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Biological and Molecular Characteristics of a Novel Partitivirus Infecting the Edible Fungus *Lentinula edodes*

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Abstract

A new partitivirus named *Lentinula edodes partitivirus 1* (LePV1) was isolated from a diseased *L. edodes* strain with severe degeneration of the mycelium and imperfect browning in bag cultures. The nucleotide sequences of LePV1 dsRNA-1 and dsRNA-2 were determined; they were 2,382 bp and 2,245 bp in length, and each contained a single ORF encoding RNA-dependent RNA polymerase (RdRp) and coat protein (CP), respectively. The purified virus preparation contained isometric particles 34 nm in diameter encapsidating these dsRNAs. Phylogenetic analyses showed LePV1 to be a new member of *Betapartitivirus*, with the RdRp sequence most closely related to *Grapevine partitivirus*. RT-PCR analysis showed that 27 of the 56 Chinese *L. edodes* core collection strains carry LePV1, with the virus being more common in wild strains than cultivated strains. In addition, qPCR analysis suggested that coinfection with *L. edodes mycovirus HKB* (LeV-HKB) could increase replication of the *RdRp* gene of LePV1. This study may be essential for the development of more accurate disease diagnostics and the formulation of control strategies for viral diseases in *L. edodes*.

Mycovirus research was pioneered in 1962 with the report of three distinct virus-like particles (VLPs) linked to La France disease in *Agaricus bisporus* mushrooms (Goodin et al. 1992; Hollings 1962). A number of new fungal viruses were subsequently found, and their molecular characteristics and genomic organization were partially described (Bevan et al. 1973; Boland 1992; Jiang and Ghabrial 2004; Yu et al. 2010). The genomes of mycoviruses are made of double-stranded (ds) RNAs or single-stranded (ss) RNA or DNA; they are assembled into virus particles or exist as naked nucleic acid in fungal vesicles (Ghabrial et al. 2015).

In the majority of cases, mycoviruses with potential for biological control of plant and fungal diseases has been the subject of much research (Milgroom and Cortesi 2004; Xie and Jiang 2014). However, viral diseases of mushrooms are also important in mushroom cultivation, especially as the number of cultivated mushroom species and mushroom farms increases. Several mushroom species, including *A. bisporus*, *Pleurotus* spp., *Lentinula edodes*, and *Flammulina velutipes*, have been reported as infected with viruses (Grogan et al. 2003; Magae 2012; Magae and Sunagawa 2010; Ro et al. 2007; Tavantzis et al. 1980; Yu et al. 2003). Some of these viruses are associated with considerable morphological and physiological changes, resulting in crop losses and economic implications for the growers. For instance, two important viral diseases in *A. bisporus* cultivation, La France disease caused by the *La France isometric virus* (LIV) and/or *Mushroom bacilliform virus* (MBV) and "patch disease" caused by *Mushroom virus X* (MVX), result in serious morphological deformities and are considered limiting factors in commercial *A. bisporus* cultivation, reducing both the quality and quantity of the mushrooms (Goodin et al. 1992; Rao et al. 2007; Tavantzis et al. 1980). Other symptomatic mushroom viruses such as *Oyster mushroom spherical virus* (OMSV), *P. ostreatus spherical virus* (PoSV), *P. eryngii spherical virus* (PeSV), and *F. velutipes browning virus* (FvBV) have been associated with *P. ostreatus* die-back disease and mycelial degradation (Yu et al. 2003), *P. eryngii* mycelial degradation and deformed basidiocarps (Ro et al. 2007), and brown discolored basidiocarps in *F. velutipes* (Magae and Sunagawa 2010), respectively.

Lentinula edodes (Berk.) Pegler, also known as Xianggu and Shiitake mushroom, is well known for its culinary uses and medicinal properties and is the second most important mushroom in terms of total world production (Bedigian 2004). Since the 1970s, many morphologically distinct VLPs or dsRNAs have been detected in *L. edodes* in China, South Korea, Japan, and the U.S.A. (Magae 2012; Rytter et al. 1991; Ushiyama et al. 1977; Won et al. 2013; Yao et al. 2010). The presence of viruses has been confirmed in some abnormal *L. edodes* strains, such as strains with mycelial degeneration, inadequate or imperfect substrate browning, and malformations of the fruiting body (Kim et al. 2013; Magae 2012; Won et al. 2013; Yao et al. 2010). However, the relationships of these mycoviruses to any harmful effects on *L. edodes* have not been determined because these mycoviruses are also found in asymptomatic strains (Kim et al. 2013). Among the viruses infecting *L. edodes*, *L. edodes mycovirus HKB* (LeV-HKB) was the first to have its whole genome sequenced. LeV-HKB was characterized as a linear unencapsidated dsRNA element with an 11-kb-long viral genome by direct visualization of the nucleic acid using atomic force microscopy; its viral genome contains two open reading frames (ORFs) (Magae 2012). *L. edodes spherical virus* (LeSV), isolated from a diseased *L. edodes*, was subsequently reported to be related to LeV-HKB because part of its genome showed very high similarity with the *RdRp* gene of LeV-HKB, and the N-terminal sequence of its viral coat protein was also found in the middle of the ORF1 gene of LeV-HKB (Won et al. 2013).

In this study, we report a new mycovirus, *L. edodes partitivirus 1* (LePV1), which was isolated from a symptomatic *L. edodes* strain presenting degeneration of mycelia, abnormal browning of the bag cultures, and inability to form a fruiting body. We determined the sequence and some molecular characteristics of the two-segmented dsRNA genome and the characteristics of the LePV1 virus particles. We also investigated the occurrence of LePV1 among Chinese *L. edodes* core collections by RT-PCR detection. In addition, quantitative PCR (qPCR) was conducted to evaluate *RdRp* and *CP* gene expression in *L. edodes* infected with LePV1 and coinfected with LePV1 and LeV-HKB.

