of the FsV genome and cloning of useful genes.

A virus infecting marine filamentous brown alga, *Feldmannia* species (FsV) is large icosahedral dsDNA virus and their virus particle is about 120 to 150 nm diameter. The virus has genome of two different sizes (158 and 178 kb) depend on the temperature at which the algal culture was incubated. We have constructed a genomic DNA library from the 158kb genome and completed sequencing of the 153,259 bp, which encodes about 128 major open reading frames on both strands. About 90% genes revealed the amino acids sequence identities to other genes of algae viruses including EsV-1, FirrV-1 and PBCV-1, (Richard et al., 1996). Among the identified genes, we have cloned the DNA adenine methyltransferase, expressed in *E. coli* and confirmed their activities. The results have not been published and will be presented in the meeting.

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