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Short communication

# Curing viruses in *Pleurotus ostreatus* by growth on a limited nutrient medium containing cAMP and rifamycin

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A B S T R A C T

Oyster mushroom spherical virus (OMSV) and oyster mushroom isometric virus (OMIV) are the causative agents of a fruiting body deformation disease in the edible mushroom *Pleurotus ostreatus*. The curing of these mycoviruses was facilitated by a serial transfer of infected mycelia onto a limited nutrient medium containing 1 mM of cAMP and 75 μg/ml of rifamycin (cAMP-rifamycin plate). The mycelia were grown on cAMP-rifamycin plates for 5 successive passages. ELISA and RT-PCR showed that the amount of mycoviruses inside the mycelia decreased significantly with increasing numbers of passages. The mycelia became free of viruses after 5 successive passages. Cultivation of the virus-cured mycelia on a mushroom compost medium produced a normal harvest, whereas the spawn infected with viruses failed to produce any fruiting bodies.



Mycoviruses are a group of viruses that infect fungi, including mushrooms. The infection often causes severe mushroom diseases, which lead to a significant reduction in the production yield and commercial value of mushroom products. La France disease is the most well-known mushroom disease caused by mycovirus in the button mushroom, *Agaricus bisporus* (Hollings, 1962). The causative agent of the disease is a spherical virus with a double-stranded DNA (dsDNA) genome, which is known as La France isometric virus (LIV) (Goodin et al., 1992; Van der Lende et al., 1994). Infection with LIV results in the degeneration of the mycelium and premature fruiting body formation. PeSV (*Pleurotus eryngii* spherical virus) is a recently discovered mushroom virus that infects the king oyster mushroom, *P. eryngii*. PeSV is a single-stranded RNA (ssRNA) virus encapsulated by a spherical coat (Ro et al., 2007). Infection with PeSV is not as severe as with LIV, but PeSV infection is usually accompanied by deformation of the fruiting body, leading to the reduction in the mushroom's commercial value.

Oyster mushroom spherical virus (OMSV) and oyster mushroom isometric virus (OMIV) are two of the reported viruses found in the oyster mushroom, *Pleurotus ostreatus* (Ro et al., 2006; Yu et al., 2003). Both viruses are spherical RNA viruses but differ in their size and genome structure. OMSV is a 27 nm spherical virus with a 5.8 kb ssRNA genome (Yu et al., 2003), while OMIV is a 43 nm dsRNA virus containing four segmented dsRNA genomes (1.7, 1.9, 2.0, and 2.1 kbp) (Ro et al., 2006). These viruses are both found together in the diseased fruiting bodies causing retarded mycelial growth, undeveloped stipe, and deformed flat caps. The effects appeared to be exacerbated in certain cultivars, such as *P. osteratus* cv. Chunchu, which is one of the most cultivated cultivars in Korea. Infection of both viruses in this cultivar often causes total loss of harvest as shown in Fig. 1A andC.

A recent study on a novel mushroom virus from *Lentinula edodes* demonstrated that the mycovirus can be transmitted by both vertical and horizontal (lateral) transmission (unpublished data). In general, mycoviruses can be infected by progeny during sporulation (vertical transmission; Dalzoto et al., 2006) and also spread to neighboring mycelia through anastomosis (hyphal fusion; horizontal transmission). For this reason, the detection and removal of the virus-infected spawn has been considered to be the best way to control outbreaks of mushroom viral diseases. Accordingly, research in this field has been generally conducted to develop detection techniques targeting viral nucleic acids and coat proteins (Kim et al., 2008a,b; Ro et al., 2006). However, this detection and removal strategy is not the ultimate solution for the virus problem in the mushroom industry. Therefore, in this study, emphasis was placed on the elimination of viruses by treating with chemicals that impose physiological stresses on the infected mushroom mycelia.

