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Infection of *Rosellinia necatrix* with purified viral particles of a member of *Partitiviridae* (RnPV1-W8)

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Summary. Isolate W8 of the white root rot fungus, *Rosellinia necatrix*, harbors three dsRNA segments, L1-, L2- and M-dsRNAs, and showed an irregular colony margin, slow growth, and moderate virulence. The M-dsRNA was previously shown to be the genome of a partitivirus, RnPV1-W8. Here a transfection protocol was developed for RnPV1-W8. Protoplasts of two virus-free isolates of *R. necatrix* were inoculated with purified viral particles using a polyethylene glycol-mediated method. Virus infection was confirmed by electrophoresis and Northern analysis. RnPV1-W8 introduced into the new host isolates was transmissible via hyphal anastomosis. However, the infection had no effect on the morphology and virulence of infected isolates of *R. necatrix*. This is the first report on the transfection of a partitivirus for *R. necatrix*.

Introduction

Mycoviruses are found in a broad range of fungal species including Ascomycetes, Basidiomycetes, and Deuteromycetes [2, 6, 15, 19, 25]. Most mycoviruses have dsRNA genomes and are classified into five families, *Partitiviridae*, *Totiviridae*, *Chrysoviridae*, *Reoviridae*, and *Hypoviridae* [13, 14, 16, 26, 41]. Mixed viral infections are often observed [5]. Mycoviruses lack an extracellular phase to their life cycles, and viral transmission is generally limited to the intercellular route [5].

In plant pathogenic fungi, mycoviruses are usually neutral without any appreciable effect on the host fungi, but several viruses are known to reduce host virulence and have been studied extensively [2, 4, 17, 19, 21, 23, 33]. The horizontal transmission of viruses between strains of the same species is often hampered by somatic incompatibility of the host fungus. Therefore, the development of

inoculation methods is important for elucidation of cause-effect relationships and the utilization of mycoviruses as biocontrol agents. Artificial infection has been successful in only a few species such as *Cryphonectria parasitica* and *Diaporthe ambigua* where transformation with viral cDNA or transfection with in-vitro transcribed single stranded, plus-sense RNA was used [8, 10, 11, 27]. *Cryphonectria parasitica hypovirus 1*-EP713 (CHV1-EP713) introduced by the two methods, replicated not only in *C. parasitica* but also in other species belonging to *Diaporthales* and reduced the virulence of infected fungi, indicating its potential as an efficient biocontrol agent [9, 31]. Transfection with purified viral particles has been reported in several fungal species [7, 15, 24, 34]. Recently, purified viral particles from two plant pathogenic fungi, a 6.8-kbp dsRNA in *Botrytis cinerea* and Mycoreovirus 1(MYRV1-Cp9B21) in *C. parasitica*, infected isolates of their respective host species belonging to different vegetative compatible groups and reduced virulence. However, mycoviruses are still regarded as difficult to transfect using purified viral particles as an inoculum [5]. Therefore, the development of artificial methods of inoculation should pave the way for the efficient utilization of mycoviruses as agents for the biocontrol of fungal pathogens.

The family *Partitiviridae* is composed of three genera, *Partitivirus* in fungi, and *Alphacryptovirus* and *Betacryptovirus* in plants [13]. Virions are isometric, nonenveloped, and 30–40 nm in diameter, with two separate, monocistronic segments encoding an RNA-dependent RNA polymerase and coat protein. Mycoviruses in this family are ubiquitous among fungal species. Symptoms caused by members of this family are cryptic, or ambiguous. *Nectria radicicola* virus L1 is an exception [1]. This virus causes an up-regulation of host virulence by changing host signal transduction pathways.

Rosellinia necatrix Prillieux causes white root rot in 171 plant species belonging to 63 genera including fruit trees [36, 37]. In fruit tree orchards in Japan, 50–100 liters of fungicide per tree are drenched at least every other year to control the disease [20], which raises concerns about soil pollution. *R. necatrix* propagates mainly through mycelial spread in the soil. Ascospores are never found in the orchard and conidia do not develop into mycelia [28]. Therefore, mycoviruses are unlikely to be lost during sporulation as is the case with other fungal species [2, 12, 30] and expected to persist in *R. necatrix*. More than 300 isolates of *R. necatrix* have been collected throughout Japan, and 65 were found to harbor dsRNAs varying in size and number of segment [3]. Among the 65 isolates, W370 contains Mycoreovirus 3 (MYRV3-RnW370) which shows sequence similarity to mammal-pathogenic coltiviruses and *Cryphonectria parasitica mycoreovirus* 1 [15, 29, 35, 39, 40]. MYRV3-RnW370 infection results in reduced mycelial growth, causing hypovirulence in *R. necatrix* [21]. Other dsRNA-containing strains include W8, which is studied less extensively, compared with the isolate W370.

