RESEARCH ARTICLE

Detection of RNA Mycoviruses in Wild Strains of *Lentinula edodes* in Korea

Eunjin Kim, Mi-Jeong Park, Yeongseon Jang*, Rhim Ryoo, and Kang-Hyeon Ka
Forest Microbiology Division, Forest Bioresources Department, National Institute of Forest Science, Suwon, 16631, Korea

*Corresponding author: idjys@korea.kr

ABSTRACT

In general, mycoviruses remain latent and rarely cause visible symptoms in fungal hosts; however, some viral infections have demonstrated abnormal mycelial growth and fruiting body development in commercial macrofungi, including *Lentinula edodes*. Compared to other cultivated mushrooms, *L. edodes* is more vulnerable to viral infections as it is still widely cultivated under near-natural conditions. In this study, we investigated whether Korean wild strains of *L. edodes* were infected by RNA mycoviruses that have previously been reported in other parts of the world (LeSV, LePV1, LeV-HKB, LeNSRV1, and LeNSRV2). Using specific primer sets that target the RNA-dependent RNA polymerase genes of each of the RNA mycovirus, reverse transcription-polymerase chain reaction (RT-PCR) was used to detect viral infection. Viral infection was detected in about 90% of the 112 wild strains that were collected in Korea between 1983 and 2020. Moreover, multiple infections with RNA mycoviruses were detected in strains that had normal fruiting bodies. This work contributes to our understanding of the distribution of RNA mycoviruses in Korea and the impact of multiple viral infections in a single strain of *L. edodes*.

Keywords: *Lentinula edodes*, Multiple infection, Mushroom, Mycovirus, RT-PCR (reverse transcription-polymerase chain reaction)

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2021 September, 49(3): 285-294
https://doi.org/10.4489/KJM.20210025

Received: June 12, 2021
Revised: August 18, 2021
Accepted: August 24, 2021

INTRODUCTION

Environmental changes and mutations in specific genes can lead to the emergence of mycoviral infections [1]. Viral infections in mushrooms are caused by mycelium fusion, which also transmits the virus to healthy mycelium by spores [2]. Similar to mushroom spores, mycelium remaining in the mushroom cultivator can be a viral infection medium [3].

Lentinula edodes is a commercially important, edible mushroom that is cultivated throughout Korea. Compared to *Flammulina velutipes* and *Pleurotus ostratus*, *L. edodes* has a relatively long cultivation period, with fruiting bodies that can be harvested more than five times on the same growth medium. The cultivation of *L. edodes* in farms is often exposed to the surrounding environment, which can increase the risk of viral infections [4,5].

Since the first description of fungal viruses 50 years ago, there has been an increase in the number of mycoviruses that have been identified [6]. Viral infections in *L. edodes* was first reported in Japan during the 1970s [7], and viruses associated with *L. edodes* have also been found in Korea in more recent studies [4,8]. LeV-HKB was the first mycovirus that was identified to have a dsRNA genome, and it shares a high degree of similarity with the genomes of LeSV and LeV-HKB [8,10]. LePV1 and LeV-HKB can coinfect *L. edodes*, and studies have also been performed to evaluate how these infections affect each other [9].

It is essential to screen strains that are not infected with a virus as viral infections have a significant impact on mushroom quality and productivity [11]. Since infection of the mycovirus is difficult to eradicate and it is necessary to use virus-free strains to prevent damage caused by the mycovirus [12,13].

The cultivation conditions of some mushrooms can help to prevent the impact of viral infections, even if they are strains that contain viral particles [14-16]. If viral particles are found but no disease appears, the viral infection may be overlooked; however, the same strain can cause problems under different conditions. Therefore, viral detection is strains used for breeding is paramount to the successful cultivation of mushrooms.

