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FULL PAPER

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Transmissibility of viral double-stranded RNA between strains of the violet root rot fungus *Helicobasidium mompa* and the potential for viral dsRNA infection to this fungus using monokaryotic strains

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Abstract To examine the potential of a method of double-stranded (ds) RNA infection to *Helicobasidium mompa*, the transmissibility of dsRNA between strains of this fungus was investigated. Strain V70 was used as a dsRNA donor. The dsRNA recipients were six strains that were mycelially incompatible with V70, plus two monokaryotic strains. Random amplified polymorphic DNA analysis suggested that the mycelially incompatible strains were genetically different mutually; however, the analysis also suggested that V70 was genetically homogeneous with the two monokaryotic strains. When V70 was paired with either of the mycelially incompatible strains, the dsRNAs did not transmit to the recipients at all. When V70 was paired with the two monokaryotic strains, the dsRNAs were transmitted to the monokaryotic strains. The two monokaryotic strains, which had acquired dsRNAs from V70 in the previous experiment, were used as new dsRNA donors in a next experiment so that we could investigate dsRNA transmission from these monokaryotic strains to the six strains used in the previous experiment. One of the monokaryotic strains permitted dsRNA transmission to two of the six recipients. We conclude that we can infect genetically different strains of *H. mompa* with dsRNA using the monokaryotic strains.

Key words Double-stranded (ds) RNA · dsRNA transmission · *Helicobasidium mompa* · Monokaryon · Mycelial incompatibility

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Introduction

The basidiomycetous fungus *Helicobasidium mompa* Tanaka causes violet root rot in many woody and herbaceous plants (Ito 1949). Among fruit trees, apple trees are particularly damaged by this fungus (Segawa and Harada 1990), and no effective control method has been established.

In several plant pathogenic fungi, double-stranded (ds) RNA viruses that reduce the virulence of host fungi have been discovered (Anagnostakis and Day 1979; Hammar et al. 1989; Smit et al. 1996; Jian et al. 1997), and phytopathologists have taken advantage of such dsRNA viruses to control chestnut blight (Anagnostakis 1982). Application of hypovirulence-associated dsRNA viruses to control violet root rot has been proposed (Matsumoto 1998), and such dsRNA viruses have been searched for intensively in *H. mompa*. Various dsRNA elements have been detected in various field-isolated strains of *H. mompa* (Matsumoto 2001); however, the relationship between these dsRNA elements and hypovirulence in *H. mompa* is still unclear. Evaluation of the virulence-reducing ability of these dsRNA elements requires a method to infect virulent strains of *H. mompa* with various dsRNA elements.

Because fungi generally have rigid cell walls and it may be impossible to damage the cell walls without killing the fungal cells, mechanical infection methods using purified virions or dsRNA elements via extracellular routes are ineffective (Hollings et al. 1963; Hollings 1978). To overcome the cell wall barrier, viral infectivity assays using fungal protoplasts succeeded in a few virus–fungi systems (Stanway and Buck 1984; Choi and Nuss 1992). However, it is difficult to prepare protoplasts of *H. mompa*, and we have not succeeded in obtaining enough protoplasts for a viral infectivity assay.

It is already known that, in hyphal anastomosis, fungal viruses are transmitted only within the same fungal strain or between closely related strains (Ghabrial 1998). Although viral dsRNA is transmitted within the same fungal strain or between closely related strains, for the present we consider

that the viral dsRNA infection method using hyphal anastomosis is likely the most effective for *H. mompa*. Thus far, mycelial incompatibility (Katsumata et al. 1996; Akahira et al. 2000) and alterations in karyotype (Aimi et al. 2001) have been reported as cytological characteristics in *H. mompa*. In this report, we first conducted pairing experiments to determine whether the mycelial incompatibility of *H. mompa* would prevent dsRNA transmission between strains. We then used pairing experiments to investigate the transmissibility of dsRNA when monokaryotic strains were used, and considered the probability of a dsRNA infection method using monokaryotic strains that would transcend the mycelial incompatibility of *H. mompa.*

Materials and methods

Strains of *Helicobasidium mompa*

All strains used in this research are listed in Table 1. The strains were stocked at 4°C on oatmeal agar (OMA; Difco, NJ, USA) slants and cultured on OMA plates at 25°C before the experiment. Osaki et al. (2002) already showed that strain V70 possesses three dsRNA segments: dsRNA-1, dsRNA-2, and dsRNA-3 (numbered in order of size), and that these dsRNA segments originate from partitivirus, a fungal virus. They also showed that dsRNA-1 codes the RNA-dependent RNA polymerase (RDRP) gene from nucleotide sequencing data (DDBJ accession no. AB025903). Because the RDRP gene is indispensable in viral replication (Ghabrial 1998), we used strain V70 as a dsRNA donor and investigated the transmission of dsRNA-1 between strains of *H. mompa*. Karyotypes of two strains, V1M and V3M, were already reported as monokaryotic (Aimi et al. 2001), and we reconfirmed that these strains were monokaryotic before the experiment.