Isolate W8 has an irregular colony margin, grew slowly on PDA, and is moderately virulent, containing three dsRNA segments, i.e., L1, L2 and M-dsRNA

[32]. M-dsRNA was recently identified as a partitivirus, *Rosellinia necatrix partitivirus 1*-W8 (RnPV1-W8), but L-dsRNAs are regarded as other viral species [32]. However, it is still not clear whether the assumed hypovirulence is incited singly by RnPV1-W8, or L1- and L2-dsRNAs were involved in the phenotype of isolate W8. This paper describes the transfection of protoplasts generated from virus-free *R. necatrix* isolates with RnPV1-W8 in the form of purified viral particles.

Materials and methods

Fungal isolates and culture conditions

Isolate W8 of *Rosellinia necatrix* contained three dsRNA segments as previously described [32]. Isolates W37 and W97 were virus-free and originated from diseased roots of Japanese pear (*Pyrus pyrifolia* Nakai var *culta*) in Chiba and Saga, respectively [3]. W37 and W97 were selected for transfection experiments, because both isolates were virus-free and strongly virulent. The three isolates used belonged to different mycelial compatibility groups. All isolates were grown on PDA (DIFCO, DIFCO laboratories, Detroit, USA) at 20 °C and kept at 4 °C until used. Mycelia used for dsRNA extraction were cultured on cellophane-overlaid PDA (cellophane-PDA) plates. Mycelia for the purification of viral particles were cultured in Czapeck-Dox broth and stored at −20 °C.

Transfection with purified viral particles

Purified viral particles of RnPV1-W8 prepared as described previously [32] were filtered with Ultrafree-MC sterile centrifugal filter units (Millipore, Tokyo Japan) and subjected to a viral infection assay. The filter-sterilized fraction containing 2 μg of M-dsRNA was introduced into 1.2–3.6 × 10^7 protoplasts with PEG according to the method described by Kanematsu et al. [21]. Transfected protoplasts were transferred on YCDA (0.1% yeast extract, 0.1% casein hydrolysate, 0.5 M glucose and 1.5% agar) and kept for one week at 20 °C until regenerated mycelia covered the surface. Eight mycelial plugs (referred to as regenerants) chosen at random were grown on cellophane-PDA to assay for viral infection. Colonies of regenerants containing dsRNA were subcultured three times to confirm the stability of dsRNA in each culture.

Extraction of dsRNA

Cultured mycelia were collected with cellophane (cellophane-mycelia) and stored at −20 °C. Total nucleic acids were extracted from disrupted cellophane-mycelia in liquid nitrogen with extraction buffer (0.1 M Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, 0.6 M NaCl, 3% SDS, 28 mM 2-mercaptoethanol), subjected to two rounds of phenol-chloroform extraction and a single round of chloroform extraction, and then, precipitated with 1/10 vol of 3 M NaOAc and 3 vol of EtOH. Total nucleic acids were recovered after centrifugation and single-stranded (ss) and dsRNAs were precipitated by adding LiCl to adjust the final concentrate to 4 M. ssRNA and a small amount of genomic DNA in the RNA fraction was removed by S1 nuclease and DNase as described by Sasaki et al. [32].

Northern analysis

Northern hybridization analysis was performed as described by Sasaki et al. [32]. NaOH to denature dsRNA in native gel was modified to 0.1 M. RNA probes to detect complementary

strands were prepared using the Dig RNA labeling kit (Roche diagnostics, Tokyo, Japan) as described previously [32].