In this study, we use reverse transcription-polymerase chain reaction (RT-PCR) to detect RNA mycoviral infection in wild strains of *L. edodes* that can be used as a breeding resource in the future. The presence of five RNA mycoviruses (LePV1, LeSV, LeV-HKB, LeNSRV1, and LeNSRV2) were tested in the wild strains of *L. edodes*, and we observed the occurrence of single and multiple viral infections within a single wild strain.

MATERIALS AND METHODS

Fungal strains

One hundred and twelve wild strains of *L. edodes* were examined in this study (Table 1). They were isolated from samples that were collected from various locations across Korea from 1983 to 2020 and maintained at 4℃ through successive subcultures by the National Institute of Forest Science. The strains were cultured on potato dextrose agar (PDA, Difco, MD, USA) media at 25℃ for two weeks under dark conditions before use.

Table 1. The sampling information and RT-PCR analysis of the occurrence of RNA mycoviruses in wild strains of *Lentinula edodes* in Korea.

Strain No.[a]	Sample location	Year	Viruses				
			LeSV	LePV1	LeHKB	LeNSRV1	LeNSRV2
NIFoS 36	Gyeongsangnam-do	1983	+		+		
NIFoS 37	Gyeongsangnam-do	1983		+			
NIFoS 38	Gyeongsangnam-do	1983				+	
NIFoS 39	Gyeongsangnam-do	1983					
NIFoS 40	Gyeongsangnam-do	1983					

Table 1. Continued

Strain No.[a]	Sample location	Year	Viruses				
			LeSV	LePV1	LeHKB	LeNSRV1	LeNSRV2
NIFoS 41	Gangwon-do	1983			+		
NIFoS 42	Jeju-do	1983		+	+	+	
NIFoS 43	Jeju-do	1983					
NIFoS 45	Gangwon-do	1984		+		+	
NIFoS 46	Gangwon-do	1984		+	+		
NIFoS 47	Gangwon-do	1984		+	+		
NIFoS 48	Gangwon-do	1984				+	
NIFoS 49	Gangwon-do	1984		+	+		
NIFoS 50	Gangwon-do	1984	+	+	+		
NIFoS 51	Gangwon-do	1984	+	+	+	+	+
NIFoS 52	Gangwon-do	1984	+		+	+	
NIFoS 53	Gangwon-do	1984		+	+		
NIFoS 55	Gyeongsangnam-do	1984	+	+	+		
NIFoS 56	Gyeongsangnam-do	1984		+			
NIFoS 57	Gyeongsangnam-do	1984		+			
NIFoS 58	Gyeongsangnam-do	1984	+	+	+		
NIFoS 59	Gyeongsangnam-do	1984	+		+		
NIFoS 60	Gyeongsangnam-do	1984	+			+	
NIFoS 62	Gangwon-do	1985		+	+	+	
NIFoS 63	Gangwon-do	1985	+	+	+	+	+
NIFoS 64	Gangwon-do	1985			+	+	
NIFoS 65	Gangwon-do	1985	+		+		
NIFoS 66	Gangwon-do	1985	+	+	+		
NIFoS 67	Gangwon-do	1985	+	+	+	+	
NIFoS 68	Gangwon-do	1985				+	
NIFoS 128	Jeollabuk-do	1986	+	+	+		
NIFoS 129	Chungcheongbuk-do	1986	+		+		
NIFoS 130	Chungcheongbuk-do	1986	+		+		
NIFoS 135	Gyeongsangnam-do	1986		+			
NIFoS 136	Gyeongsangnam-do	1986	+			+	
NIFoS 177	Jeju-do	1989					+
NIFoS 188	Gangwon-do	1989					
NIFoS 221	Gangwon-do	1991					
NIFoS 299	Gangwon-do	1995			+		
NIFoS 351	Gangwon-do	1996				+	
NIFoS 352	Gangwon-do	1996	+		+	+	
NIFoS 353	Gangwon-do	1996	+	+	+	+	
NIFoS 354	Gangwon-do	1996	+			+	
NIFoS 369	Gangwon-do	1998	+		+	+	
NIFoS 370	Gangwon-do	1998	+		+		
NIFoS 411	Gangwon-do	1999		+		+	
NIFoS 663	Gangwon-do	2004				+	
NIFoS 664	Gangwon-do	2004	+	+	+	+	