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*The ***e-X***tra logo stands for "electronic extra" and indicates that two supplementary tables are available online.

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Materials and Methods

Strains and culture conditions. *L. edodes* strain SX12, LMLH13, and 56 *L. edodes* core collection strains (containing 21 cultivated strains and 35 wild strains) were used in this study. SX12 is a cultivated strain grown on many farms in central and eastern China, occasionally showing serious symptoms (Figs. 1A and B). In this study, two samples of SX12 were used as materials to extract dsRNA, including mycelial isolation from sawdust substrate in an abnormal cultivated bag and asymptomatic fruiting bodies. LMLH13 is a virus-free strain that presents normal mycelial performance and normal substrate browning and was used as the healthy control. The 56 strains in the Chinese *L. edodes* core collection are listed in Table 1 (Liu et al. 2015a). These 56 strains underwent LePV1 detection by RT-PCR. All of the strains were cultured in malt yeast glucose broth (MYG, containing 2% malt extract, 2% glucose, 0.1% yeast extract, and 0.1% peptone) at 150 rpm and 25°C for 1 to 2 weeks (Xiang et al. 2016). Their mycelia were collected and stored at –80°C for dsRNA extraction, virus particle isolation, and RT-PCR detection.

dsRNA extraction and purification. Approximately 1 g of frozen mycelia were ground to a fine powder in liquid nitrogen and dsRNA was obtained by phenol extraction and purified through a cellulose CF-11 column (Morris and Dodds 1979). The dsRNA was dissolved in RNase-free water and digested with DNase I and S1 Nuclease (TaKaRa, Dalian, China) to remove genomic DNA and ssRNAs. The resulting dsRNAs were electrophoresed on a 0.8% agarose gel. Two bands were obtained, excised, and purified separately using a gel extraction kit for DNA (BioTeke, Beijing, China). The quality and concentration of purified dsRNA was evaluated by electrophoresis and a DS-11 Spectrophotometer (DeNovix, Wilmington, U.S.A.).

Full-length cDNA cloning and sequencing. Purified dsRNA was used as the template for cDNA cloning. The cDNA was cloned using the method reported by Xie et al. (2011) with a minor modification. cDNA was synthesized using Moloney murine leukemia virus (M-MLV) reverse transcription (TaKaRa, Dalian, China) with the tagged random primer dN6 (5′-CGATCGATCATGATGCAATGCNNNNNN-3′). The cDNAs obtained were further purified with a silica column (BioTeke, Beijing, China) and amplified using the tagged oligonucleotide (5′-CGATCGAT CATGATGCAATGC-3′) in combination with end-filling with rTaq (TaKaRa, Dalian, China). The amplification products (>500 bp) were cloned into the *pEASY* T1 vector (TaKaRa, Dalian, China), and then transformed into competent *Escherichia coli* T1 cells. The transformants were then verified by PCR and sequenced (Tsingke, Wuhan, China). The gaps between two different clones were determined by RT-PCR using primers designed for the specific cDNA sequence obtained. The 5′ and 3′ terminal sequences of dsRNA were determined following the method of Liu et al. (2015b) with some modifications. An anchor primer PC3-T7 loop (5′-GGATCCCGGGAATTCGGTAATACGACTCACTATATTTT TATAGTGAGTCGTATTA-3′) was ligated to dsRNA using T4 RNA ligase (TaKaRa, Dalian, China), and then the mixture was purified with chloroform and a reverse transcription process was performed. Amplified PCR of the cDNA was implemented using the anchor-complemented primer PC2 (5′-CCGAATTCCCGGGATCC-3′) and sequence-specific primers corresponding to the 5′ or 3′ terminal sequences of the dsRNA, respectively. The nucleotides were determined by sequencing three times and were then assembled. Finally, the full sequence of the cDNA was verified by RT-PCR.

Sequence analysis. Sequence similarity searches were performed using the National Center for Biotechnology Information (NCBI) databases with the blastp program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence assembly and multiple-sequence alignments were carried out with DNAMAN version 8 and CLUSTAL X2, respectively. The ORF of the virus genome was deduced through the ORF Finder program of the NCBI (https://www.ncbi.nlm.nih.gov/orffinder/). Phylogenetic trees were constructed using the amino acid sequences of RdRp and CP by the neighbor-joining method using MEGA version 6.0 (Tamura et al. 2013). Amino acid sequences of other mycoviruses were used for comparative analysis and were retrieved from the NCBI database with the Batch Entrez program (http://www.ncbi.nlm.nih.gov/sites/batchentrez). The secondary structures of the terminal sequences of the dsRNAs were determined with RNAstructure version 5.8 (Reuter and Mathews 2010).

Electron microscopy and SDS-PAGE analysis of virus particles. Virus particles were purified using a sucrose density gradient centrifugation method as previously described (Liu et al. 2015b). Approximately 30 g of frozen mycelia were ground to a fine powder in liquid nitrogen and added to 200 ml of 0.1 M sodium phosphate buffer (PBS, pH 7.4) containing 0.2 M KCl and 0.5% β-mercaptoethanol. The mixture was homogenized by shaking at 100 rpm for 50 min at 10°C and then centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was subsequently centrifuged at 26,000 rpm at 4°C for 2 h with an ultracentrifuge (Beckman Coulter, U.S.A.). The resulting pellets were resuspended in 0.05 M PBS. The virus preparations were further purified using sucrose gradients ranging from 20 to 60% (w/v) and centrifuged at 30,000 rpm at 4°C for 2 h. Each fraction was individually subjected to dsRNA verification. To remove the sucrose, the gradient containing virus particles was diluted with 0.05 M PBS and recentrifuged at 36,000 rpm at 4°C for 2 h. Finally, the pellets were resuspended using 150 μl 0.01 M PBS and stained with 2% (w/v) phosphor tungstic acid (PTA) for observation with a transmission electron microscope (TEM) (Hitachi, Japan). In addition, the virus particle suspension was subjected to electrophoresis analysis by 12% SDS-PAGE using the method described by Wang et al. (2014) and the gels were stained with Coomassie brilliant blue R-250 (Biosharp, Hefei, China).