Viral transmission

A virus-infected mycelial plug (donor) and a virus-free plug (recipient), which was transformed with a vector containing a bacterial hygromycin B-resistance gene (pSH75) [22] according to Kanematsu et al. [21], were co-transferred into 12-cm square dishes containing 30 ml of PDA at a distance of 2.5 cm apart and incubated at 20 °C. Mycelial plugs were recovered from colonies of the recipient, and subcultured on PDA with hygromycin B to eliminate contamination of donor mycelia. Mycelial plugs from the donor were transferred to PDA. Three mycelial plugs each from the margin of a subcultured colony were inoculated onto cellophane-PDA plates to observe the change in culture morphology and to determine the presence of RnPV1-W8.

Virulence assay

Virulence was assayed as described by Uetake et al. [38] with a slight modification. Seeds of yellow lupine (*Lupinus luteus* L.) were sown in plastic cups containing 1:1 of vermiculite and a soil medium for horticulture (Kureha-Engeibaid, Kureha chemical industry, Tokyo, Japan), thinned to two plants per cup, and grown in a greenhouse for about three weeks. Fungal cultures, which were kept on PDA at 20 °C for two weeks in the dark, were overlaid with 1.5-cm long sterilized apple twigs, and incubated for three additional weeks. Apple twigs covered with mycelia were used as inoculum. One twig fragment was buried at the base of the stem in the soil and kept in the greenhouse. The mortality of plants was determined 2 and 4 weeks after inoculation.

Results

Transfection to virus-free isolates

Electrophoretic analysis indicated that dsRNA with the same mobility as RnPV1-W8 was detected in most subcultures (31 out of 32) derived from virus-transfected, regenerated mycelia (Fig. 1). Viral infection was then confirmed by Northern analysis with RNA probes specific to the complementary strand of RdRp and CP (Fig. 1). Hybridization signals were observed in isolate W8 and in virus-transfected strains of isolates W37 and W97, but not in virus-free strains. RnPV1-W8 was readily detected in infected strains after repeated subculturing.

Effect of viral infection on fungal growth

Isolate W8, originally infected with RnPV1-W8, exhibited an irregular colony morphology and slow growth as compared to W37 and W97 (Fig. 2) seven days after inoculation. On the other hand, virus-transfected regenerants of W37(RnPV1-W8) and W97(RnPV1-W8) showed the same growth rate and smooth colony margin as their respective original isolates on PDA (Fig. 2). Several other transfected regenerants obtained from independent experiments were also indistinguishable from the respective original isolates (data not shown).

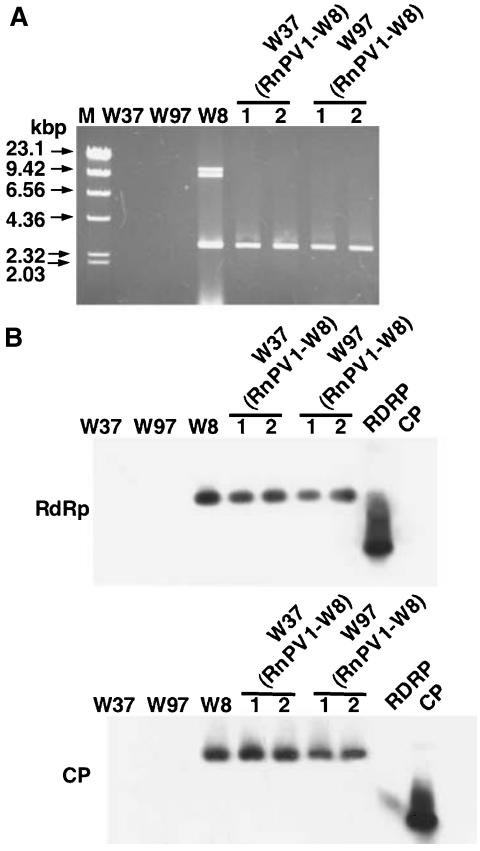

Fig. 1. Agarose gel electrophoresis (**A**) and Northern hybridization analysis (**B**) of dsRNA in strains W37 and W97 transfected with RnPV1-W8. Extracted dsRNA was electrophoresed on 0.7% agarose in TAE. Isolates and strains are indicated above the lanes. Lane M, lambda-phage digested with *Hin*d III as a molecular marker. Virus-transfected strains are indicated as the isolate number with (RnPV1-W8). Probes to detect RdRp and CP segments are indicated in the left of **B**. RdRp, 20 ng of PCR product of probe containing RdRp sequence; CP, 20 ng of PCR product of probe containing CP sequence

