Plant Pathol. J. 22(1) : 68-74 (2006)

The Plant Pathology Journal

A New Double-Stranded RNA Mycovirus from *Pleurotus ostreatus* (ASI 2504)

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(Received on November 18, 205; Accepted on December 5, 2005)

A new virus with a dsRNA genome was isolated and characterized from the Suhan-neutari strain (ASI 2504) of *Pleurotus ostreatus*, which was characterized as long and slightly bent with small caps on the stipe of fruit body. Thirty nm isometric viruses with three dsRNA segments (approximately 2.0, 1.84 and 1.82 kb in sizes) were isolated by ultracentrifugation in sucrose gradients. Western analysis of protein extracted purified viruses with anti-virus polyclonal antibody confirmed that viruses have two specific proteins (36 and 68 kDa). Computer analysis of 2.0 kb segment shows that high sequence identity with RNA-dependent RNA polymerase (RdRp) of partitiviruses, respectively. When compared to other dsRNA mycoviruses in a phylogenetic analysis, OMDV was most related to *Pleurotus ostreatus virus 1*.

Keywords : dsRNA, *Pleurotus ostreatus*, virus

Several fungi have been shown to contain cytoplasmatically inheritable viruses. Many of these viruses are called mycoviruses, which have segmented dsRNA genome with nonenveloped isometric particles (Castro et al., 1999; Harmsen, 1958; Nogawa et al., 1996; Yu et al., 2003). The isometric dsRNA mycoviruses are classified into two families, *Totiviridae* and *Partitiviridae*, consisting of viruses that are enclosed in nonenveloped isometric particles of 25 to 50 nm in diameter and typically cause latent infections in their host fungi (Ghabrial, 1998). In most hosts, these remain latent or cryptic, since many mycovirus-infected fungi do not show symptoms (Vilches & Castillo, 1997). However, several mycovirus-associated fungal diseases have recently been reported in *Nectria radicicola*, *Sclerotinia sclerotiorum*, *Fusarium graminearum*, *Cryphonectria parasitica*, and others. The presence of mycovirus in fungal mycelia is often detected by electron microscopy as viruses or through the isolation of distinct species of nucleic acid from the fungal mycelia (Preisig et al., 2000). In recent years, however, it has become evident that the mere detection of dsRNA predicts neither an association with viruses, nor even a viral origin (Romaine et al., 1994).

In the mushroom, the first mycovirus was discovered in *Agaricus bisporus* (white-button mushroom). The resultant disease called La France disease, the symptoms of which included malformed fruit bodies with a reduced yield (Goodin et al., 1992; Laemmli, 1970). *A. bisporus virus* was highly infectious, although there were differences in infectivity and symptom severity depending upon environmental conditions and host cultivars (Go et al., 1992b). Among 56 mushrooms examined for the presence of virus in 1978, none were detected in *Pholiota nameko*, *Volvariella volvacea*, *Tremella fuciformis* or *Flammulina velutipes* (Magae & Hayashi, 1999). Since then, mycoviruses have been found in two major edible mushrooms, *Lentinus edodes* and *Pleurotus ostreatus* (oyster mushroom) (Yu et al., 2003), as well as in *Agrocybe aegerita*, another cultivated basidiomycete.

The available mycoviral sequence data related to virus genomes is limited, although there were a few reports which addressed dsRNA sequences (Harmsen, 1958) and ssRNA sequences for a bacilliform particle from the button mushroom *Agaricus bisporus* (Revill et al., 1994), and ssRNA sequences for a spherical particle from the oyster mushroom *Pleurotus ostreatus* (Yu et al., 2003) (Table 1). Although much effort has been put into the characterization of the viruses which caused oyster mushroom malformation, the etiology has not yet been elucidated.

Suhan-neutari is one of the most popular oyster mushrooms (*Pleurotus ostreatus*) in Korea (Go et al., 1992a), but its productivity and quality has proven to be unstable according to the culture condition. Recently farmhouses of

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Table 1. Characteristics of viruses in *Pleurotus spp.* and *Agaricus bisporus* up to the present-day