Determination of mycelial incompatibility and karyotype

To determine mycelial incompatibility, all the strains were paired by turns on OMA plates 9 cm in diameter. Two agar blocks containing mycelia of the testing strains were inoculated in the center of each OMA plate at intervals of 2 cm and cultured at 25°C for 1 month. Strains between which a distinct brown interaction zone emerged were regarded as mycelially incompatible.

Table 1. Strains of *Helicobasidium mompa* used in this research

Strain	Karyotype	Source
V1M	Monokaryon	*Malus pumila* (apple)
V2	Dikaryon	*Malus pumila* (apple)
V3M	Monokaryon	*Malus pumila* (apple)
V18	Dikaryon	*Asparagus officinalis* (asparagus)
V21	Dikaryon	*Malus pumila* (apple)
V26	Dikaryon	*Malus pumila* (apple)
V38	Dikaryon	*Malus pumila* (apple)
V48	Dikaryon	*Malus pumila* (apple)
V70	Dikaryon	*Malus pumila* (apple)

Fungal nuclei were stained with 0.1% (w/v) acridine orange (Sigma, St. Louis, MO, USA) in 50 mM sodium phosphate buffer (pH 7.0) for 15 min in the dark. Stained nuclei were observed using a Leica DMR fluorescent microscope (Leica Microsystems, Bensheim, Germany) equipped with a Leica L5 filter-set at a magnification of 400×.

Random amplified polymorphic DNA analysis

To investigate the genetic background of the fungal strains, random amplified polymorphic DNA (RAPD) analysis was performed. Fungal DNA extraction was carried out following the methods described by Wagner et al. (1987) and Bousquet et al. (1990). Polymerase chain reaction (PCR) was performed using three oligonucleotide primers (F-01, F-04, and F-09 in RAPD 10-mer Kit F; Qiagen-Operon, CA, USA). The contents of the reaction mixture and the PCR conditions followed the manufacturer's protocol (Qiagen-Operon). The reaction products were separated by 5% polyacrylamide gel (acrylamide : bisacrylamide, 40 : 0.96, w/w) electrophoresis in TAE [40 mM Tris, 4 mM sodium acetate, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0] buffer, and were visualized by ethidium bromide staining (1 μg/ml ethidium bromide in distilled water).

Pairing of strains and observation of hyphal anastomosis

dsRNA transmission between strains was investigated by pairing the dsRNA donor strain with each recipient strain on an OMA plate. Two agar blocks, containing the donor strain and a recipient, respectively, were inoculated in the center of an OMA plate (9 cm in diameter) at a distance of 2 cm apart and cultured at 25°C for 35 days. A small section of the recipient colony was sampled at the end of pairing and used for dsRNA extraction. Each pairing was repeated ten times. To observe hyphal anastomosis, strains were paired on clarified oatmeal agar (COMA; Adams et al. 1990) plates 9 cm in diameter and 3 mm thick. Hyphal anastomoses were observed from the reverse side of the COMA plate at the junction of the two colonies by a Nikon TE300 inverted microscope (Nikon Instech, Kawasaki, Japan), at a magnification of 400×, every 5 days from the beginning of the pairing. Anastomosis was distinguished by referring to the anastomosis of *Rhizoctonia solani* (Yokoyama et al. 1983; Yokoyama and Ogoshi 1986). The pairings for observation of hyphal anastomoses on COMA plates were begun simultaneously with the pairings for dsRNA transmission on OMA plates and continued for 35 days.

dsRNA extraction and purification

A small section of agar block (5 mm square) was sampled from the recipient colony along the edge of the plate and was subcultured temporarily on a fresh OMA plate. A small piece of growing mycelia on the OMA plate was inoculated in 100 ml oatmeal broth medium (75 g commercial oatmeal in 1 l distilled water) and cultured for 14 days at 25°C in a

rotary shaker (90 rpm). The harvested mycelia were lyophilized, and dsRNA was extracted from 0.5 g mycelia according to the method described by Morris and Dodds (1979). The extracted dsRNA sample was dissolved in 100 µl diethylpyrocarbonate (DEPC)-treated water and treated with 50 µl nuclease mixture [5 units of DNase I (Amersham Pharmacia Biotech, NJ, USA), 20 units of S1 nuclease (Takara, Ohtsu, Japan), 15 mM $MgCl_2$, 300 mM NaCl, 3 mM $ZnSO_4$, and 90 mM sodium acetate, pH 4.6] overnight at 37°C to remove residual DNA and single-stranded RNA (ssRNA). The purified dsRNA was dissolved in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) buffer and separated by 5% polyacrylamide gel electrophoresis as already described.