Table 1. Continued

Strain No.[a]	Sample location	Year	Viruses				
			LeSV	LePV1	LeHKB	LeNSRV1	LeNSRV2
NIFoS 665	Gangwon-do	2004	+		+		
NIFoS 666	Gangwon-do	2004	+		+		
NIFoS 667	Gangwon-do	2004			+		
NIFoS 668	Gangwon-do	2004	+	+	+	+	
NIFoS 669	Gangwon-do	2004					+
NIFoS 670	Gangwon-do	2004	+	+			+
NIFoS 672	Gangwon-do	2004	+	+		+	
NIFoS 673	Gangwon-do	2004	+	+	+		
NIFoS 674	Gangwon-do	2004	+	+		+	
NIFoS 675	Gangwon-do	2004		+		+	
NIFoS 676	Gangwon-do	2004	+	+	+		
NIFoS 1520	Gangwon-do	2011	+	+		+	+
NIFoS 1522	Gangwon-do	2011	+		+	+	+
NIFoS 1651	Jeollanam-do	2011	+	+	+		
NIFoS 1652	Jeollanam-do	2011	+		+		
NIFoS 2063	Gangwon-do	2013	+	+			+
NIFoS 2064	Gangwon-do	2013	+				
NIFoS 2101	Gangwon-do	2013	+	+	+	+	
NIFoS 2290	Gangwon-do	2013		+			+
NIFoS 2497	Gangwon-do	2014	+				
NIFoS 2498	Gangwon-do	2014	+				
NIFoS 2521	Gangwon-do	2014	+		+		
NIFoS 2783	Gangwon-do	2014	+		+		
NIFoS 3010	Gangwon-do	2015	+		+	+	+
NIFoS 3125	Jeollanam-do	2015	+		+	+	
NIFoS 3166	Gangwon-do	2016	+		+		
NIFoS 3168	Gangwon-do	2016			+		
NIFoS 3169	Gangwon-do	2016	+	+			
NIFoS 3170	Gangwon-do	2016				+	
NIFoS 3172	Gangwon-do	2016	+			+	
NIFoS 3173	Jeju-do	2016	+		+	+	
NIFoS 3175	Gangwon-do	2016		+	+		
NIFoS 3177	Gangwon-do	2016	+			+	
NIFoS 3198	Gangwon-do	2016	+	+		+	
NIFoS 3199	Gangwon-do	2016			+	+	
NIFoS 3201	Gangwon-do	2016	+	+	+	+	
NIFoS 3202	Gangwon-do	2016		+	+	+	
NIFoS 3203	Gangwon-do	2016	+	+		+	
NIFoS 3983	Gyeonggi-do	2017					+
NIFoS 3984	Gyeonggi-do	2017					
NIFoS 3986	Gyeonggi-do	2017					+

Table 1. Continued

Strain No.[a]	Sample location	Year	Viruses				
			LeSV	LePV1	LeHKB	LeNSRV1	LeNSRV2
NIFoS 3987	Gyeonggi-do	2017					
NIFoS 3989	Gyeonggi-do	2017					
NIFoS 3990	Gyeonggi-do	2017					
NIFoS 3991	Gyeonggi-do	2017					
NIFoS 4367	Gyeonggi-do	2018					+
NIFoS 4368	Gyeonggi-do	2018					+
NIFoS 4617	Gyeonggi-do	2018					+
NIFoS 4618	Gyeonggi-do	2018					+
NIFoS 4619	Gyeonggi-do	2018					
NIFoS 4961	Gangwon-do	2019					
NIFoS 4962	Gyeongsangnam-do	2019	+				
NIFoS 5241	Jeju-do	2020		+	+	+	+
NIFoS 5242	Gangwon-do	2020	+			+	+
NIFoS 5243	Gangwon-do	2020		+	+	+	
NIFoS 5244	Gangwon-do	2020				+	+
NIFoS 5245	Gangwon-do	2020		+		+	+
NIFoS 5246	Gangwon-do	2020		+	+	+	
NIFoS 5252	Gangwon-do	2020	+		+	+	
NIFoS 5253	Gangwon-do	2020	+	+		+	
NIFoS 5254	Gangwon-do	2020	+	+	+	+	+
NIFoS 5255	Gangwon-do	2020		+	+	+	
NIFoS 5256	Gangwon-do	2020		+	+	+	+
NIFoS 5257	Gangwon-do	2020	+		+	+	+

