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Study of Viral Effects of the Mycovirus (LeV) and Virus-Free Commercial Line in the Edible Mushroom *Lentinula edodes*

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dsRNA was found in malformed cultures of *Lentinula edodes* strain FMRI0339, one of the three most popular sawdust cultivated commercial strains of shiitake, and was also found in healthy-looking fruiting bodies and actively growing mycelia. Cloning of the partial genome of the dsRNA revealed the presence of the RdRp sequence of a novel *L. edodes* mycovirus (LeV), and sequence comparison of the cloned amplicon showed an identical sequence to known RdRp genes of LeV found in strain HKA. The meiotic stability of dsRNA was examined by measuring the ratio of the presence of dsRNA among sexual monokaryotic progeny. More than 40% of the monokaryotic progeny still contained the dsRNA, indicating the persistence of dsRNA during sexual reproduction. Comparing the mycelia growth of monokaryotic progeny suggested that, although variations in the growth rate existed among progeny and virus infection was observed in highly actively growing progeny, there appeared to be a tendency toward a lower frequency of virus incidence in actively growing progeny.

This study attempted to cure the edible mushroom *L. edodes* strain FMRI0339 of the *L. edodes* mycovirus (LeV) in order to obtain an isogenic virus-free fungal strain as well as a virus-infected strain for comparison. Mycelial fragmentation, followed by being spread on a plate with serial dilutions resulted in a virus-free colony. Viral absence was confirmed with gel electrophoresis after dsRNA-specific virus purification, Northern blot analysis, and PCR using reverse transcriptase (RT-PCR). Once cured, all of fungal cultures remained virus-free over the next two years. Interestingly, the viral titer of LeV varied depending on the culture condition. The titer from the plate culture showed at least a 20-fold higher concentration than that grown in the liquid culture. However, the reduced virus titer in the liquid culture was recovered by transferring the mycelia to a plate containing the same medium. In addition, oxygen-depleted culture conditions resulted in a significant decrease of viral concentration, but not to the extent seen in the submerged liquid culture. Although no $discernable phenotypic changes in colony morphology were observed, virus-cured strains showed significantly higher growth rates and mycelial mass than virus-infected strains. We were also explored effects of LeV on fruiting body formation and mushroom yield. The fruiting body formation yield of virus-free *L. edodes* was larger than virus-infected *L. edodes*. These results indicate that LeV infection has a deleterious effect on mycelial growth and fruiting body formation. In addition, we have been investigated host-parasite interaction between *L. edodes* and its mycovirus interaction to study viral mechanism by establishment of proteomics.