Host	Genome	Morphology	Particle size (nm)	Genome configuration (segment(s))	Genome Size (kb)	CP (kD)	Reference
P. ostreatus	dsRNA	Isometric	30	3	2.0, 1.84, 1.82	36, 68	This study
P. ostreatus	dsRNA	Isometric	24, 30	5	1.7, 1.9, 21, 2.4, 8	–	Lende et al., 1995
P. ostreatus	ssRNA	Spherical	27	1	5.784	28.5	Yu et al., 2003
P. florida	dsRNA	Spherical	21, 26, 36, 47	1	2.0	70	Park and Kim, 1996
P. florida	ssRNA	Bacilliform	16 × 47, 4 × 23	1	0.7	20	Park and Kim, 1996
P. florida	–	Spherical	30	–	–	–	Go et al., 1992a
A. bisporus	dsRNA	Isometric	34	9	3.6, 3.0, 2.8, 2.7, 2.5, 1.6, 1.35, 0.86, 0.78	90, 115, 120	Lende et al., 1996

P. ostreatus have suffered from viral disease of cultivated mushroom. DsRNA mycoviruses from *Pleurotus ostreatus* in Korea were isolated and characterized (Kim et al., 2005; Lim et al., 2005; Seo et al., 2004). Also, in preliminary experiments, we found the viral genome (dsRNA) in the malformed fruit bodies of Suhan-neutari strains stored at the National Institute of Agricultural Science and Technology. Thus, this study to characterize the viruses was purposed. Among them, Suhan-neutari showed different dsRNA patterns than the mycoviruses published in previous papers (Lende et al., 1995). Therefore, in this paper, we characterized the viruses in the malformed fruit body of Suhan-neutari according to morphology, genome, coat protein, and nucleotide sequence of viruses. We obtained 30 nm isometric viruses, which contained the three dsRNA conducted segments (approximately 2.0, 1.84 and 1.82 kb in sizes) and showed two protein bands (36 and 68 kDa).

Materials and Methods

Mushroom strain and culture condition. Fruit bodies of the Suhan-neutari (ASI 2504) strain among *P. ostreatus* were obtained from oyster mushroom farms of the National Institute of Agricultural Science and Technology in Korea. Rice bran and poplar sawdust were used as substrates for cultivation of the oyster mushroom. Poplar sawdust and rice bran were mixed at 80:20 ratios (v/v) and the mixture was adjusted to a water content of 65%, in a polypropylene bottle (Go et al., 1992b). Cultured fruit bodies were stored at –70°C for further analysis.

Purification of viruses and Sucrose density gradients centrifugation. Viruses from mushrooms were purified by the modified method published by Harmsen et al. (1989). Frozen fruit bodies (100 g) were homogenized in two volumes (w/v) of 0.01 M sodium phosphate buffer (pH 7.5) containing 0.5% 2-mercaptoethanol in a Nissen-AM7 homogenizer for 5 to 10 min at high speed on an ice bath. The mixture was centrifuged at 10,000 × g for 90 min in a Hithachi RPR9-2-1213 rotor, and cell debris was subsequently removed. The supernatant solution was collected and, for precipitation of the virus like-particles, polyethylenglycol (PEG) M.W 8,000 and NaCl were added to a final concentration of 10% and 0.6 M, respectively, and stirred for overnight at 4. The resulting precipitate was collected by centrifugation at 10,000 × g for 30 min and resuspended in 8 ml of 0.1 M sodium phosphate buffer (pH 7.5). Two ml of supernatant was overlaid on continuous sucrose density gradients prepared by layering successively filtrated 1 ml of 10% and 1.5 ml of 20-70% (w/v) each sucrose in distilled water. Gradients were centrifuged at 102,000 × g for 16 hr (Beckman, SW 41 Ti rotor). A specific light scattering band was produced by VIRUS preparation in sucrose density gradient centrifugation. Each gradient fraction (500 μl) was centrifuged at 197,000 × g for 1 hr. The pellets were resuspended in 100 μl of 0.1% DEPC-ddw and monitored at 260 nm with a Beckman DU 650 spectrophotometer.

Electron microscopy. The carbon films were layered onto 300 mesh copper grids. Samples were dropped on 300 mesh copper grids and incubated for 5 min. After removing excess water, samples were negatively stained with 0.5% (w/v) uranyl acetate for 5 min. Grids were air-dried and examined at 100 kV with a Model Reo 912ab transmission electron microscope.