[a]NIFoS, National Institute of Forest Science.

RNA extraction and cDNA synthesis

The mycelium grown on each PDA medium was collected by a cell scraper and frozen in liquid nitrogen. Total RNA was extracted using the RNeasy Mini Kit (Qiagen, CA, USA), following the manufacturer's instructions. The quantity and quality of the RNA were examined using an Epoch Multi-volume Spectrophotometer (BioTek, VT, USA). Single strand cDNA synthesis was carried out using Moloney murine leukemia virus reverse transcriptase (New England Biolabs).

RT-PCR

To confirm viral infection, RT-PCR was conducted with specific primers to amplify the RNA-dependent RNA polymerase (RdRp) genes of each mycovirus (Table 2). Amplification of the β-tubulin gene was used as a positive control to confirm successful RNA extraction and cDNA synthesis. All the primers used for RT-PCR are listed in Table 2. RT-PCR for β-tubulin and LeSV were performed under the following conditions: 95℃ for 5 min; 25 cycles of 95℃ for 30 s, 55℃ for 30 s, and 72℃ for 1 min; 72℃ for 5 min. RT-PCR for LePV1, LeV-HKB, LeNSRV1, and LeNSRV2 were performed under the following

Table 2. The specific primer sets targeting RNA-dependent RNA polymerase genes of each RNA mycovirus.

Primer set	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
β-tubulin	GACCGTATGATGTGCACGTAC	CACAAGATGGTTGAGGTCACC	[9]
LeSV	GCGATGATGACATACAGTAGGC	CGACGTCGGATAACATTGCGTC	This study
LePV1	AGCCTTTGACGATGTATCCGACTAC	GGGTTATGATTGCGAGAGGCATT	[9]
LeV-HKB	TGTTGTATAAGACAGGCGGTGTGGG	GGGTATATCTCAGCAAGCCTATGC	[9]
LeNSRV1	CGAGACATCCTCGCGGCTGTAGAGG	CCGAGGTTACCAGCTCCGATTGTC	[9]
LeNSRV2	AAGTATGGGGTAGTGATGATAGTGG	GAGGCTCCACCTTCCAATGTCTGAG	[9]

conditions: 95℃ for 5 min; 35 cycles of 95℃ for 30 s, 60℃ for 30 s, and 72℃ for 1 min; 72℃ for 5 min. The PCR products were confirmed using 1.5% (w/v) agarose gel electrophoresis.

RESULTS AND DISCUSSION

We isolated 112 wild strains of *L. edodes* from different times and locations in Korea (Table 1); there were 75 strains from Gangwon province, 14 strains from Gyeongsangnam province, 12 strains from Gyeonggi province, 5 strains from Jeju province, 3 strains from Jeollanam province, 2 strains from Chungcheongbuk province, and 1 strain from Jeollabuk province.

Specific primer sets (LeSV, LePV1, LeV-HKB, LeNSRV1, and LeNSRV2) were used (Table 2) to detect viral infection. According to the RT-PCR analysis, products of 328 to 394 bp in length were amplified from the wild strains when infected with the viruses (LeSV 386 bp, LePV1 389 bp, LeV-HKB 394 bp, LeNSRV1 377 bp, and LeNSRV2 328 bp) (Fig. 1).