Identification of dsRNA-1

The dsRNA segment that was newly detected in each recipient after pairing was then identified by reverse transcription-polymerase chain reaction (RT-PCR) using RNA PCR Kit Ver. 2.1 (Takara) with the following primer set: forward primer, 70-1N (5′-CACATCTACGAGCACA ACTTCC-3′) and reverse primer, 70-3N (5′-AAGGGCCA GAGAGTAGAAGGG-3′). Each dsRNA sample was denatured at 99°C for 10 min and chilled quickly. Reverse transcription was performed following the manufacturer's protocol; however, PCR was performed for 30 cycles according to the following condition: 1 min at 94°C, 1 min at 64°C, and 2 min at 72°C. The reaction products were separated by 1.5% agarose gel electrophoresis in TAE buffer and visualized by ethidium bromide staining. The nucleotide sequence of the amplified DNA segment was further analyzed. The amplified DNA segment was ligated to *Escherichia coli* plasmid vector (pGEM-T vector system; Promega, Madison, WI, USA), and *E. coli* JM109-competent cells (Takara) were transformed with the recombinant plasmids. The cloned DNA segments were sequenced by the dideoxy method with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, CA, USA). The sequencing reaction products were analyzed with a Model 310 Genetic Analyzer (Applied Biosystems), and the resulting sequences were compared using Genetyx-Mac software (Software Development, Tokyo, Japan).

Monomycelial isolation

When the recipients acquired their dsRNA segments from the donor, to negate the possibility that the recipient colonies might be contaminated with donor mycelia, monomycelial isolation was done from the recipient colony, and both the dsRNA banding pattern and the karyotype of the monomycelial-isolated strain were investigated. Small pieces of the recipient colonies, which were subcultured temporarily after pairing on OMA plates, were transferred to 1.6% water agar medium (12 cm square) and cultured for 30 days at 25°C. Ten apexes of growing hyphae were picked up with a needle under a Nikon SMZ-2T stereoscopic microscope and cultured on fresh OMA plates. Karyotype observation and dsRNA detection were performed following the methods already described.

Results

Karyotype, mycelial incompatibility, and RAPD analysis of the strains

Acrydine orange staining of nuclei showed that all the strains except two strains, V1M and V3M, were dikaryotic. All the strains were paired by turns on OMA plates, and distinct brown interaction zones emerged between the colonies in all the pairings of different dikaryotic strains (Table 2). Mycelial growth was sparse in such interaction zones. No pairings within the same strain formed such interaction zones. This observation suggested that the mycelia of all the dikaryotic strains used here were mutually incompatible.

Table 2. Summary of dsRNA transmission

Donor (karyotype)[a]	Recipient (karyotype)	Emergence of interaction zone	Hyphal anastomosis	Frequency of dsRNA transmission[b]
V70 (dikaryon)	V2 (dikaryon)	+	Imperfect fusion	0/10
V70 (dikaryon)	V18 (dikaryon)	+	Imperfect fusion	0/10
V70 (dikaryon)	V21 (dikaryon)	+	Imperfect fusion	0/10
V70 (dikaryon)	V26 (dikaryon)	+	Imperfect fusion	0/10
V70 (dikaryon)	V38 (dikaryon)	+	Imperfect fusion	0/10
V70 (dikaryon)	V48 (dikaryon)	+	Imperfect fusion	0/10
V70 (dikaryon)	V1M (monokaryon)	−	Perfect fusion	1/10
V70 (dikaryon)	V3M (monokaryon)	−	Perfect fusion	3/10
V3M(ds+) (monokaryon)	V2 (dikaryon)	−	Imperfect fusion	1/10
V3M(ds+) (monokaryon)	V18 (dikaryon)	−	Imperfect fusion	2/10
V3M(ds+) (monokaryon)	V21 (dikaryon)	−	Imperfect fusion	0/10
V3M(ds+) (monokaryon)	V26 (dikaryon)	−	Imperfect fusion	0/10
V3M(ds+) (monokaryon)	V38 (dikaryon)	−	Imperfect fusion	0/10
V3M(ds+) (monokaryon)	V48 (dikaryon)	−	Imperfect fusion	0/10