RT-PCR verification of LePV1 from purified virus particles. To confirm that the virions were generated by LePV1, RNA was extracted from the purified virions with 10% SDS and phenol/chloroform/isoamyl alcohol (PCI, 25:24:1, v/v/v) and precipitated with ethanol. The cDNA was synthesized using M-MLV reverse transcription (TaKaRa, Dalian, China) and oligo $(dT)_{18}$ primer (Fan et al. 2014). RT-PCR was carried out with primers designed to amplify the full-length cDNA of LePV1 (common forward primer: GAACATCTGTCCACACGCC; dsRNA-1 reverse primer: GTTATGATTGCGAGAGGCATTC; dsRNA-2 reverse primer: AGCATTCGCTTCTTCGCAGT). The PCR reaction conditions were 95°C for 5 min, 30 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1.5 min, and 72°C for 10 min. PCR products were visualized by electrophoresis on a 1% agarose gel with ethidium bromide.

RT-PCR investigation of the occurrence of LePV1 in the *L. edodes* core collection. Total RNA was extracted from mycelia using the STE method (Shui et al. 2008). Approximately 0.5 g of mycelium powder was added to 300 μl extraction buffer (RNase-free H_2O: 10 × STE: 10% SDS = 7:2:1), then 600 μl PCI was added. The mixture was homogenized via vortex and centrifuged at 12,000 rpm at 4°C for 10 min. RNA was re-extracted from the supernatant with isovolumetric PCI. Then, a one-tenth volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of ethanol were added to

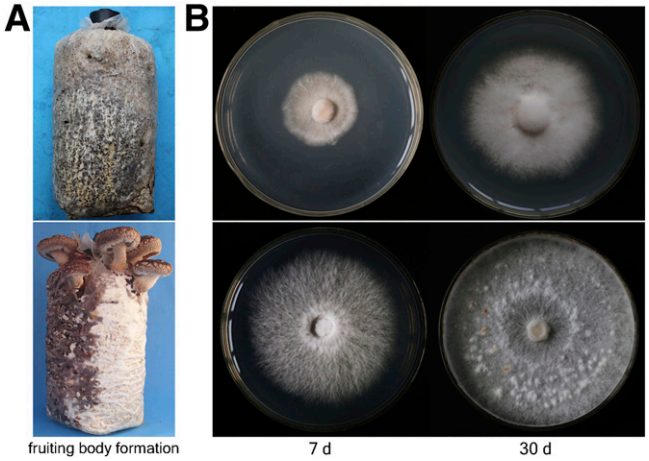

Fig. 1. Culture characteristics of a diseased strain SX12 (top) and healthy strain LMLH13 (bottom) of *Lentinula edodes*. **A,** Abnormal browning of the bag cultures and inability to form a fruiting body (top); normal browning and normal fruiting body formation (bottom). **B,** Degenerate mycelia (top) and normal mycelia (bottom) cultured on PDA medium for 7 days and 30 days at 25°C in the dark, respectively.

precipitate the genomic RNA (Wang and Vodkin 1994). The genomic DNA in the total RNA preparation was removed using recombinant DNase I (TaKaRa, Dalian, China). The cDNA was synthesized following the protocol described by Fan et al. (2014). RT-PCR was carried out with specific primers for the *RdRp* gene of LePV1 (LePV1-F: 5′-CGCTTTTCTTATTCTTGCCCG-3′; LePV1-R: 5′-GTTTCGAGCC CATACATAATACAG-3′) resulting in a 315-bp product. The *β-actin* gene (forward primer: 5′-GGAGAAGATTTGGCATCACACA-3′; reverse primer: 5′-GAAGAGCGAAACCCTCGTAGA-3′) was used as positive control to confirm the successful extraction of all RNA from *L. edodes*. The PCR reaction conditions were 95°C for 5 min followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 50 s, and a final step of 72°C for 10 min. PCR products were visualized by electrophoresis on a 1% agarose gel stained with ethidium bromide.

Quantitative analysis of the *RdRp* and *CP* genes of LePV1. To evaluate the *RdRp* and *CP* gene quantity, a quantitative PCR (qPCR) process was conducted on all *L. edodes* cDNA using AceQ qPCR SYBR Green Master Mix (Vazyme, Nanjing, China). Quantification of both gene targets was performed in triplicate. Primers for the *RdRp* used in qPCR were RdRp-F (GTACCCTACTTACCCCGACC) and RdRp-R (CGGAACAGCCAAATTCGCAGA). Primers for the *CP* were CP-F (AACTTACAAGCAATCCCCACGTT) and CP-R (ACAGCACCTAGTTGAGTAGTTCC). The *β-actin* gene, described above, was used as the reference gene. Analysis of gene expression were carried out using CFX Manager (Bio-Rad, U.S.A.).