Approximately half of the strains were infected with LeSV (50.9%, 57/112), LeV-HKB (50.9%, 57/112), LeNSRV1 (45.5%, 51/112), or LePV1 (42.9%, 48/112) viruses. The LeNSRV2 virus had a lesser infection than the other viruses (20.5%, 23/112) (Fig. 2A).

Most of the infected strains were co-infected with different viruses (Fig. 2B). Among them, 30 strains (26.8%) were infected with two different viruses and 29 strains (25.9%) were infected with three different viruses. One viral infection was observed in 26 (23.2%) strains. Only three strains, NIFoS 51, 63, and 5254, that were collected from Gangwon province had five mycoviruses. On the other hand, 12 strains (10.7% of the total wild strains) were not infected by the viruses.

In this study, we observed multiple infections in which two or more types of virus were present in a single strain. Although multiple viral infections are often found in plants and fungi, much research is still needed on the consequences of multiple infections [17,18]. For example, the African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) are commonly found to co-infect cassava plants with cassava mosaic disease (CMD) symptoms [19]; viral symptoms were more severe when ACMV and EACMV were present in *Nicotiana benthamiana* plants [19]. These results indicate that the two viruses have synergistic interactions [19]. The single infection of sweet potato feathery mottle virus (SPFMV, genus Potyvirus) and sweet potato chlorotic stunt virus (SPCSV, genus Crinivirus) in sweet potato do not show