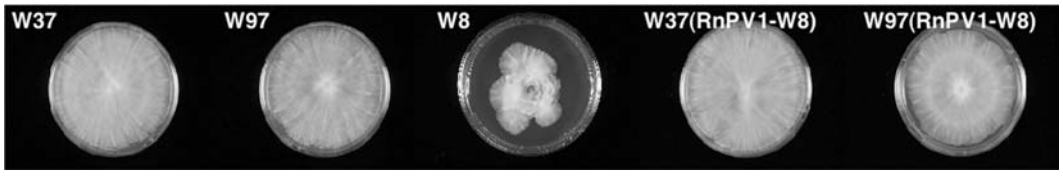

Fig. 2. Culture morphology of *R. necatrix* infected with RnPV1-W8. Isolate designation is indicated above the culture. Photos were taken 7 days after inoculation

Viral transmission by hyphal anastomosis

Virus-transfected regenerants and their respective, virus-free isolates had the same growth rate and did not show incompatible reactions along colony junctions when cultured side-by-side on the same plate (Fig. 3A). All the colonies produced by the recipient subcultures contained viral dsRNA (data not shown). Recipient subcultures with dsRNA did not differ in morphology from the donors (Fig. 3B). On the other hand, the colony of isolate W8 when surrounded by isolate W37 or W97 in same plate, an incompatible reaction (black pigmented line) was observed between the isolates (Fig. 3A). The transmission of RnPV1-W8 from W8 to W37 or W97 by hyphal anastomosis did not occur, and subcultures from isolates W37

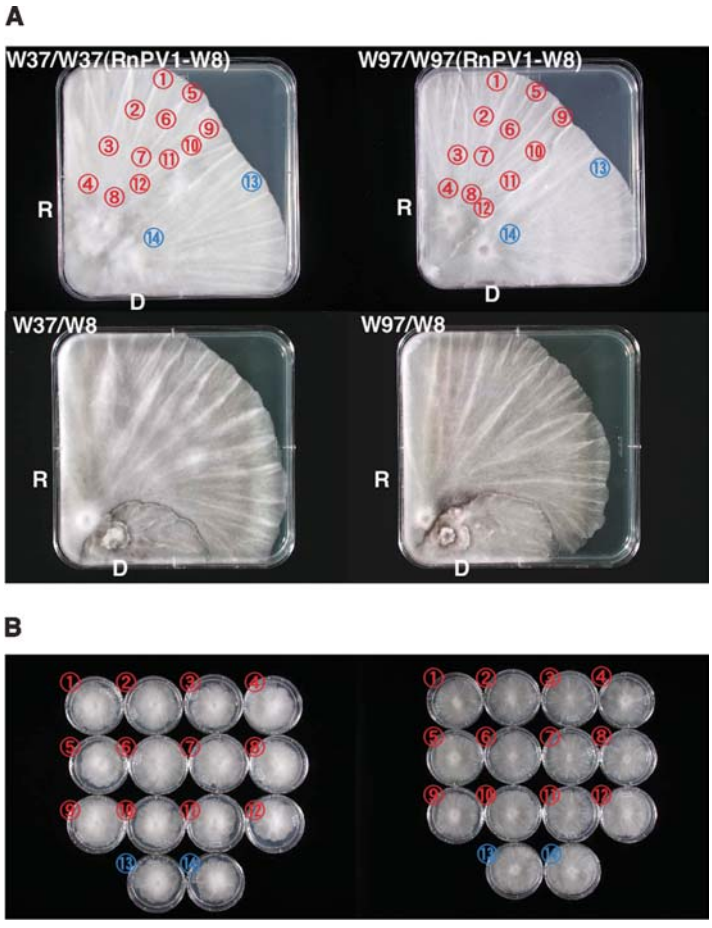

Fig. 3. Virus transmission via hyphal anastomosis. Mycelial plugs of virus-free isolate (W37 or W97) and their respective virus-transfected regenerants were grown side-by-side on PDA for 17 days. **A**, isolate designation is indicated in the upper left corner of each culture plate. *R*: virus-free recipients transformed with the *hph* gene, *D*: donor regenerants infected with RnPV1-W8. Mycelial plugs numbered 1 to from the recipient colonies (indicated in red) and plugs numbered 13 to 14 from donor colonies (indicated in blue) were subcultured on PDA with and without hygromycin, respectively. **B**, five-day-old PDA subcultures originated from plugs, as indicated in panel **A**

and W97 did not show any appreciable change in colony morphology (data not shown).