[a] Donor strain V3M(ds+) represents the strain V3M, which acquired dsRNA segments from strain V70
[b] Numerator represents number of plates in which dsRNA transmission has been permitted and denominator represents number of replications in pairing tests

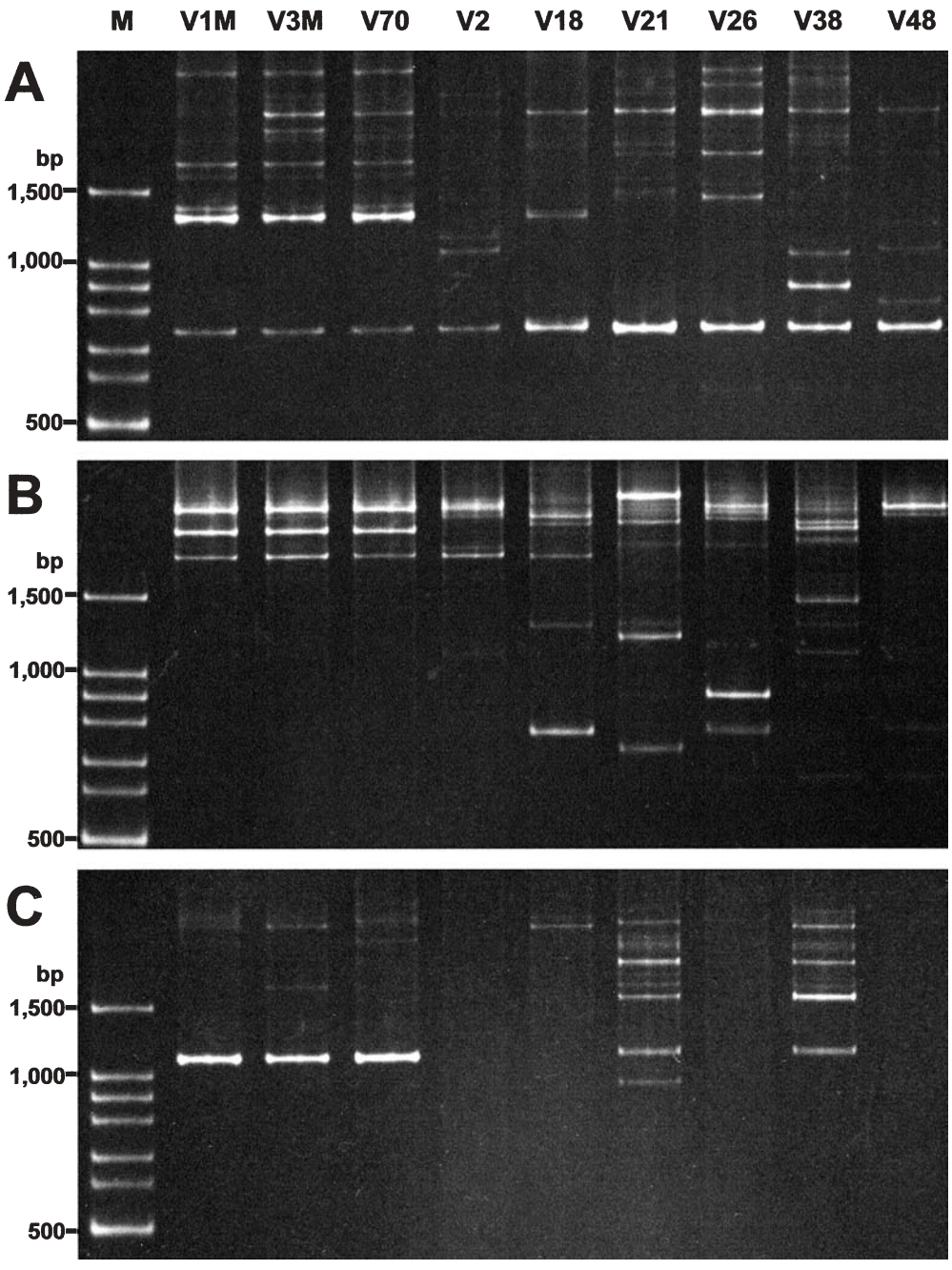

Fig. 1. Random amplified polymorphic DNA (RAPD) analysis: RAPD patterns using F-01 primer (**A**), F-04 primer (**B**), and F-09 primer (**C**). *Strain numbers* are shown above the respective lanes. *M*, 100-bp DNA ladder marker (Takara)

Distinct interaction zones emerged between colonies of different dikaryotic strains, but such interaction zones did not emerge in pairings of dikaryotic and monokaryotic strains (Table 2). RAPD analysis using three PCR primers revealed that each mycelial compatibility group had a unique RAPD pattern; however, strains V1M, V3M, and V70 showed similar RAPD patterns (Fig. 1).

dsRNA segments in the strains

dsRNA segments were detected in eight of the nine strains and were not detected in strain V3M (Fig. 2). Each host strain had a unique banding pattern of the dsRNA segment. Because the smaller segment in strain V21 and the larger segment in strain V48 showed the same electrophoretic mobility to dsRNA-1, these dsRNA segments were identified by RT-PCR using primers specific to dsRNA-1. No DNA segments were amplified from these dsRNA segments (data not shown); this result indicated that dsRNA-1 was unique to strain V70.

