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RNA viruses in *Pleurotus ostreatus*

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Introduction

Mycoviruses are widespread in fungi, including plantpathogenic fungi. In most cases, they have been reported to be cryptic or show few symptoms leading to latent infection in host cells (Buck, 1986). However, several mycoviruses associated with fungal diseases were also reported.

Most mycoviruses reported have dsRNA genomes, either encapsidated in particles (Buck, 1986), present as unencapsidated forms in cytoplasm (Ahn and Lee, 2001; Hansen et al., 1985), or found in mitochondrial fractions (Hong et al., 1998; Lakshman et al., 1998). It is unusual in some ways that the genome of mycovirus is single-stranded RNA (ssRNA). Until now only three ss-RNA mycoviruses have been reported.

Mycoviruses present in mushrooms were also found in Agaricus bisporus (white-button mushroom) The viral disease of A. bisporus was first described in 1950 and subsequently shown to be related to the presence of virus particles in 1962.

Oyster mushroom (*Pleurotus ostreatus*) is one of the most popular mushrooms in Korea where it is grown by industrial scale. Recently, this species has been cultivated nationwidely as the mushroom is being estimated as a high value crop by part-and full-time farmers. Oyster mushroom malformation (OMM) is one of the most severe diseases of unknown etiology. The symptom was rather complex, its spread is fast, and the disease recurs on the same farm. Therefore, the outbreak of the OMM in a commercial farm leads to complete yield loss in affected areas and difficult to control.

Result and Discussion

The disease (OMM) always accompanied the presence of isometric virus particles. Using purification procedures involving Tris-EDTA buffer extraction, PEG-NaCl precipitation, differential centrifugation, and equilibrium centrifugation in CsCl gradient (1.585 g/cm^3), we have obtained four isometric viral particles; one of 27nm, two of 34 nm and one of 43nm in diameter. The smallest one, we named Oyster Mushroom Spherical Viruses (OMSV) encapsidated ssRNAs of 5.8 kb. The others, we named Oyster Mushroom Isometric Viruses (OMIV) encapsidated dsRNA as their genomes ; OMIV-I encapsidated 12 different dsRNAs of 2.65, 2.45, 2.40, 2.20, 2.15, 2.10, 2.05, 2.00, 1.90, 1.80, 1.00 and 0.80 kb. OMIV-II encapsidated 3 different dsRNAs of 2.25, 2.15, and 2.05 kb and OMIV-III encapsidated 4 different dsRNAs of 2.1, 2.0, 1.9 and 1.7 kb. The virus particles did not crossreact with each other to monoclonal and polyclonal antibodies against OMIV-I OMIV-II and OMIV-III, respectively Coomassie Brilliant Blue stained polyacrylamide gel (12%) electrophoresis showed different polypeptides of coat proteins of Mr 28.5, 71, 62 and 53 kD in viral coat proteins of OMSV, OMIV-I, OMIV-II and OMIV-III particles, respectively. These results demonstrated that there are at least 4 different kinds of isometrc viruses whose genomes were ssRNA or dsRNA in oyster mushroom.

We were focused on the ssRNA virus, OMSV. The nucleotide sequence of OMSV revealed that its genomic RNA was positive strand, containing 5784 bases with seven open reading frames (ORF). ORF1 had the motifs of RNA-dependent RNA polymerases (RdRp) and helicase. ORF2 encoded a coat protein. ORF3 to 7 could encode putative polypeptides of approximately 12, 12.5, 21, 14.5, and 23 kDa, respectively, but none of them showed significant similarity to any other known polypeptides. The 5' end of the viral RNA was uncapped and the 3' end was polyadenylated with 74 bases. Genomic structure and organization and the derived amino acid sequence of RdRp and helicase domain were similar to those of tymoviruses, a plant virus group(Yu et all., 2003).

To examine a correlation between the presence of OMSV and the disease, we eliminated OMSV from diseased isolates by Adenosine 3',5'-cyclic monophosphate (cAMP) treatment. Curing of the virus converted abnormal phenotypes into normal phenotypes involving normal mycelial growth, the formation of normal fruiting bodies, and increased yield. To determine that conversion of malformed phenotypes into normal phenotypes is due to elimination of the ssRNA virus, we carried out reverse transcription-polymerase chain reaction (RT-PCR) using total RNA from the virus-cured strain. RT-PCR analysis of primers targeted to the coat protein gene of 5.8 kb viral genome of OMSV showed that the virus-cured strains lost the 5.8 kb RNA of the virus. This suggests that OMSV could be a causative agent for the disease symptoms.

References

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