Virulence assay

Yellow lupin plants inoculated with either W37 or W97 started to wilt 1 and 2 weeks after inoculation respectively, and became severely wilted after 2 and 4 weeks with rot in the lower hypocotyls (Fig. 4). Finally, they were completely destroyed. Regenerants transfected with RnPV1-W8 exhibited the same level of virulence. Plants inoculated with W8 wilted slightly, and retarded in the growth

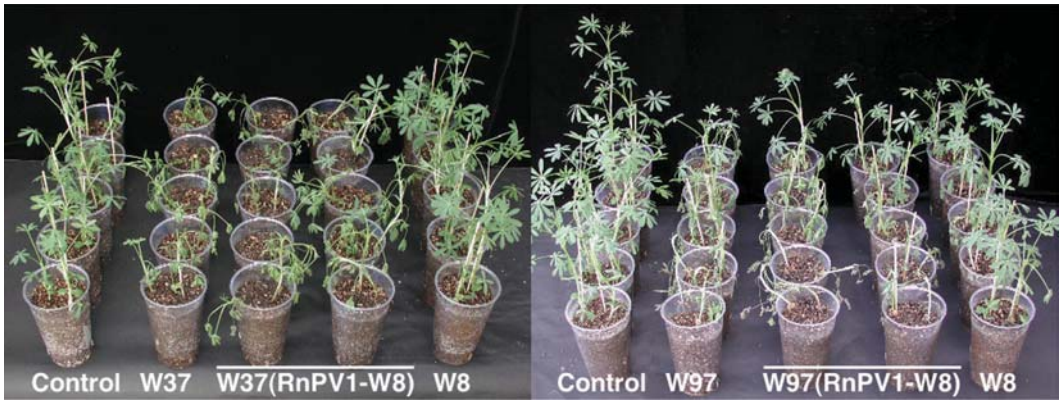

Fig. 4. Virulence assay of *R. necatrix* transfected with RnPV1-W8. Isolate designation is indicated at the bottom of the panel. W37 and W97: virus-free, virulent isolates; W8: weakly virulent isolate W8; W37(RnPV1-W8) :W37 transfected with RnPV1-W8 and W97(RnPV1-W8): RnPV1-W8 transfected with RnPV1-W8. Photographs were taken 2 weeks after inoculation for strains W37 (left half) and 4 weeks after inoculation for strains W98 (right half)

as compared to control plants (Fig. 4). They survived four weeks after inoculation.

Discussion

RnPV1-W8 is a member of the family *Partitiviridae* [32] and this is the first report to demonstrate the infectivity of its purified viral particles to *R. necatrix*. We purified RnPV1-W8 particles and used them to transfect protoplasts in the presence of PEG. In dsRNA viruses, replication starts on transcription of +ssRNA in viral particles after introduction into the host cells [5], and so the viral particles need to remain intact for infection. The procedures used for purification and transfection in this study were found to be appropriate for RnPV1-W8. Other studies applied the PEG method for transfection with purified mycoviruses [7, 15]. The use of PEG was also suitable for the combination of *R. necatrix* and RnPV1-W8.

Electrophoretic bands of approximately 2.3 kbp in isolate W8 and protoplast regenerants W37(RnPV1-W8) and W97(RnPV1-W8) were resistant to DNase and S1 nuclease but sensitive to RNase A under low-salt conditions (data not shown), implying that they were dsRNA. We used a filter-sterilized viral particle fraction for protoplast inoculation, and as a consequence, all mycelial fragments of W8 were removed. In addition, since isolates W8, W37 and W97 belong to different mycelial compatibility groups, the mixing of cytoplasm is not likely to occur (Fig. 4). The bands detected from regenerants were obviously derived from mycelia-infected RnPV1-W8. Results of the Northern analysis also support successful viral transfection (Fig. 1B). RNA probes detected signals for RdRp and CP of RnPV1-W8 at almost the same level of intensity in virus-transfected regenerants and isolate W8.