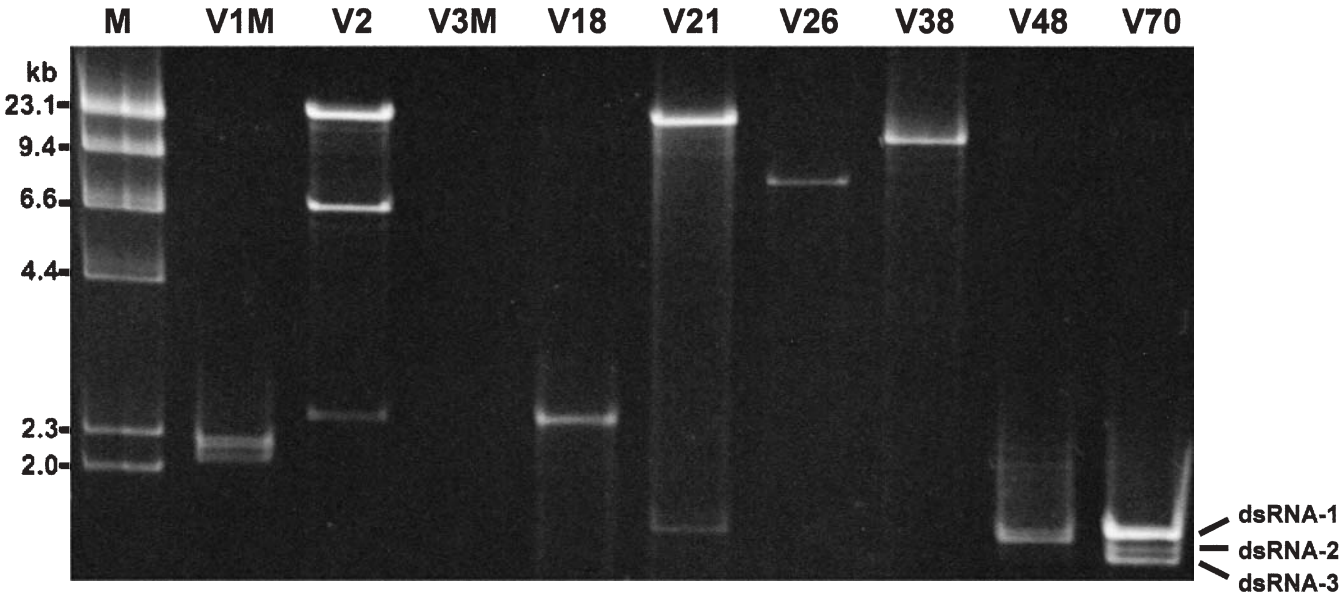

Fig. 2. dsRNA segments in the strains used in this research. *Strain numbers* are shown above the respective lanes. *M*, lambda/*Hind*III DNA ladder marker (Takara). Strain V70 (donor of dsRNA) possessed three dsRNA segments: *dsRNA-1*, *dsRNA-2*, and *dsRNA-3*

Transmissibility of dsRNA-1 from donor to mycelially incompatible strains

When strain V70 was paired with the six mycelially incompatible strains, no alteration in dsRNA banding patterns was observed in any of the recipient strains, which suggested that dsRNA-1 was not transmitted between mycelially incompatible strains (Table 2). Hyphae of strain V70 frequently formed anastomoses with hyphae of mycelially incompatible strains 15 days after the beginning of culture, and anastomosed hyphae were always accompanied by cellular deterioration (Fig. 3). Cellular deterioration in anastomosed hyphae was considered a sign of imperfect fusion.