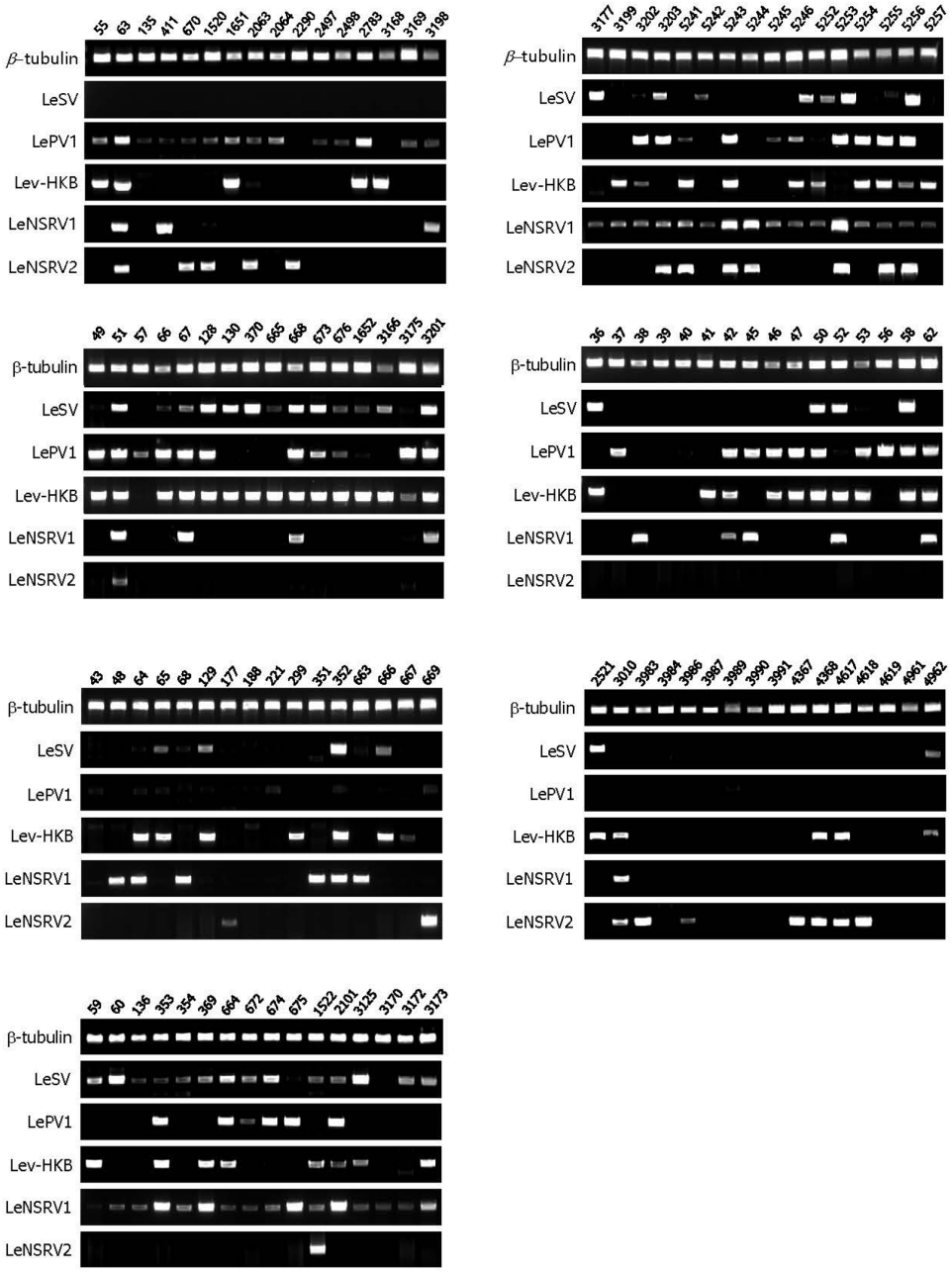

Fig. 1. Reverse transcription-polymerase chain reaction (RT-PCR) detection of viral infection from 112 wild strains of *Lentinula edodes*. Specific primer sets targeting RNA-dependent RNA polymerase genes of each RNA mycovirus were adopted for RT-PCR. Numbers represent each of the wild strains. Amplification of the *β*-tubulin gene was used as the positive control to confirm the RNA extraction.

significant symptoms [20]. In contrast, coinfection with the two viruses is caused by the sweet potato virus disease (SPVD), with severe symptoms evident in plants [20]. With the discovery of LePV1 and LeV-HKB coinfection in *L. edodes*, studies have also been conducted to assess their influence on each other [9]. When