RnPV1-W8 is considered to be highly compatible with the host fungus, *R. necatrix*. RnPV1-W8 had a high infection rate in regenerated mycelia, was transmitted efficiently by hyphal anastomosis to virus-free isolates (Fig. 3), and was stable during subculturing for about 2 years. The high compatibility of RnPV1-W8 was also supported by the fact that efforts to generate a virus-free strain from W8 were all unsuccessful (data not shown). These features are contradictory to those of MYRV3-RnW370, a hypovirulent mycoreovirus in isolate W370. MYRV3-RnW370 was sometimes lost during subculturing and infection, and its transmission by hyphal anastomosis was sometimes limited to areas close to colony junctions [21]. Viruses are transmissible if they have no deleterious effect on the host fungus. Proteins related to cell-to-cell movement encoded by plant viruses are not reported for mycoviruses. If mycoviruses spread in mycelia through plasma streaming only, replication rate may be the limiting factor of transmission.

Because RnPV1-W8 exhibited high affinity for *R. necatrix*, RnPV1-W8 and closely-related viruses are likely to be commonly distributed in *R. necatrix*. Other dsRNA bands with electrophoretic profiles similar to RnPV1-W8 were found in several different isolates [3], and RnPV1-W8 dsRNA was hybridized with direct-labeled W659 dsRNA [18]. Isolate W8 and W659 were obtained from different locations (Okayama and Tokyo, respectively) and belong to different MCGs. Because ascospore progenies of *R. necatrix* completely lack dsRNAs contained in the parental strains [18], the possibility of infection for ancestral isolates of W8 and W659 with RnPV1-W8 is unlikely. Further analysis is required to explain the ubiquity of the partitiviruses in *R. necatrix*.

Transfection with purified viral particles is a powerful tool with which to determine the influence of a virus on the host fungus. Isolate W8 showed irregular colony morphology, slow mycelial growth, and moderate virulence (Figs. 2 and 4). We could not completely exclude the possibility that these attributes were indigenous to W8. Because these traits are not associated with virus-free *R. necatrix*; the hypovirulence-related phenotype is assumed to result from viral infection. However, RnPV1-W8 did not contribute to these properties; it is cryptic and typical of partitiviruses [13]. This is supported by the finding that isolates W37 and W97 exhibited no appreciable change in colony morphology or virulence after transfection with RnPV1-W8. The indistinguishable morphology of virus-transfected regenerants (donor) and virus-transmitted transformants (recipient) suggested that the symptoms of RnPV-1W8 were not masked by somatic mutation during protoplast production and/or viral transfection. The abnormal morphology and reduced virulence of W8 are more likely to be incited by L1 or L2-dsRNA or both. Alternatively, the combination of three segments, L1, L2 and RnPV1-W8, may be responsible for the slow, irregular growth and hypovirulence of isolate W8. The three dsRNAs in W8 are stable, and therefore, considered competent as control agents if their involvement in the reduced virulence of isolate W8 is confirmed. There is further need to clarify the relationship between other dsRNAs in W8.