Extraction of viral nucleic acids and gel electrophoresis. The procedure of RNA purification was followed by methods of Maniatis et al. (1982) with some modifications. Fifty μl of the fractions was mixed with 50 μl of 0.5 M Tris-HCl (final 100 mM), 50 μl of 0.1 M EDTA (pH 8.0) (final 10 mM), 5 μl of 5 M NaCl (final 50 mM), 50 μl of 10% SDS (final 1%) and 290 μl of 0.1% DEPC-ddw (final total volume 500 μl). Five μl of Proteinase K (10 mg/ml) was mixed. The mixtures were incubated at 37°C for 30 min and one vol. of phenol/chloroform/isoamyl alcohol was added

(25:24:1, v/v/v). The resultant emulsion was centrifuged at 10,000 × g for 20 min at 4°C and the upper aqueous phase was recovered. The resultant emulsion was centrifuged at 10,000 × g for 20 min at 22-25°C and the upper aqueous phase was recovered. The nucleic acids were precipitated with 1/10 vol. of 7.5 M NH4OAC and 2.5 vol. of 95% ethanol at −20°C for a minimum of 1 hr. The precipitate was collected by centrifugation at 10,000 × g for 30 min at 4°C, washed with 70% ethanol, dried in vacuum, and resuspended in 20 μl of TE (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). The viral nucleic acids were separated by 5% polyacrylamide gel electrophoresis (PAGE) using the continuous buffer system (Castro et al., 1999).

Viral protein extraction and western blot analysis. The viral proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using the discontinuous buffer system (Laemmli, 1970) and transferred to a PVDF membrane (Bio-Rad). Membranes were probed with a 1:100 dilution of the anti-virus specific polyclonal antibody which was prepared from the purified viruses in this study and a 1:3000 dilution of the secondary antibody. The antigen-antibody complexes were visualized with the West pico chemiluminescence substrate supersignal (Pierce) and exposed to Kodak X-OMAT film. Immunodetection determined with goat anti-mouse antibodies conjugated with horseradish peroxidase (Sigma) using diaminobenzidine-H_2O_2 as a substrate.

Analysis of viral nucleic acids. To determine the type of virus genomic nucleic acid, the sensitivity of nucleic acids to nucleases were examined. The characteristic sensitivity of the dsRNAs was determined by treating 1 μg of dsRNA with 2 μg/μl DNase I and 0.5 U/μl S1 nuclease, respectively. The reaction conditions of DNase I and S1 nuclease were as described by Sambrook et al. (1989). Each sample was incubated at 37°C for 1 hour. The nucleic acids of the viruses were analyzed by 1.8% agarose gel electrophoresis.

RT-PCR, cloning and sequencing. The cDNA synthesis was performed following the method of Gubler & Hoffmann (1983) and using the cDNA synthesis kit (Takara). The first-strand cDNA was synthesized by priming with a random nonamer in heat-denatured dsRNA (for 10 min at 99°C) with RAV-2 Reverse transcriptase. The second strand was synthesized with *Escherichia coli* DNA polymerase I from nicks introduced by RNase H in the RNA strand of the RNA-cDNA hybrid. The cDNA products, blunt-ended with T4 DNA polymerase, were purified by phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v) extraction and ethanol precipitation, as before. Products were then cloned into the EcoRV site of pBluescript II KS(+) vector and transformed into *E. coli* Top 10 (Invitrogen). Plasmid DNA was prepared from the transformants using the alkaline lysis method. Sequencing of the cloned cDNA products and of the RT-PCR products was performed with the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer).

Sequence analysis. Analysis was performed with an ABI PRISM 377 DNA Sequencer (Perkin-Elmer). Multiple alignments for sequence comparison with other viruses were performed by the program DNAstar and identified using the Basic Local Alignment Search Tool (BLAST) search program of GenBank. Sequences were analyzed using the CLUSTAL W program, which is listed at the ExPASY home page (http://www.expasy.ch/tools). MultAline, by Florence Corpet (http://prodes.toulouse.inra.fr/multalin/multalin. html), was also used for its published sequences of other mushroom viruses. In addition, the phylogenetic tree was obtained by the Megalign program.

Results and Discussion

Isolation and identification of mushroom strain. Fruit bodies of the healthy-looking Suhan-neutari strain mushroom (ASI 2504) showed a typical phenotype of the oyster mushroom, such as a straight, short, thick stipe with a middle-size cap of a gray color. Fruit bodies of unhealthy-looking mushrooms which are determined to be infected with specific viruses produced a bent, long, thin stipe with small caps which was somewhat darker in color than the healthy-looking Suhan-neutari strain (Fig. 1A, B). The stipes of the fruit body of Suhan-neutari appeared thin and long when poorly cultivated, but sometime strips were still quite big and short. The symptoms showed a variety of malformations depending on strain and the environmental conditions of cultivation. The mechanisms regarding such malformed fruit bodies remain undiscovered. One or more dsRNA viruses may remain latent in many fungi without producing any symptom expression. Fungal viruses spread by cell-to-cell fusion or anastomosis. No natural extracellular infection cycle of a fungal virus has been demonstrated, despite numerous attempts to do so. Due to the lack of a reliable infectivity assay to satisfy Koch's postulate, host-parasite interaction of each virus in the development of malformed oyster mushrooms is still unclear. Therefore, we thought that it was necessary for these studies to introduce viral particles in *P. ostreatus* in order to investigate whether or not these viruses are responsible for symptom expression.