For the treatment experiments, the fruiting bodies of OMSV- and OMIV-infected *P. ostreatus* cv. Chunchu were collected from commercial mushroom farms in the southern regions of Korea. The presence of OMSV and OMIV was confirmed by reverse transcriptase-PCR (RT-PCR). Primers targeting the RNA dependent RNA polymerase (RdRp) of OMSV were designed using the whole genome sequence of OMSV (GenBank ID: AY182001; Yu et al.,

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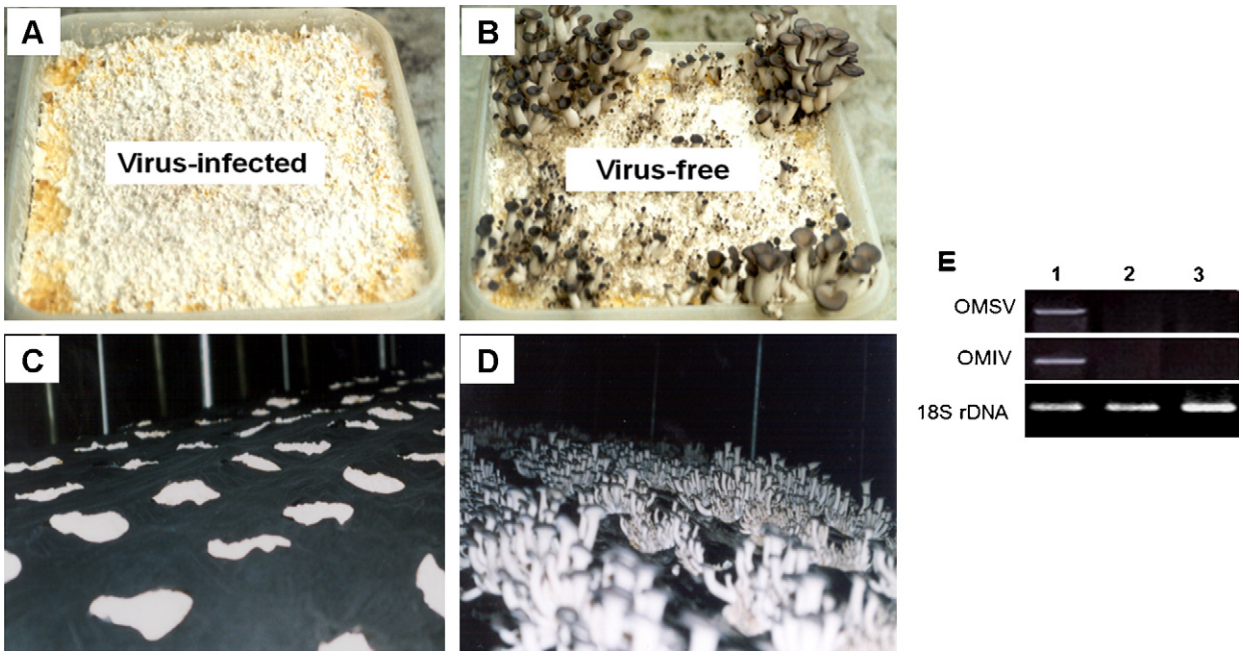


**Fig. 1.** Field test of virus-infected and virus-cured *P. ostreatus*. (A) and (C) Small and large scale tests, respectively, on the fruiting body formation of virus-infected *P. ostreatus*. (B) and (D) The same tests on the virus-cured *P. ostreatus* derived from the 5th passage on a cAMP-rifamycin plate. (E) RT-PCR screening for the presence of mycoviruses from the field test samples. Lane 1: RNA extracted from the virus-infected mycelia shown in panel (A); lane 2: RNA from the virus-free mycelia shown in panel (B); lane 3: RNA extracted from the fruiting bodies shown in panel (D).