Results

Nucleotide sequence of LePV1 genomic dsRNAs. Two dsRNA bands were detected in SX12 both in the mycelial isolation from sawdust substrate in an abnormal cultivated bag and in asymptomatic fruiting bodies; they were confirmed to be dsRNA by DNase I and S1 nuclease digestion (Fig. 2A). Their full-length cDNA sequences were obtained and deposited in the GenBank database with accession numbers KX354971 (dsRNA-1) and KX354972 (dsRNA-2), respectively. The full-length cDNA sequence of dsRNA-1 is 2,382 bp in length and has a GC-content of 44% excluding the poly(A) tail; the dsRNA-2 is 2,245 bp in length and has a GC-content of 45%. The 5′- and 3′-untranslated regions (UTRs) are 75 and 159 bp long in dsRNA-1 and 93 and 235 bp long in dsRNA-2, respectively. Moreover, the 5′-UTRs and 3′-UTRs of the dsRNAs shared 71.28 and 42.19% similarity, respectively. They both have an adenine-rich region approximately 50 bp in length in their 3′ terminus (Fig. 2B). The conserved sequence of their 5′-UTRs was predicted to fold into a stem-loop structure (Fig. 2C); a similar stem-loop structure was also observed in RNA1 from *Red clover cryptic virus 2* (RCCV-2) (Lesker et al. 2013), and was found to play an important role in dsRNA replication and virion assembly. In addition, the genome of LePV1 possesses the conserved sequence GAA at the 5′ terminus in both dsRNA-1 and dsRNA-2 (Fig. 2D). This 5′ terminal conserved GAA was also found in some fungal viruses in the genus *Betapartitivirus* (Fig. 2D) and the 5′ terminal conserved GA was found in the *Alphapartitivirus* and *Deltapartitivirus* genera (Nibert et al. 2014).

Amino acid sequence and phylogenetic analysis. Analysis of LePV1 sequences showed that dsRNA-1 contains a single long open reading frame (ORF) (from nt 76 to nt 2223) on its positive strand (Fig. 3A). The ORF encodes a 715-amino-acid (aa) protein with a predicted molecular mass of 84 kDa, containing a RdRp conserved domain (from nt 1170 to nt 1740, cd01699, 1.26e-03) belonging to

Table 1. The source of 56 strains and RT-PCR analysis of the occurrence of LePV1 in Chinese *Lentinula edodes* core collections[a]

Cultivated strains				Wild strains			
Number	Strain name	Source	LePV1	Number	Strain name	Source	LePV1
1	S605	Shanghai	+	22	EFISAAS0229	Jingdong	-
2	Cr04	Sanming	-	23	EFISAAS0351	Jingdong	+
3	L12	Sanming	+	24	00167	Yaoan	+
4	L135	Sanming	-	25	00168	Xianggelila	+
5	L856	Sanming	-	26	ACCC50786	Huangshan	-
6	S602	Shanghai	-	27	GAN059	Kang couty	+
7	Guangxiang-51	Guangdong	-	28	HUB039	Shennongjia	-
8	Hunong-1	Shanghai	+	29	HUB040	Shennongjia	-
9	Huaxiang-8	Wuhan	-	30	HUB091	Changyang	+
10	Qingke-20	Qingyuan	+	31	HN002	Chenzhou	-
11	Qiu-6	Wuhan	-	32	SHX044	Liuba	-
12	Rifeng-34	Henan	-	33	NO.41	Xiushui	-
13	Senyuan-10	Yichang	-	34	LeQc743s	Qingchuan	-
14	Senyuan-1	Yichang	-	35	LeWs735	Dechang	+
15	Senyuan-2	Yichang	+	36	LHLy14	Huili	+
16	430	Wuhan	-	37	LHLy217	Huili	-
17	908	Henan	-	38	LMLH14	Mianning	-
18	868	Zhejiang	+	39	LMLH36	Mianning	+
19	Xiangjiu	Guangdong	-	40	LMLH52	Mianning	+
20	Yuhua-2	Biyang	-	41	LMLH59	Mianning	+
21	Yuhua-4	Biyang	-	42	LMLH116	Mianning	+
				43	LMLHA18	Mianning	+
				44	LMLHA36	Mianning	+
				45	LMLHL22	Mianning	+
				46	LMLHL26	Mianning	-
				47	LMLHL210	Mianning	+
				48	LMYP62	Miyi	+
				49	LPG82	Puge	-
				50	YAASM234	Nanhua	+
				51	YAASM358	Chuxiong	+
				52	YAASM366	Chuxiong	+
				53	YAASM1515	Yangbi	-
				54	YAASM1518	Yangbi	-
				55	YAASM3334	Longling	+
				56	YAASM3353	Yiliang	+

[a] + indicates LePV1 infected; - indicates no LePV1 infected.

the poly(A)-type retrotransposons. Multiple comparison analysis revealed that the 715-aa protein contains six conserved motifs of RdRp (from III to VIII) (Fig. 3B). These motifs have also been described in other dsRNA viruses (Bruenn 1993; Jiang and Ghabrial 2004; Koonin 1991; Liu et al. 2015b). According to the phylogenetic tree of the amino acid sequences of RdRp, LePV1 is most closely related to the *Grapevine partitivirus* (AFX73019) with 59% similarity; they are placed in a distinctive cluster of the genus *Betapartitivirus* (Fig. 4A). dsRNA-2 contains a single ORF (from nt 94 to nt 2010) on its positive strand (Fig. 3A). The deduced amino acid sequence includes 638-aa and encodes a protein with a predicted molecular mass of 72 kDa that is highly homologous to CP from the family *Partitiviridae*. Furthermore, the phylogenetic tree constructed using the amino acid sequences of CP indicate that LePV1 is a new member of