Purification and molecular characterization of viruses.

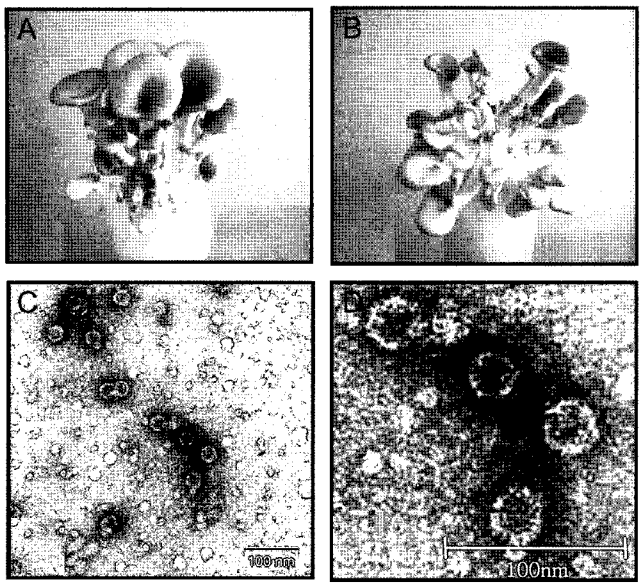

Fig. 1. Symptoms of fruiting bodies of malformed in Suhan (ASI 2504) cultivar of *P. ostreatus* and electron micrographs of virus. Malformed fruit bodies (B) showed bent, long stipes with small caps compared to healthy fruit bodies (A) Electron microscopic observation of the viruses from malformed fruit bodies of *P. ostreatus* after negative staining with 0.5% uranyl acetate. The predominant virus particle has a diameter of 30 nm particles and is visible at low levels in purified preparations. The bar represents 100 nm. (C) Original photograph. (D) Partially enlarged photograph of the box inside photograph C.

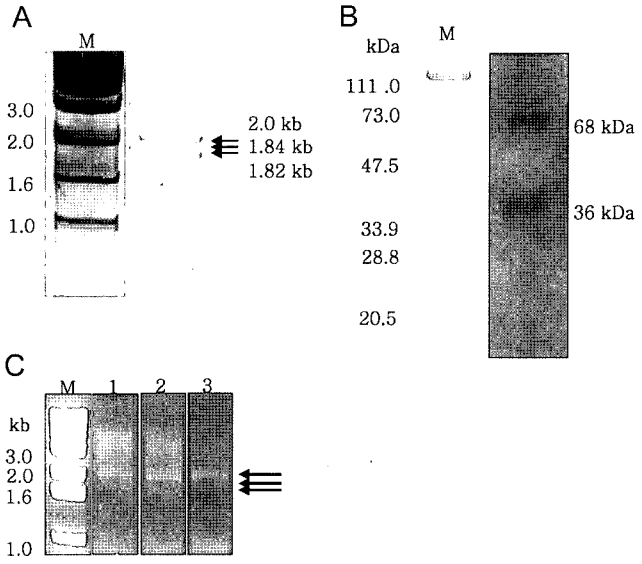

Fig. 2. Molecular characterization of virus isolated from Suhan (ASI 2504) cultivar of *P. ostreatus*. (a) Polyacrylamide gel electrophoresis of dsRNA extracted after equilibrium centrifugation. Electrophoresis was carried out in 5% polyacrylamide gel 150 V until the dye reached the bottom of the gel (about 50 min) and was silver-stained. The distribution of the three dsRNAs following equilibrium centrifugation in sucrose. M: size marker (1 kb DNA ladder). (b) Western blots of protein extracted from viruses, separated on a SDS-polyacrylamide gel. Molecular weight standards included phosphorylase B (111 kDa), bovine serum albumin (73 kDa), ovalbumin (47.5 kDa), carbonic anhydrase (33.9 kDa), soybean trypsin inhibitor (28.8 kDa) and lysozyme (20.5 kDa). M: size marker (low protein marker). Lane 1: Results of immunofluorescence. (c) Agarose gel analysis of dsRNA extracted in 25% sucrose gradient after equilibrium centrifugation. Lane 1 contains phenol-extracted nucleic acids from PEG-precipitated virus particles. M: 1kb DNA ladder. Lane 1: RNA only. Lane 2: RNA treated with DNase I. Lane 3: RNA treated with S1 nuclease