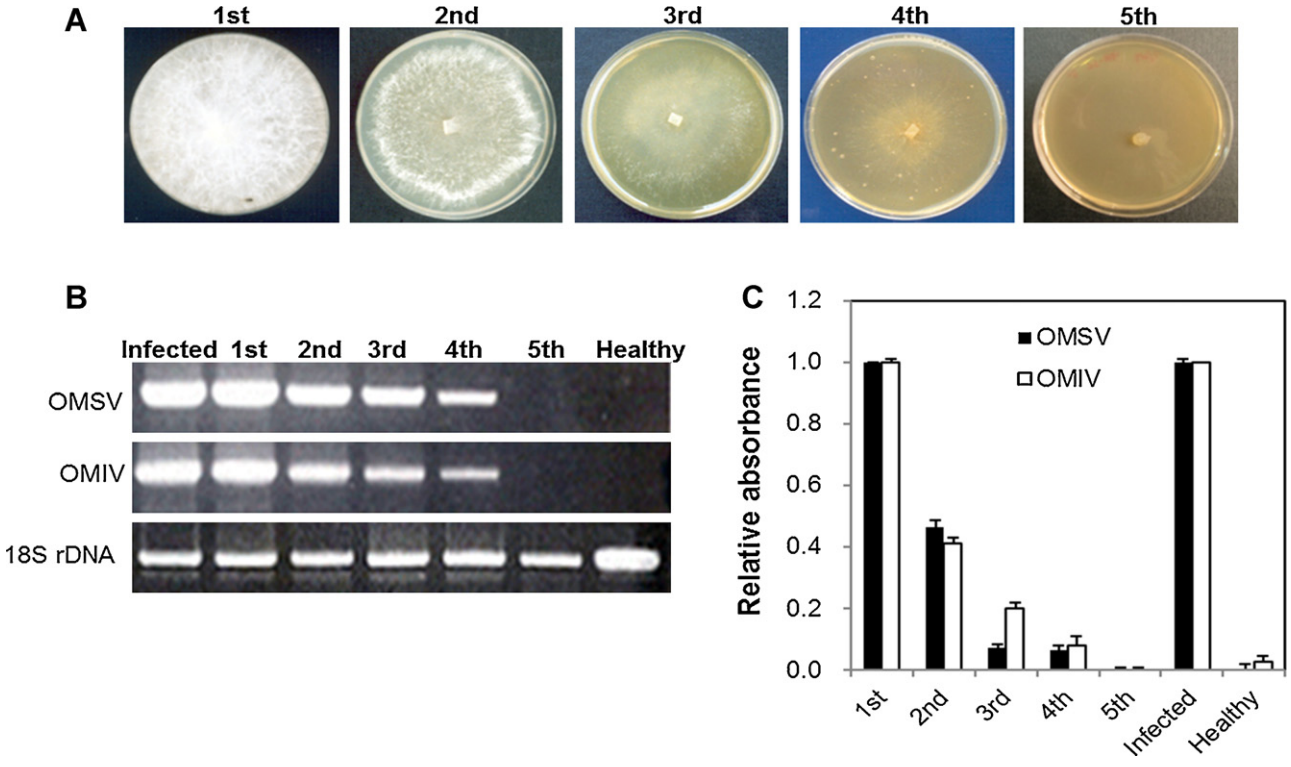


**Fig. 2.** Curing of OMSV and OMIV from P. ostreatus on cAMP-rifamycin plates. (A) The virus-infected mycelia were successively transferred to new plates after full growth. Full growth took 18 days for the first passage while taking more than 25 days for the fifth passage. (B) Semi-quantitative RT-PCR analysis of the mycelial RNAs extracted from each passage. Primer sets targeting OMSV and OMIV RNA genomes are described in the main text. 18S rDNA was included as control experiment. The "healthy" sample was from the virus-cured mycelia. (C) Triple antibody sandwich-ELISA(TAS-ELISA) analysis on the proteins extracted from the same samples. TAS-ELISA detected the RdRp protein and the coat protein for OMSV and OMIV, respectively.