Transmissibility of dsRNA-1 from donor to monokaryotic strains

When strain V70 was paired with the two monokaryotic strains, new dsRNA segments were detected in each monokaryotic strain (Fig. 4A). In the new dsRNA segments of strains V1M and V3M, RT-PCR amplified DNA segments about 550 bp in length (Fig. 5A). The nucleotide sequences of the amplified DNA segments agreed with the nucleotide sequence between 159 and 700 bases in dsRNA-1 (data not shown). Therefore, dsRNA-1 was considered the largest segment in the newly detected dsRNA segments in both monokaryotic strains. dsRNA-1 was always detected together with smaller dsRNA segments, which were thought to be dsRNA-2 and dsRNA-3. Hyphae of strain V70 anastomosed with hyphae of both the monokaryotic strains 20 days after pairing was initiated. Anastomosed hyphae were not accompanied by cellular deterioration, and they remained stable until the end of observation, suggesting perfect fusion (Fig. 6). Monomycelial isolation was performed, and ten monomycelial-isolated strains were obtained from the colonies of both monokaryotic strains. dsRNA-1 was redetected together with dsRNA-2 and dsRNA-3 in all the monomycelial-isolated strains, all of which remained monokaryotic. Because the hyphae of both V1M and V3M were still monokaryotic after acquisition of the dsRNA segments and could be definitely distinguished from the hyphae of V70, dsRNA segments including dsRNA-1 certainly were transmitted from V70 to both V1M and V3M without contamination by mycelia of the donor strain. No alteration in either colony morphology or growth rate as a result of the acquisition of dsRNA segments from strain V70 was seen in V1M or V3M.

Transmissibility of dsRNA-1 from monokaryotic strains to dikaryotic strains

The two monokaryotic strains, which acquired dsRNA segments from strain V70 in the previous experiment, were used as the new dsRNA donors as we investigated the transmission of dsRNA-1 from them to the six dikaryotic strains: V2, V18, V21, V26, V38, and V48. For all six of these strains, pairing with V1M did not alter the banding pattern of the dsRNA segment. On the other hand, new dsRNA segments were detected in strains V2 and V18 after pairing with V3M (Fig. 4B). RT-PCR also amplified DNA segments about 550 bp in length from the new dsRNA segments in both V2 and V18 (Fig. 5B). The nucleotide sequences of the amplified DNA segments agreed with the nucleotide sequence of dsRNA-1 (data not shown). Therefore, dsRNA-1 was considered the largest segment in the newly detected dsRNA segments in V2 and V18. In strain V18, dsRNA-2 and dsRNA-3 were detected together with dsRNA-1; in V2, however, dsRNA-2 was not detected (Fig. 4B). Hyphal anastomoses were observed 20 days after the