Partially purified viruses from malformed fruit bodies were purified through 10-70% sucrose density gradients. Following equilibrium centrifugation from malformed fruit bodies, the 260 nm absorbance profile of the 10-70% sucrose density gradients was carried out (data not shown). Negatively stained viruses purified from those mushrooms were analyzed by electron microscopy. The viruses were visible and were estimated to have a diameter of 30 nm as isometric particles found at low levels in purified preparations (Fig. 1C, D). Double-stranded RNA (dsRNA) mycoviruses have been described in a wide variety of fungi. The majority of dsRNAs founded in filamentous fungi were generally enclosed in a coat protein, as isometric viruses (Howitt et al., 2001; Magae and Hayashi, 1999; Preisig et al., 2000). The viruses of these studies had dsRNA enclosed in a coat protein, also isometric. This was a very typical virus particle in the oyster mushroom. The white button mushroom, *Agaricus bisporus*, contaminated several kinds of isometric particles, such as those 25 and 34 nm in diameter, and bacilliform particles of 19 nm wide and 50 nm long. The oak mushroom, *Lentinus edodes*, has 25, 29, and 30 nm virus particles including flexible rod types of 15-17 × 1500 nm in maximum length and rigid rod types of 25-28 × 280-300 nm (Go et al., 1992b; Park and Kim, 1996). Also, the oyster mushroom, *P. ostreatus*, had isometric particles such as 24 and 30 (Lende et al., 1995), spheric particles such as 21, 26, 36, 47 and bacilliform particles such as 16 × 47 and 4 × 23 nm (Park and Kim, 1996) in diameter. In our studies, purified viruses from malformed fruit bodies of Suhan-neutari were of an isometric type about 30 nm in diameter. Isometric viruses of 30 nm in diameter were observed in gradient fractions containing the specific dsRNAs and proteins. The size of these isometric particles was found to be similar to that of viruses from other mushrooms mentioned above.

Each nucleic acid of the viruses which had been isolated from malformed fruit bodies was extracted from each fraction of the 10-70% sucrose density gradients and then separated on a 5% polyacrylamide gel. Gels were silver-stained and RNA was shown on the fraction 11, 14-16 and 23-24 (data not shown). The three different sizes of RNAs

from viruses were 2.0, 1.84 and 1.82 kb, on the same fractions mentioned above (Fig. 2A). The 2.0, 1.84 and 1.82 kb of dsRNAs showed in the fraction No. 11, 14-16 and 23-24. Fraction No. 23-24 appeared as dsRNAs, and it is presumed that PEG-complex isometric viruses were in the lower part of the ultra-centrifuged tube. The size and segment of the genome from these viruses were different from that of previously described viruses at 5.78 kb in *P. ostreatus* (Yu et al., 2003) and 2.0 and 0.7 kb in *P. florida* (Park and Kim, 1996) in Korea and 1,7, 1.9, 2.1, 2.4 and 8 kb in *P. ostreatus* in Netherlands (Lende et al., 1995). Lim et al. (2005) reported that PoV-1 dsRNA and PoV-2 dsRNA which were isolated from *Pleurotus ostreatus* in Korea had genomes of 2296 and 2223 nucleotides, respectively. We could not sequence all genomes of three dsRNAs from *P. ostreatus* (ASI2504) so that the sizes of genomes were approximately calculated. However, the sizes and numbers of ASI2504 dsRNAs were different from those from dsRNA mycoviruses isolated from other *P. ostreatus* strains.