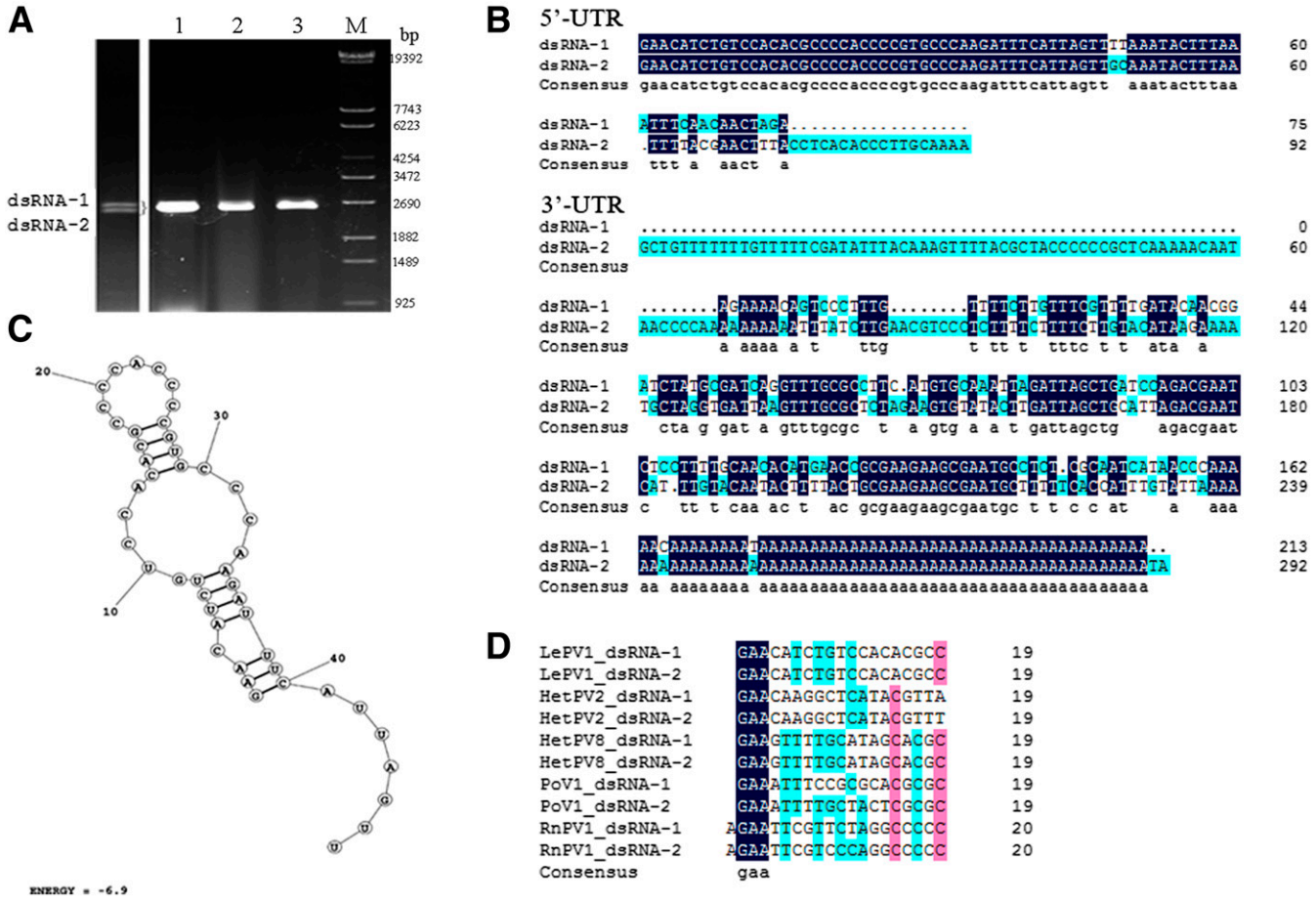

Fig. 2. Agarose gel electrophoresis analysis and molecular characteristics of the genomic dsRNA in LePV1. **A,** 0.8% agarose gel electrophoresis analysis of the dsRNAs extracted from mycelia of SX12. Lane 1: dsRNA without enzyme digestion; lanes 2 and 3: dsRNA digested by DNase I and nuclease S1, respectively; M: λ-*Eco*T14 I digest DNA marker. **B,** Molecular characteristics of conserved 5′-UTRs and 3′-UTRs (identical sequences of the 5′-UTR and 3′-UTR of the two dsRNAs are highlighted in navy blue). **C,** Predicted secondary structures of the conserved 5′-UTR sequence of LePV1. **D,** Molecular characteristics of the conserved sequence at the 5′ terminus on the plus-strand of dsRNA-1 and dsRNA-2 segments among five betapartitivirus genomes (the conserved GAA is highlighted in navy blue). The viruses used in alignment are listed in Supplementary Table S1.

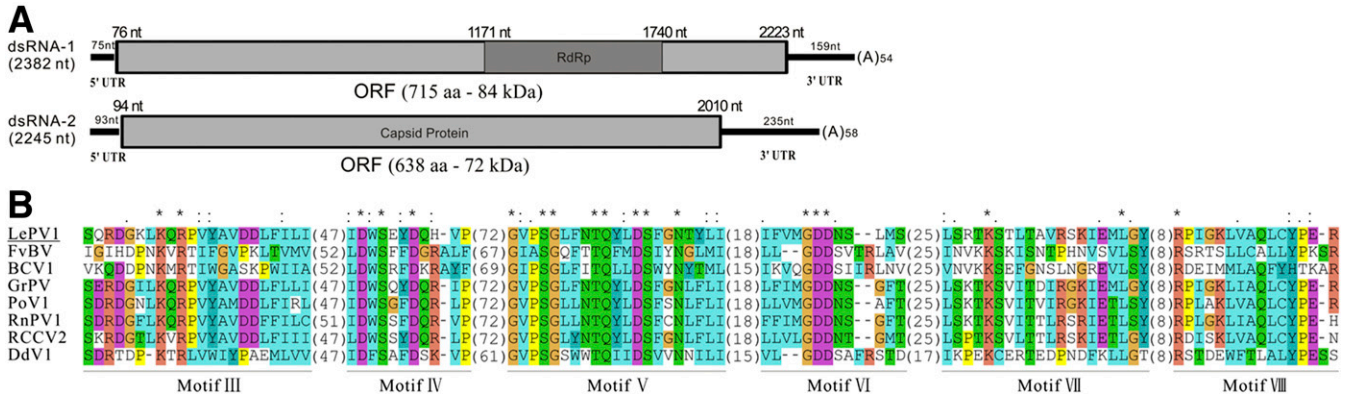

Fig. 3. Genomic organizations and conserved motifs of amino acid sequences in dsRNA-1 and dsRNA-2 from LePV1. **A,** Genome organization of dsRNA-1 and dsRNA-2 in LePV1. The open reading frame (ORF) and the untranslated regions (UTRs) are indicated by a gray block and a single bold line, respectively. The dark shadowing shows the conserved RdRp domain in dsRNA-1. **B,** Conserved motifs of amino acid sequences of RdRp (from motif III to VIII) among LePV1 and seven selected viruses in the family *Partitiviridae*. Detailed information of those selected viruses are described in the Supplementary Table S2. "*" indicates identical amino acid residues; ":" and "." indicate higher and lower chemically similar residues, respectively. Numbers in brackets represent the number of amino acid residues separating the motifs.