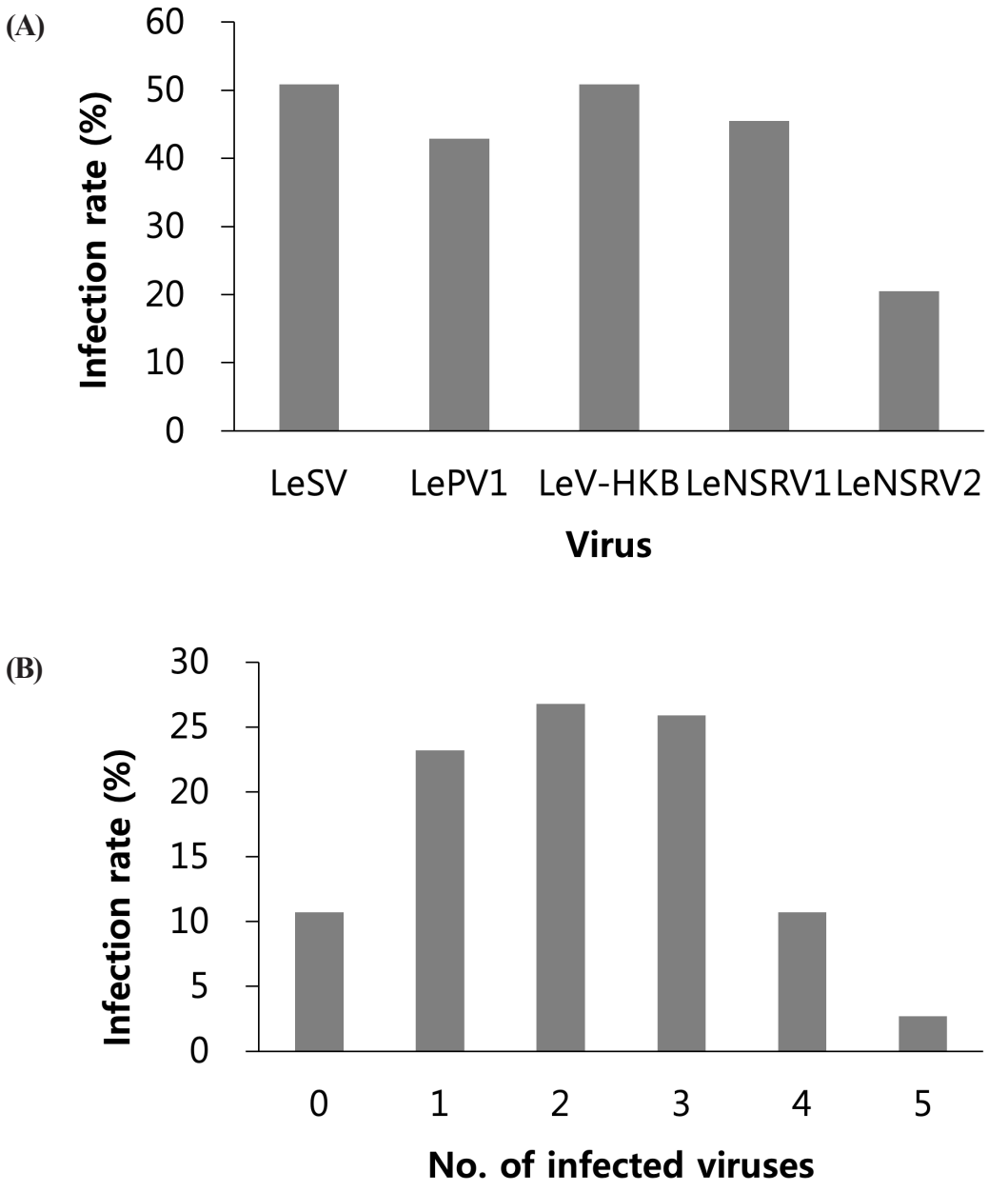

Fig. 2. Analysis of viral infections of *Lentinula edodes*. (A) Infection rate of each RNA mycovirus in the 112 wild strains used in this study. (B) Rate of viral multiple infection from 112 wild strains. The number on the horizontal axis represent the number of infected viruses.

the LePV1 virus is coinfected with LeV-HKB, the RdRp gene expression has been reported to be higher than that of a single infection [9].

Furthermore, how multiple infections of the virus found in *L. edodes* affect a viral disease should be

further studied. Despite the difficulty in evaluating the effects of each virus when several viruses infect one strain, a virus-cured strain can be used as an important control to investigate the individual effects. It is also necessary to further analyze whether a virus is present in the fruiting body by single spore isolation in the event of hybridization.

Although the number of strains collected from provinces other than Gangwon province was small, it is interesting to note that geographically LeSV, LePV1, LeV-HKB, and LeNSRV1 were not present in the strains collected from Gyeongsangnam province. Further, only the LeNSRV2 virus was detected in the samples collected from Gyeonggi province. These phenomena suggest the possibility that the infection of each virus is related to regional characteristics. To increase the diversity of wild strains of *L. edodes* in the future, we must investigate forest areas throughout the country, and the confirmation of viral infection in these collected strains will be essential.

ACKNOWLEDGEMENT

This study was supported by a grant from the Golden Seed Project of 'Breeding of new strains of shiitake for cultivar protection and substitution of import (213007-05-5-SBH10)' of the National Institute of Forest Science, Republic of Korea.