Twenty-five percent sucrose density gradient centrifu-

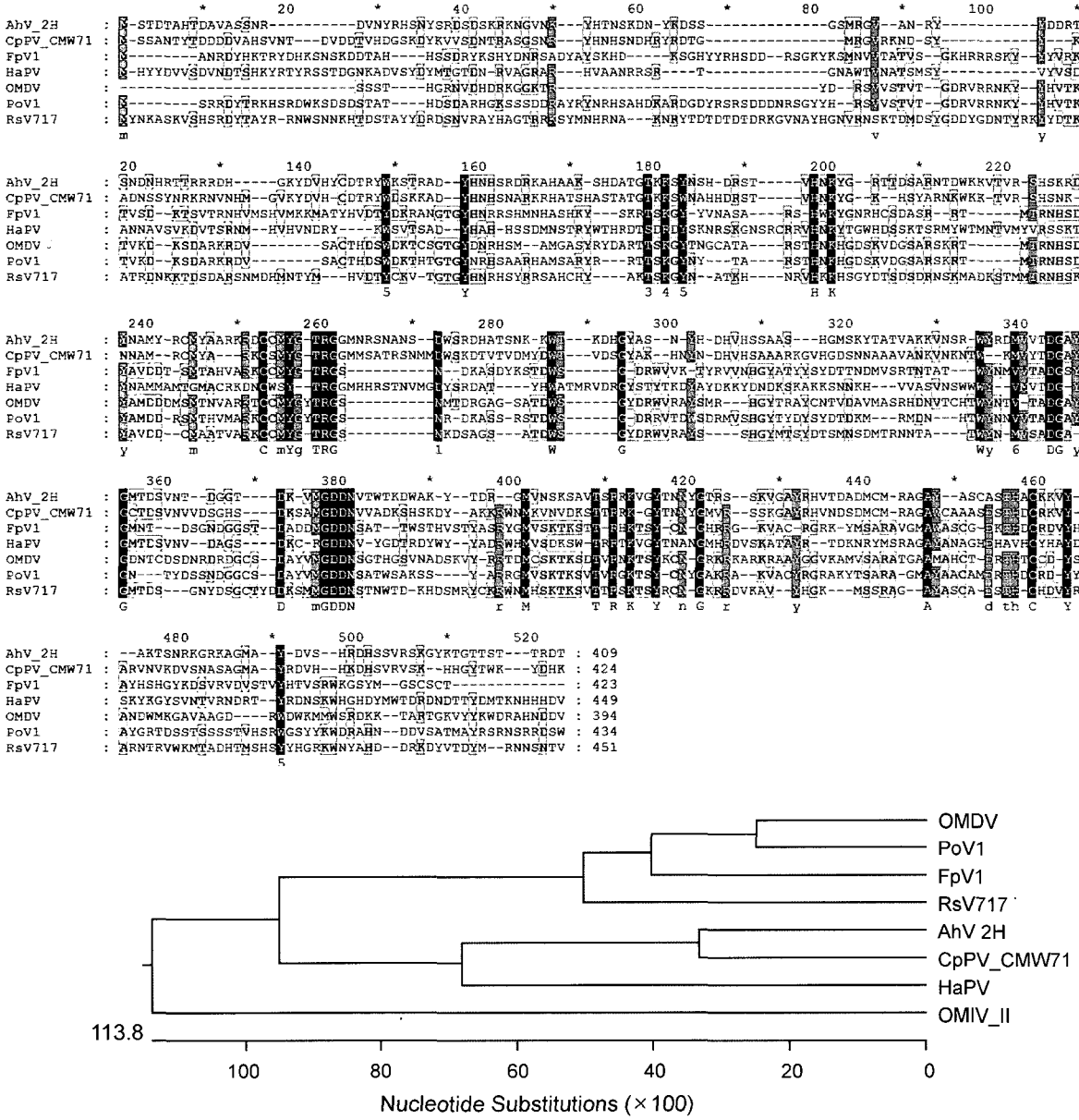

Fig. 3. Comparison of amino acids of RNA-dependent RNA polymerase between *oyster mushroom dsRNA virus* (OMDV) and other mushroom viruses. (a) Alignment of amino acid sequence of RDRP domains from OMDV, AhV2H, CpPV CMW71, FpV1, HaPV, PoV1, RsV 717. (b) The phylogenetic tree was drawn with the Megalign program. The length of each pair of branches represents the distance between sequence pairs, while the units at the bottom of the tree indicate the number of substitution events.

gation was carried out to obtain an appropriate amount of viruses. Viruses isolated in 25% sucrose density gradient from malformed fruit bodies were used to characterize viral nucleic acids and to detect viral proteins.

Fig. 2B illustrates the reaction of these undegraded proteins extracted from 25% sucrose density gradient with the antisera (dilution 1:100). The two undegraded proteins of 36 and 68 kDa extracted from malformed fruit bodies could be detected by SDS-PAGE and western blots analyzed by the antiserum of antibody. The viruses in the strain of Suhan-neutari had coat proteins of molecular weights of 36 and 68 kDa. The size of the proteins from these viruses was different from those of previously described viruses, including 28.5 kDa in *P. ostreatus* (Yu et al., 2003) and 70 and 20 kDa in *P. florida* in Korea (Park and Kim, 1996). As a result, the size and segment of genome and protein were different to that of the viruses mentioned above.