the genus *Betapartitivirus* (Fig. 4B). Abbreviations of names and viral protein accession numbers for the individual viruses used in phylogenetic tree construction are listed in Supplementary Table S2.

Characterization of mycovirus particles. The virions of LePV1 consisted of spherical particles 34 nm in diameter (Fig. 5A) found to be concentrated in the 30% fraction. SDS-PAGE analyses of purified viral proteins from SX12 and a healthy control (strain LMLH13) were conducted. A protein band of 72 kDa was obtained with SX12 (Fig. 5B), in accordance with our predicted molecular mass of 72 kDa of the protein encoded by dsRNA-2. To confirm that the virions were generated by LePV1, total RNA was extracted from purified virions and RT-PCR was conducted; the results showed that products with the expected size of 2,380 bp (dsRNA-1) and 2,228 bp (dsRNA-2) were amplified from purified virions (Fig. 5C).

Occurrence of LePV1 among the *L. edodes* core collection. To investigate whether LePV1 is widespread in *L. edodes*, we surveyed 56 core collections of Chinese *L. edodes* cultivars constructed by our laboratory using ISSR and SRAP markers (Liu et al. 2015a). The results showed that many of the strains were infected with LePV1. The infection rates of cultivated strains and wild strains were 28.6% (6/21) and 60.0% (21/35), respectively (Fig. 6A and B). The infection rate of LePV1 among all strains in the *L. edodes* core collection was 48.2% (27/56). The infection characteristics of LePV1 are summarized in Table 1.

Coinfection with LeV-HKB. During our virus dsRNA detection of *L. edodes* strains, we found that LePV1 and LeV-HKB can infect *L. edodes* separately or together (Fig. 7A). In addition, the brightness of the dsRNA-1 band was nearly equivalent to the dsRNA-2 band when LePV1 infected on its own; however, the dsRNA-1 band became brighter than that of dsRNA-2 when LePV1 was coinfected with LeV-HKB, which indicates that coinfection with LeV-HKB may promote the expression of the *RdRp* gene in LePV1. To confirm this possibility, qPCR was conducted to detect the expression of *RdRp* and *CP* genes of LePV1 in the strain infected with LePV1 alone and in the strain coinfected with LePV1 and LeV-HKB, respectively. The results showed that the expression of *RdRp* was 3.3-fold higher than *CP* in the strain infected with LePV1 alone, while it was 18.5-fold higher in the strain coinfected with LePV1 and LeV-HKB (Fig. 7B).

Discussion

Partitiviridae viruses have been found in two other mushroom species, i.e., *P. ostreatus virus 1* (PoV1) isolated from *P. ostreatus*, and FvBV isolated from *F. velutipes* (Lim et al. 2005; Magae and