REFERENCES

1. Ridley SP, Sommer SS, Wickner RB. Superkiller mutations in *Saccharomyces cerevisiae* suppress exclusion of M2 double-stranded RNA by L-A-HN and confer cold sensitivity in the presence of M and L-A-HN. Mol Cell Biol 1984;4:761-70.
2. Ghabrial SA. Origin, adaptation and evolutionary pathways of fungal viruses. Virus Genes 1998;16:119-31.
3. Magae Y. Characterization of a novel mycovirus in the cultivated mushroom, *Lentinula edodes*. Virol. J 2012;9:1-6.
4. Kim J, Yun S, Ko H, Kim D. Occurrence of dsRNA mycovirus (LeV-FMRI0339) in the edible mushroom *Lentinula edodes* and meiotic stability of LeV-FMRI0339 among monokaryotic progeny. Plant Pathol J 2013;29:460-4.
5. Rytter, JL, Royse DJ, Romaine CP. Incidence and diversity of double stranded RNA in *Lentinula edodes*. Mycologia 1991;83:506-510.
6. Hollings, M. Viruses associated with a die-back disease of cultivated mushroom. Nature 1962;196:962-5.
7. Inouye T, Furuya K, Nisikado Y. Virus-like particles found in shiitake. Ann Phytopathol Soc Japan 1970;36:356.
8. Lin YH, Fujita M, Chiba S, Hyodo K, Andika IB, Suzuki N, Kondo H. Two novel fungal negative-strand RNA viruses related to mymonaviruses and phenuiviruses in the shiitake mushroom (*Lentinula edodes*). Virol 2019;533:125-36.
9. Guo M, Bian Y, Wang J, Wang G, Ma X, Xu Z. Biological and molecular characteristics of a novel partitivirus infecting the edible fungus *Lentinula edodes*. Plant Dise 2017;101:726-33.

10. Ro HS, Lee NJ, Lee CW, Lee HS. Isolation of a novel mycovirus OMIV in *Pleurotus ostreatus* and its detection using a triple antibody sandwich-ELISA. Virol Methods J 2006;138:24-9.
11. Won HK, Park SJ, Kim DK, Shin MJ, Kim N, Lee SH, Kwon YC, Ko HK, Ro HS, Lee HS. Isolation and characterization of mycovirus in *Lentinula edodes*. Micro J 2013;51:118-22.
12. Sahin E, Akata I. Viruses infecting macrofungi. Virus Disease 2018;29:1-18.
13. Wang JJ, Guo MP, Sun YJ, Bian YB, Zhou Y, Xu ZY. Genetic variation and phylogenetic analyses reveal transmission clues of *Lentinula edodes* partitivirus 1(LePV1) from the Chinese *L. edodes* core collection. Virus Res 2018;255:127-32.
14. Ro HS, Kang EJ, Yu JS, Lee CW, Lee HS. Isolation and characterization of a novel mycovirus, PeSV, in *Pleurotus eryngii* and development of a diagnostic system for it. Biotechnol Lett 2007;29:129-35.
15. Wang L, He H, Wang SC, Chen XG, Qiu DW, Kondo H, Guo LH. Evidence for a novel negative-stranded RNA mycovirus isolated from the plant pathogenic fungus *Fusarium graminearum*. Virol 2018;518:232-40.
16. Ghabrial SA, Caston JR, Jiang DH, Nibert ML, Suzuki N. 50-plus years of fungal viruses. Virol 2015;479:356-68.
17. Harrison BD, Zhou X, Otim-Nape GW, Liu Y, Robinson DJ. Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. Ann Appl Biol 1997;131:437-48.
18. Wang L, Jiang Y, Hong N, Zhang F, Xu W, Wang G. Hypovirulence of the phytopathogenic fungus *Botryosphaeria dothidea*: Association with a coinfecting chrysovirus and partitivirus. J Virol 2014;88:7517-27.
19. Fondog VN, Pita JS, Rey MEC, de Kochko A, Beachy RN, Fauquet CM. Evidence of synergism between African cassava mosaic virus and a new double-recombinant geminivirus infecting cassava in Cameroon. J Gen Virol 2000;81:287-97.
20. Karyeija RF, Kreuze JF, Gibson RW, Valkonen JPT. Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. Virology 2000;269:26-36.