Characterization and sequencing of dsRNA as a genome. In order to characterize the property of the dsRNAs isolated from malformed fruit bodies, dsRNA was separated on a 1.8% agarose gel according to their resistance to DNase I and S1 nuclease. Viral RNA treated with DNaseI and S1 nuclease is shown to be double-stranded because RNA was not digested by two enzymes. The result was confirmed that viral RNA extracted from fruit bodies was dsRNA (Fig. 2C).

To obtain some sequence information from this dsRNA element, cDNA was prepared by randomly priming cDNA synthesis from heat-denatured dsRNA and mixed hexanucleotide based on the method of Gubler and Hoffmann (1983). cDNA products were cloned into the compatible EcoRV site of the pBluescript II KS (+) vector and transformed into *E. coli* Top 10 (Invitrogen).

A preliminary sequence analysis suggested that inserts might have been derived from a viral genome. Each of the seven cDNA sequences, 711, 1833, 594, 1014, 245, 248 and 712 bp in length, were obtained from overlapping cDNA clones sequencing the number of 18, 4, 3, 2, 2 and 2, respectively, by the program Seqman (DNAstar). The amino acid sequence translated from the 2.0 kb segment (oyster mushroom dsRNA virus (OMDV) in this study) contained the GDD motif of RDRP (Koonin and Dolja, 1993). An alignment of conserved RNA-dependent RNA polymerase (RDRP) sequence motifs for the *Oyster mushroom dsRNA virus* (OMDV), *Pleurotus ostreatus virus* (PoV1; GenBank accession number AY533038), *Fusarium poae virus 1* (FpV1; GenBank accession number AF047013), *Rhizoctonia solani virus* isolate 717 (RsV 717; GenBank accession number AF133290), *Heterobasidion annosum P-type partitivirus* (HaPV; GenBank accession number AAL79540), *Ceratocystis polonica partitivirus* isolate CMW71 (CpPV CMW71; GenBank accession number AAP79988) and *Atkinsonella hypoxylon virus 2H* (AhV 2H; GenBank accession number NC_003470) is shown in Fig. 3A. To understand the relationship OMDV and other dsRNA mycoviruses, comparative and phylogenetic analyses were performed. OMDV showed high sequence identity to PoV1 (Fig. 3B).

As a result, viruses are observed on the malformed Suhan-neutari cultivars of *P. ostreatus*. Analysis of viral nucleic acids and coat proteins extracted from the viruses was shown in agarose gel and polyacrylamide gel, respectively. Here, we also reported the partial sequence of RdRp of viruses. The partial sequence information of RdRp was not enough to support the new dsRNA mycovirus from *P. ostreatus* but other characteristics of viruses isolated from *P. ostreatus* were different from other mycoviruses with dsRNA as a genome in *P. ostreatus* (Go et al., 1992a; Kim et al., 2005; Lim et al., 2005; Seo et al., 2004). Although no attempt was made to determine full-length sequence of dsRNAs from Suhan-neutari cultivars of *P. ostreatus* (ASI2504) in this study, it is necessary to analyze the entire genome to better characterize and understand the biology and pathology of Suhan-neutari cultivars of *P. ostreatus*.

References

Castro, M., Kramer, K., Valdivia, L., Ortiz, S., Benavente, J. and Castillo, A. 1999. A new double-stranded RNA mycovirus from *Botrytis cinerea*. *FEMS Microbiol. Lett.* 175:95-99.

Ghabrial, S. A. 1998. Origin, adaptation and evolutionary pathways of fungal viruses. *Virus Genes* 16:119-131.

Go, S. J., Cha, D. Y. and Shin, G. C. 1992a. Virus-like particles from abnormal growing oyster mushrooms, *Pleurotus florida* and *Pleurotus ostreatus*. *Kor. J. Mycol.* 20:149-153.

Go, S. J., Cha, D. Y. and Wessels, J. G. H. 1992b. Symptoms of virus infected oyster mushrooms, *Pleurotus florida*. *Kor. J. Mycol.* 20:229-233.

Goodin, M. M., Schlagnhaufer, B. and Romaine, C. P. 1992. Encapsidation of the La France disease-specific double-stranded RNAs in 36-nm isometric viruslike particles. *Phytopathology.* 82:285-290.

Gubler, U. and Hoffmann, B. J. 1983. A simple and very efficient method for generating cDNA libraries. *Gene* 25:263-269.