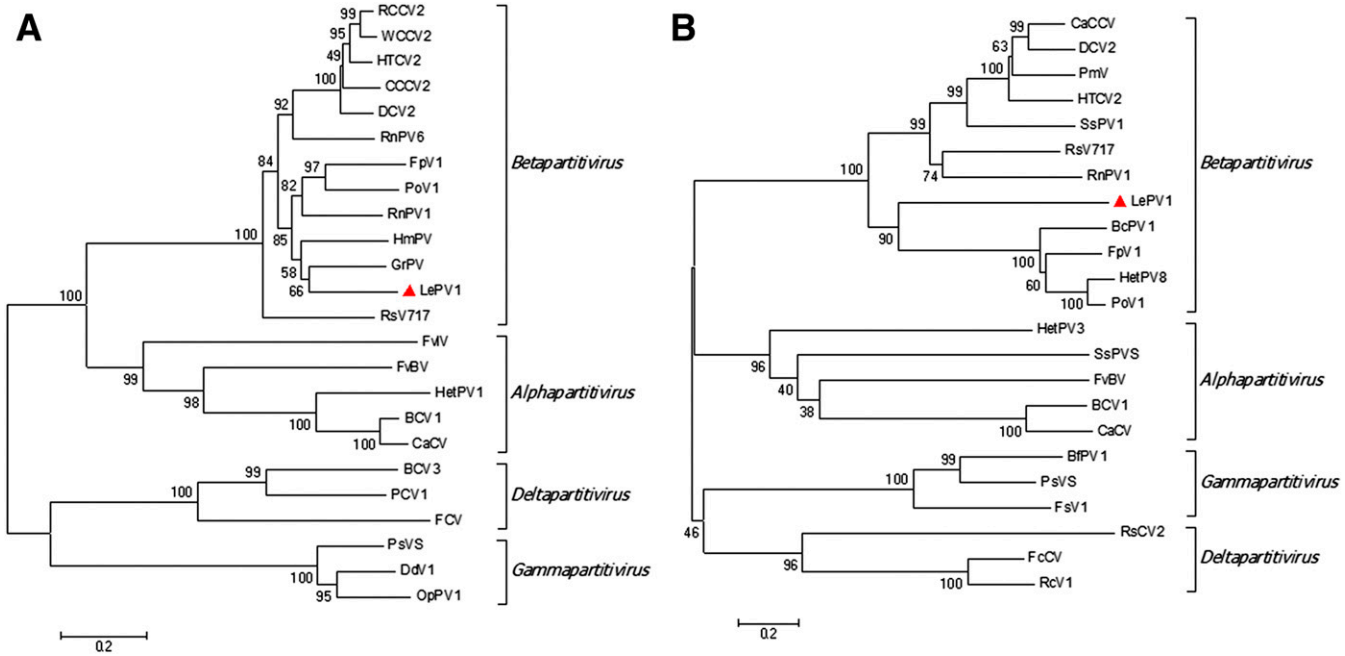

Fig. 4. Phylogenetic trees constructed based on the deduced amino acid sequences of the putative RdRp **(A)** and CP **(B)** using the neighbor-joining method (1,000 bootstrap replicates). The scale bar represents a genetic distance of 0.2 amino acid substitutions per site. The red triangle indicates the position of LePV1.

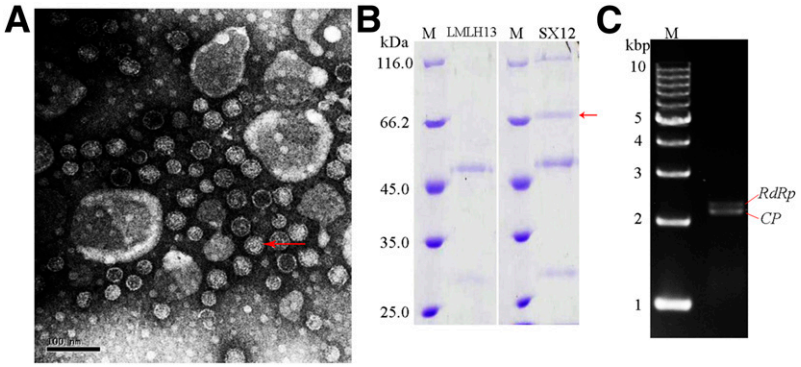

Fig. 5. Analyses of virions and capsid protein of LePV1. **A,** Electron micrographic characteristics of viral particles (the red arrow shows one of the viral particles). **B,** 12% SDS-PAGE gel electrophoresis analysis of coat protein from LePV1 virions. The red arrow shows the band (72 kDa) of capsid protein from LePV1. M: 26610 unstained protein MW marker. **C,** 1% agarose gel electrophoresis analysis of the RT-PCR products using the template of RNA extracted from the purified virus particles. M: 1 kb DNA ladder marker.

Sunagawa 2010). In this study, we report a new partitivirus (LePV1) isolated from a diseased *L. edodes* strain SX12 (Fig. 1). The genome of LePV1 showed features typical of partitiviruses (Ghabrial et al. 2008), with two segments of approximately 2.3 and 2.2 kbp containing a single ORF each (Figs. 2A and 3A). The larger segment encodes an RdRp, and the small one encodes the CP. As expected, they were also highly conserved, including an adenine-rich region in their 3′ terminus (Fig. 2B) and a stem-loop structure in the 5′ terminus (Fig. 2C and D). This conserved sequence and stem-loop structure of LePV1 seem to be related to RdRp recognition, virus replication and RNA packaging, and they merit further research (Lesker et al. 2013; Nibert et al. 2014). The phylogenetic trees of amino acid sequences from RdRp and CP suggest that LePV1 is a new member of the genus *Betapartitivirus*, within the same genus as PoV1 (Fig. 4).

Our experiments cannot directly demonstrate that LePV1 causes degeneration of mycelia and imperfect browning during cultivation of *L. edodes* SX12. However, we found that the concentration of dsRNA extracted from the mycelia of diseased strain SX12 was significantly higher than that extracted from the same weight of other strains that carry LePV1 but present normally (data not shown). In addition, we also found that the symptoms of degeneration were alleviated as the concentration of this virus dropped during the eradication of LePV1 by ribavirin (data not shown). These results indicate LePV1 is associated with the symptoms and requires further research.

The high infection rate (48.2%) implies that LePV1 is widespread among *L. edodes* species (Fig. 6). This phenomenon was also observed for other reported *L. edodes* viruses such as LeSV, with a virus incidence of 66.7% among 84 commercially available cultivars (Won et al. 2013), and total of 14 unique electrophoretic dsRNA patterns in 23 out of 25 genetically diverse lines in the U.S.A. (Rytter et al. 1991). Although LePV1 was also found in some asymptomatic strains in this study, the possibility of latent infections exists since