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References

1. Ahn IP, Lee YH (2001) A viral double-stranded RNA up regulates the fungal virulence of *Nectria radicicola*. Mol Plant Microbe Interact 14: 496–567
2. Anagnostakis SL (1982) Biological control of chestnut blight. Science 215: 466–471
3. Arakawa M, Nakamura H, Uetake Y, Matsumoto N (2002) Presence and distribution of double-stranded RNA elements in the white root rot fungus *Rosellinia necatrix*. Mycoscience 43: 21–26
4. Brasier CMA (1983) A cytoplasmic ally transmitted disease of *Ceratocystis ulum*. Nature 305: 220
5. Buck KW (1998) Molecular variability of viruses of fungi. In: Bridge P, Couteaudier Y, Clarkson J (eds) Molecular variability of fungal pathogens. CAB International, Wallingford Oxfordshire, pp 53–72
6. Buck KW (1986) Fungal virology-an overview. In: Buck KW (ed) Fungal virology. CRC press, Boca Raton FL, pp 1–84
7. Castro M, Kramer K, Valdivia L, Ortiz S, Castillo A (2003) A double-stranded RNA mycovirus confers hypovirulence-associated traits to *Botrytis cinerea*. FEMS Microbiol Lett 228: 87–91
8. Chen B, Nuss DL (1999) Infectious cDNA clone of hypovirus CHV1-Euro7: a comparative virology approach to investigate virus-mediated hypovirulence of the chestnut blight fungus *Cryphonectria parasitica*. J Virol 73: 985–992
9. Chen B, Chen CH, Bowman BH, Nuss DL (1996) Phenotypic changes associated with wild-type and mutant hypovirus RNA transfection of plant pathogenic fungi phylogenetically related to *Cryphonectria parasitica*. Phytopathology 86: 301–310
10. Chen B, Choi GH, Nuss DL (1994) Attenuation of fungal virulence by synthetic infectious hypovirus transcripts. Science 264: 1762–1764
11. Choi GH, Nuss DL (1992) Hypovirulence of chestnut blight fungus conferred by an infectious viral cDNA. Science 257: 800–803
12. Chu YM, Jeon JJ, Yea SJ, Kim YH, Yun SH, Lee YW, Kim KH (2002) Double-Stranded RNA Mycovirus from *Fusarium graminearum*. Appl Environ Microbiol 68: 2529–2534
13. Ghabrial SA, Bozarth RF, Buck KW, Martelli GP, Milne RG (2000) Family *Partitiviridae*. In: van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RB (eds) Virus taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, Springer Wien New York, pp 503–513
14. Hillman BI, Suzuki N (2004) Viruses of the chestnut blight fungus, *Cryphonectria parasitica*. Adv Virus Res 63: 423–472
15. Hillman BI, Supyani S, Kondo H, Suzuki N (2004) A reovirus of the fungus *Cryphonectria parasitica* that is infectious as particles and related to the Coltivirus genus of animal pathogen. J Virol 78: 892–898
16. Hillman BI, Fulbright DW, Nuss DL, Van Alfen NK (2000). Family *Hypoviridae*. In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RB (eds) Virus taxonomy:

Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, Springer Wien New York, pp 515–520
17. Huang S, Ghabrial SA (1996) Organization and expression of the double-stranded RNA genome of *Helminthosporium victoriae* 190S virus, a Totivirus infecting a plant pathogenic filamentous fungus. Proc Natl Acad Sci USA 93: 12541–12546
18. Ikeda K, Nakamura H, Arakawa M, Matsumoto N (2004) Diversity and vertical transmission of double-stranded RNA elements in root rot pathogens of trees, *Helicobasidium mompa* and *Rosellinia necatrix*. Mycol Res 108: 626–634
19. Jian J, Lakshman DK, Tavantzis SM (1997) Association of distinct double-stranded RNAs with enhanced or diminished virulence in *Rhizoctonia solani* infected potato. Mol Plant Microbe Interact 10: 1002–1009
20. Kanadani G, Date H, Nasu H (1998) Effect of Fluazinam soil-drench on white root rot of grapevine. Ann Phytopath Soc Jpn 64: 139–141
21. Kanematsu S, Arakawa M, Oikawa Y, Onoue M, Osaki H, Nakamura H, Ikeda K, Kuga-Uetake Y, Nitta H, Sasaki A, Suzaki K, Yoshida K, Matsumoto N (2004) A reovirus causes hypovirulence of *Rosellinia necatrix*. Phytopathology 94: 561–568
22. Kimura N, Tsuge T (1993) Gene cluster involved in melanin biosynthesis of the filamentous fungus *Alternalria alternarta*. J Bacteriol 175: 4427–4435
23. Lakshman DK, Jian J, Tavantzis SM (1998) A double-stranded RNA element from a hypovirulent strain of *Rhizoctonia solani* occurs in DNA form and is genetically related to the pentafunctional AROM protein of the shikimate pathway. Proc Natl Acad Sci USA 95: 6425–6429
24. Lhoas P (1971) Infection of protoplasts from *Penicillium stoloniferum* with double-stranded RNA viruses. J Gen Virol 13: 365–367
25. Melzer M, Boland GH (1995) Transmissible hypovirulence in *Sclerotinia minor*. Can J Plant Pathol 18: 19–28
26. Mertens PPC, Hillman BI, Suzuki N (2005) Genus Mycoreovirus. In: Fauquet CM et al. (eds). Virus taxonomy: Eighth Report of the International Committee for the Taxonomy of Viruses. Academic Press, San Diego. pp 556–560
27. Moleleki N, van Heerden SW, Wingfield MJ, Wingfield BD, Preisig O (2003) Transfection of *Diaporthe perjuncta* with Diaporthe RNA virus. Appl Environm Microbiol 69: 3952–3956
28. Nakamura H, Uetake Y, Arakawa M, Okabe I, Matsumoto M (2000) Observations the teleomorph of the white root rot fungus, *Rosellinia necatrix*, and related fungus, *Rosellinia aquia*. Mycoscience 41: 503–507
29. Osaki H, Wei CZ, Arakawa M, Iwanami T, Nomura K, Matsumoto N, Ohtsu Y (2002) Nucleotide sequences of double-stranded RNA segments from a hypovirulent strain of the white root rot fungus *Rosellinia necatrix*: possibility of the first member of the *Reoviridae* from fungus. Virus Genes 25: 101–107
30. Rogers HJ, Buck KW (1986) Transmission of double-stranded RNA and a disease factor in *Ophostoma ulmi*. Plant Pathol 35: 277–287
31. Sasaki A, Onoue M, Kanematsu S, Suzaki K, Miyanishi M, Suzuki N, Nuss DL, Yoshida K (2002) Extending chestnut blight hypovirus host range within *Diaporthales* by biolistic delivery of viral cDNA. Mol Plant Microbe Interact 15: 780–789
32. Sasaki A, Miyanishi M, Ozaki K, Onoue M, Yoshida K (2005) Molecular characterization of a partitivirus from the plant pathogenic ascomycete *Rosellinia necatrix*. Arch Virol 150: 1069–1083
33. Smart CD, Yuan W, Foglia R, Nuss DL, Fulbright DW, Hillman BI (1999) *Cryphonectria hypovirus* 3, a virus species in the family *Hypoviridae* with a single open reading frame. Virology 265: 66–73