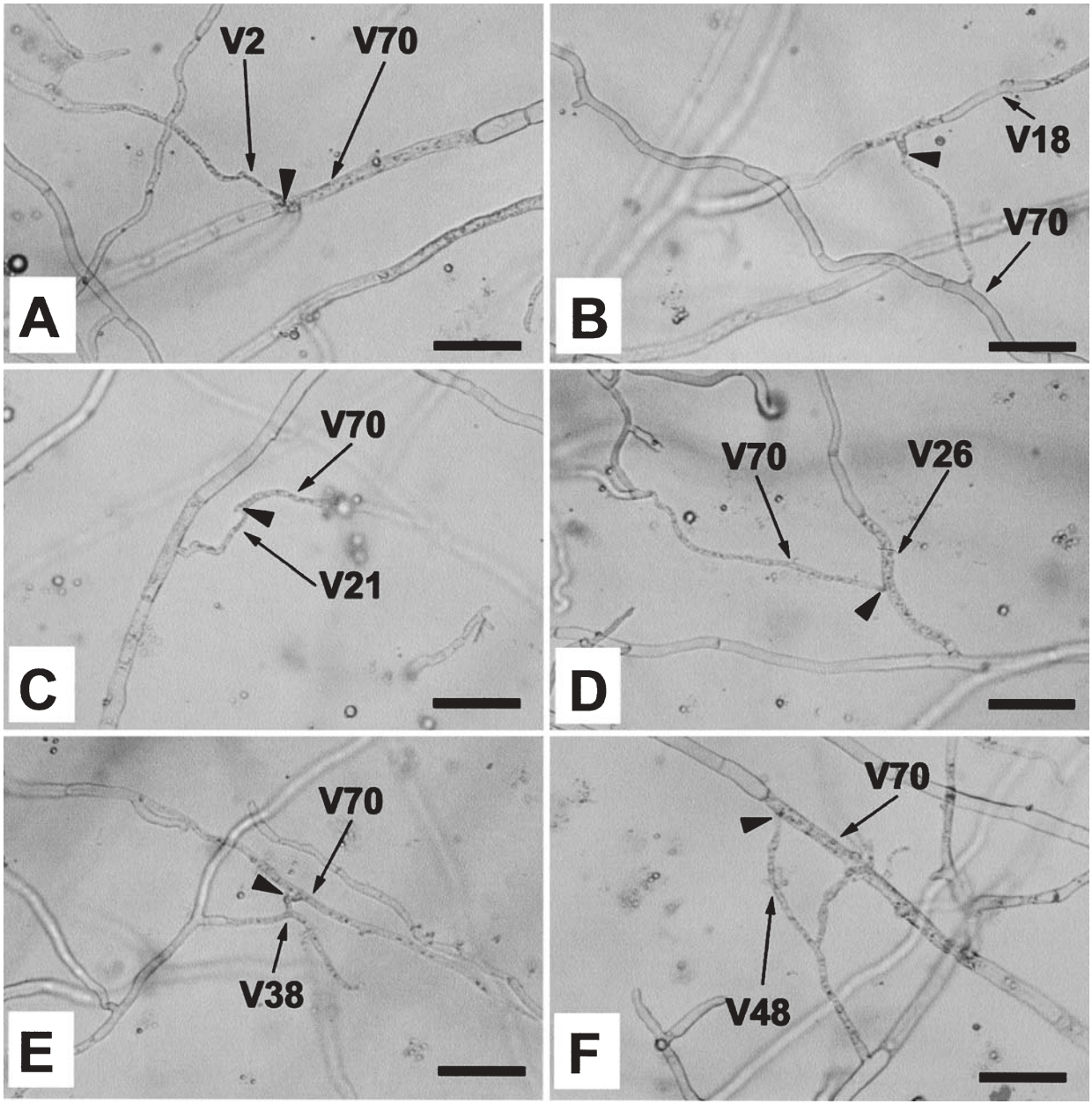

Fig. 3. Imperfect fusions (*arrowheads*) between strains V70 and mycelially incompatible strains. **A** Strains V70 and V2. **B** Strains V70 and V18. **C** Strains V70 and V21. **D** Strains V70 and V26. **E** Strains V70 and V38. **F** Strains V70 and V48. *Arrows*, points at which hyphal anastomoses occurred. *Bars* 20 μm

beginning of pairing, and hyphae of strains V2 and V18 fused imperfectly with hyphae of strain V3M (Fig. 7). Ten monomycelial-isolated strains were also obtained from colonies of V2 and V18 that had acquired the dsRNA segments from V3M. DsRNA-2 and dsRNA-3 were also redetected together with dsRNA-1 in all the monomycelial-isolated strains obtained from V18; on the other hand, all the strains obtained from V2 still lacked dsRNA-2. The hyphae of all the monomycelial-isolated strains were dikaryotic and distinguishable from the hyphae of strain V3M. Additionally, the RAPD patterns of V2 and V18 did not change from before to after acquisition of the dsRNA segments from strain V3M. These results suggested that dsRNA segments including dsRNA-1 were certainly transmitted from strain V3M to both V2 and V18, regardless of imperfect fusion. After the acquisition of dsRNA segments, neither V2 nor V18 showed any change in colony morphology or growth rate.

Discussion

Our results showed that dsRNA segments were not transmitted at all between mycelially incompatible strains of *H. mompa*. The results of RAPD analysis (see Fig. 1) showed