Kim, Y. J., Park, S. H., Yie, S. W. and Kim, K. H. 2005. RT-PCR detection of dsRNA mycoviruses infecting *Pleurotus ostreatus* and *Agaricus blazei* Murrill. *Plant Pathol. J.* 21:343-348.

Harmsen, M. C. 1958. Molecular analysis of *Agaricus bisporus* double-stranded RNA. La France disease of the cultivated mushroom (*Agaricus bisporus*). pp. 19-26.

Harmsen, M. C., van Griensven, L. J. L. D. and Wessels, J. G. H. 1989. Molecular analysis of *Agaricus bisporus* double-stranded RNA. *J. Gen. Virol.* 70:1613-1616.

Howitt, R. L. J., Beever, R. E., Pearson, M. N. and Forster, L. S. 2001. Genome characterization of *Botrytis virus F*, a flexous

rod-shaped mycovirus resembling plant 'potex-like'viruses. *J. Gen. Virol.* 82:67-78.

Koonin, E. V. and Dolja, V. V. 1993. Evolution and taxonomy of positive-strand RNA viruses: implications of comparative analysis of amino acid sequences. *Crit. Rev. Biochem. Mol. Biol.* 28:375-430.

Laemmli, U. K. 1970. Cleavage of structural proteins during the asswmbly of the head of bacteriophage T4. *Nature* 227:680-685.

Van der Lende T. R., Harmsen, M. C., Go, S. J. and Wessel, J. G. H. 1995. Double-stranede RNA mycoviruses in mycelium of *Pleurotus ostreatus*. *FEMS Microbiol. Lett.* 125:51-56.

Van der Lende T. R., Dutiman E. H., Gunnewijk M. G., Yu L. and Wessels J. G. 1996. Functional analysis of dsRNAs (L1, L3, L5 and M2) associated with isometric 34 nm virions of *Agaricus bisporus* (white button mushroom). *Virology* 217:88-96.

Lim, W. S., Jeong, J. H., Jeong, R. D., Yoo, Y. B., Yie, S. W. and Kim, K. H. 2005. Completely nucleotide sequence and genome organization of a dsRNA partitivirus infecting *Pleurotus ostreatus*. *Virus Res.* 108:111-119.

Magae, Y. and Hayashi, N. 1999. Double-stranded RNA and virus-like particles in the edible basidiomycete *Flammulina velutipes*. *FEMS Microbiol. Lett.* 180:331-335.

Maniatis, T., Fritsch, E. F. and Sambrook, J. 1982. Purification of nucleic acids. In: *Molecular Cloning. A Laboratory Manual,* pp. 458-460. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Nogawa, M., Nakatani, A., Gonda, K., Shimosaka, M. and Okazaki, M. 1996. Replication of double-stranded RNA in mycovirus from the plant pathogenic fungus, *Fusarium solani*. *FEMS Microbiol. Lett.* 137:45-49.

Park, J. S. and Kim, Y. H. 1996. Infectious RNA viruses in the edible mushroom *Pleurotus spp. Jour. Microbiol.* 61-67.

Preisig, O., Moleleki, N., Smit, W. A., Wingfield, B. D. and Wingfield, M. J. 2000. A novel RNA mycovirus in a hypovirulent isolate of the plant pathogen *Diaporthe ambigua. J. Gen. Virol.* 81:3107-3114.

Revill, P. A., Davidson, A. D. and Wright, P. J. 1994. The nucleotide stquenct and genome organization of mushroom bacilliform virus: A single-stranded RNA virus of *Agaricus bisporus* (Lnage) imbach. *Virology* 202:904-911.

Romaine, C. P., Schlagnhaufer, B. and Goodin, M. M. 1994. Vesicle-associated double-stranded ribonucleic acid genetic elements in *Agaricus bisporus*. *Curr. Genet.* 25:128-134.

Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular Cloning : A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Seo, J. K., Lim, W. S., Jeong, J. H., Yoo, Y. B., Yie, S. W. and Kim, K. H. 2004. Characterization and RT-PCR detection of dsRNA mycoviruses from Oyster mushroom, *Pleurotus ostreatus*. *Plant Pathol. J.* 20:200-205.

Vilches, S. and Castillo, A. 1997. Double-stranded mycovirus in *Botrytis cinerea*. *FEMS Microbiol. Lett.* 155:125-130.

Yu, H. J., Lim, D. B. and Lee, H. S. 2003. Characterization of a novel single-stranded RNA mycovirus in *Pleurotus ostreatus*. *Virology* 314:9-15.