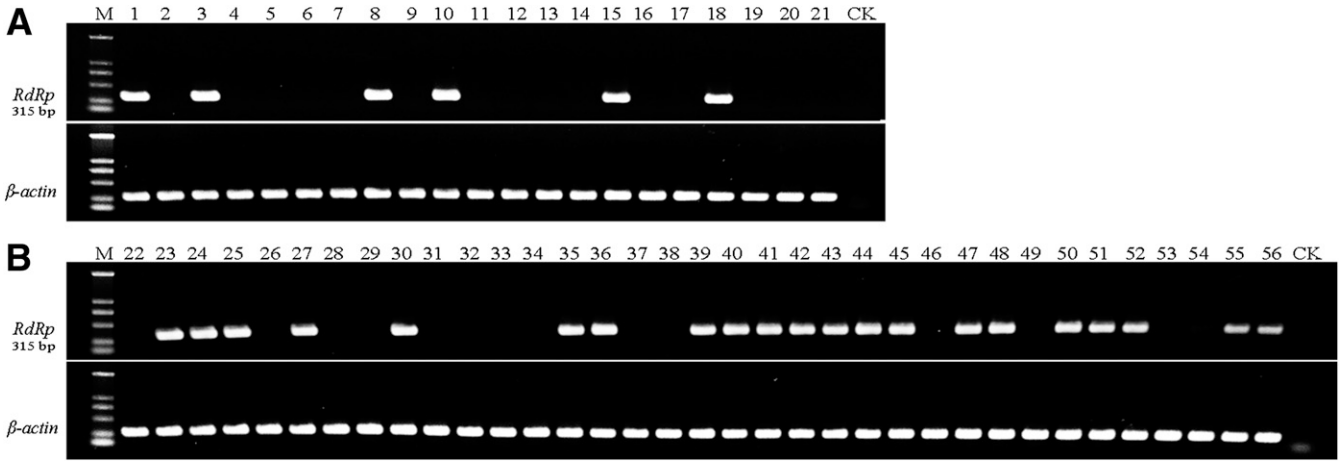

Fig. 6. RT-PCR analysis of LePV1 infection in *Lentinula edodes* core collections. **A,** Occurrence of LePV1 among 21 *L. edodes* cultivated strains. Numbers 1 to 21 represent 21 cultivated strains, respectively. **B,** Occurrence of LePV1 among 35 *L. edodes* wild strains. Numbers 22 to 56 represent each of the 35 wild strains. M: BM 2000 marker; CK: blank control using ddH_2O as template in the RT-PCR process. The *β-actin* gene was used as positive control to confirm the successful extraction of RNA.

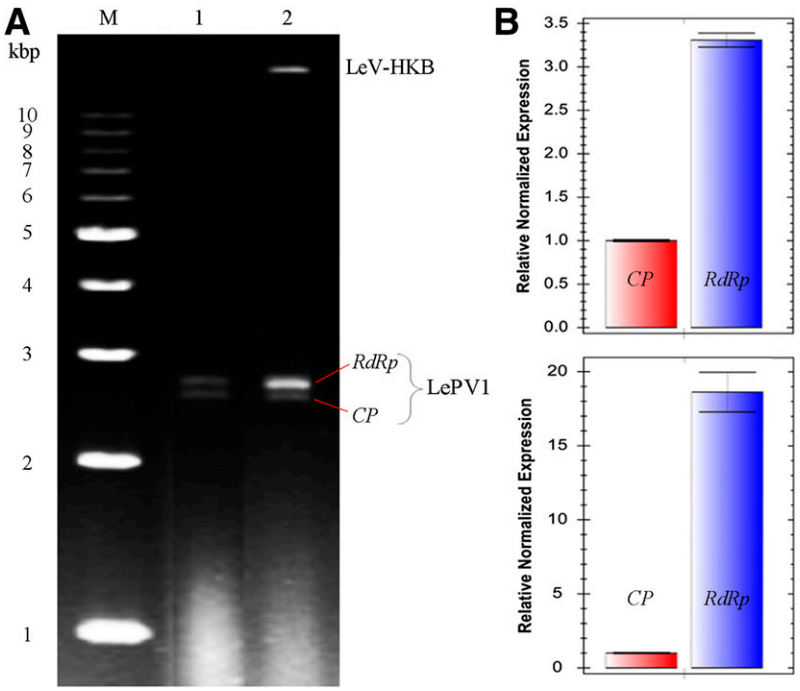

Fig. 7. Different characteristics between strain infected by LePV1 alone (lane 1 and top) and strain coinfected with LePV1 and LeV-HKB (lane 2 and bottom). **A,** Agarose gel electrophoresis analysis of the dsRNAs. Lane 1 and lane 2 show infection alone and coinfection, respectively. M: 1 kb DNA ladder marker. **B,** qPCR analysis of the gene expression of *RdRp* and *CP* genes. LePV1 infection alone (top), coinfection (bottom).

members of the family *Partitiviridae* have been reported to have conditionally significant impacts on their hosts (Xiao et al. 2014; Zheng et al. 2014). At this point, the high frequency of virus incidence is a potential threat to the cultivation of *L. edodes*; future breeding systems for *L. edodes* should take care to use virus-free germplasm resources as parental materials. It has been reported that for both LIV and LeSV, wild strains have lower infection rates compared with cultivated strains (Revill and Wright 1997; Won et al. 2013); however, in this study, we found that 60.0% of the wild strains were infected with LePV1 compared with only 28.6% of the cultivated strains. This could be because most of the cultivated strains are not grouped into the core collections because of their low level of genetic diversity (a total of 89 cultivars were used as materials to construct the core collections, but only 21 cultivars were grouped into the core collections) (Liu et al. 2015a); as a result, some potentially positive strains with close kinship to the LePV1-infecting cultivars in core collections were not detected.

Coinfection of unrelated viruses are often found in plant and fungal viral diseases (Fondong et al. 2000; Harrison et al. 1997; Rao et al. 2007; Wang et al. 2014). In this study, we observed the phenomenon of coinfection with LePV1 and LeV-HKB in *L. edodes* (Fig. 7A) and verified a significantly higher expression of the *RdRp* gene in LePV1 when it coinfects with LeV-HKB compared with when it infects alone (Fig. 7B). This phenomenon has also been described in several fungal and plant viruses (Karyeija et al. 2000; Sun et al. 2006; Vance 1991). For example, *Cryphonectria hypovirus 1* (CHV1) coinfection with *Mycoreovirus 1* (MyRV1) can elevate the replication of MyRV1, while CHV1 replication appeared to be unaffected by MyRV1 coinfection (Sun et al. 2006). This type of synergistic effect has been reported to be mediated by the papain-like cysteine protease p29 encoded by CHV1 ORF A (Sun et al. 2006). In this study, the coinfecting virus LeV-HKB contains two ORFs; its ORF1 encodes a unknown hypothetically functional protein with a NUDIX domain, and its ORF2 encodes a putative RdRp (Magae 2012). The enhancement of LePV1 replication by LeV-HKB coinfection may be mediated by the protein encoded by the LeV-HKB ORF1, which merits further research.

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