34. Stanway C, Buck KW (1984) Infection of protoplasts of the Wheat Take-all fungus, *Gaeumannomyces graminis* var. *tritici*, with double-stranded RNA viruses. J Gen Virol 65: 2061–2066
35. Suzuki N, Supyani S, Maruyama K, Hillman BI (2004) Complete genome sequence of *Mycoreovirus 1*/Cp9B21, a member of a new genus within the family *Reoviridae*, from the chestnut blight fungus *Cryphonectria parasitica*. J Gen Virol 85: 3437–3448
36. Sztejnberg A (1991) Rosellinia (Dematophora) root rot. In: Jones AL, Aldwicnckle HS (eds) Compendium of Apple and Pear Disease. American Phytopathological Society Press, St Paul MN, pp 46–47
37. Terai Y (1995) Rosellinia (Dematophora) root rot. In: Ogawa EM, Zehr EI, Bird GW, Ritchie DF, Uriu K, Umemoto JK (eds) Compendium of stone fruit disease. American Phytopathological Society Press, St Paul MN, pp 42–43
38. Uetake Y, Nakamura H, Arakawa M, Okabe I, Matsumoto N (2001) Inoculation of *Lupinus luteus* with white root rot fungus, *Rosellinia necatrix*, to estimate virulence. J Gen Plant Pathol 67: 285–287
39. Wei CZ, Osaki H, Iwanami T, Matsumoto N, Ohtsu Y (2003) Molecular characterization of dsRNA segments 2 and 5 and electron microscopy of a novel reovirus from a hypovirulent isolate, W370, of the plant pathogen *Rosellinia necatrix*. J Gen Virol 84: 2431–2437
40. Wei CZ, Osaki H, Iwanami T, Matsumoto N, Ohtsu Y (2004) Complete nucleotide sequences of genome segment 1 and 3 of *Rosellinia anti-rot virus* in the family *Reoviridae*. Arch Virol 149: 773–777
41. Wickner RB, Ghabrial SA, Bruenn JA, Buck KW, Pattersom JL, Stuart KD, Wang CC (2000) Family *Totiviridae*. In: van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RB (eds) Virus taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, Springer Wien New York, pp 491–501

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