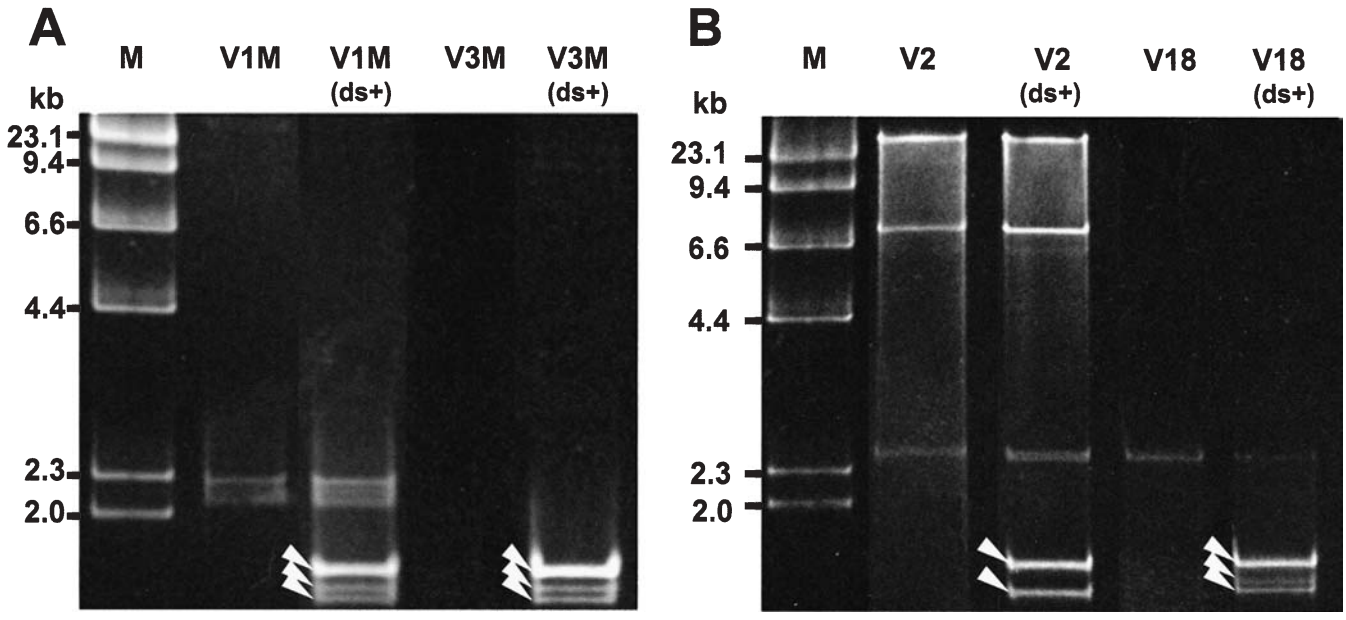

Fig. 4. dsRNA transmission from donors to recipients. **A** dsRNA transmission from strain V70 to strains V1M and V3M. *Strain numbers* are shown above the respective lanes. In each recipient, postfixed (*ds+*) means acquisition of dsRNA segments (indicated by *arrowheads*) from strain V70. **B** dsRNA transmission from strain V3M(ds+), which acquired dsRNA segments from strain V70, to strains V2 and V18. *Strain numbers* are shown above the respective lanes. In each recipient, postfixed (*ds+*) means acquisition of dsRNA segments (indicated by *arrowheads*) from strain V3M(ds+) as well. dsRNA-2 was not detected in strain V2. In both parts of the figure, *M* represents the lambda/*Hind*III DNA ladder marker (Takara)

that mycelial incompatibility reflected a difference in the genetic background of *H. mompa* as well as in *Sclerotinia sclerotiorum* (Kohn et al. 1991). Based on these results, we are convinced that dsRNA transmission hardly occurs between mycelially incompatible strains of *H. mompa*, that is, between different individuals, as reported in the other fungi (Todd and Rayner 1980). Additionally, our investigation revealed that each dsRNA banding pattern was unique to each host fungal strain (see Fig. 2). We also suppose from this observation that dsRNA in natural populations of *H. mompa* is not likely to prevail beyond mycelial incompatibility, as reported in the white root rot fungus *Rosellinia necatrix* (Arakawa et al. 2002).

Although dsRNA segments were not transmitted at all between V70 and mycelially incompatible strains, these dsRNA segments were transmitted from strain V3M, which had acquired the dsRNA segments from V70, to both V2 and V18 (see Fig. 4B, Table 2). Imperfect fusion (see Fig. 7) and RAPD analysis (Fig. 1) also suggested that both V2 and V18 were genetically different strains from V3M; that is, that they were different individuals. Based on these results, we considered that we were able to infect different individuals of *H. mompa* with dsRNA elements using the monokaryotic strains. Anagnostakis (1983; Anagnostakis and Day 1979) speculated that dsRNA could be transmitted between even vegetatively incompatible strains of *Endothia parasitica* given sufficient time before cells died in anastomosed hyphae. If this speculation is correct, we consider that anastomosed hyphae had sufficient time for dsRNA transmission in the V3M–V2 and V3M–V18 pairings. Additionally, as either dsRNA-2 or dsRNA-3 is considered a satellite or defective segment (Osaki et al. 2002), elimination of dsRNA-2 (see Fig. 4B) in the process of dsRNA transmission seems to be caused by a difference in the manner of dsRNA segment proliferations or a delay of cell death in anastomosed hyphae.

As mentioned previously, strains V3M and V70 are the same or closely related; however, V3M permits dsRNA transmission to different individuals of *H. mompa*. We speculate this is due to the monokaryotic state of *H. mompa*. Aimi et al. (2001) reported that the diploid dikaryon seems to be a normal nuclear phase of *H. mompa* in nature. They also attributed monokaryotization in *H. mompa* to the fusion of two nuclei and the unstableness of cellular ploidy in this fungus to either a lack of or a defect in mating-type factors. Raper and Raper (1973) also reported, in the tetrapolar basidiomycete *Schizophyllum commune*, that strains carrying mutations in the *B* genes showed instability in self–nonself recognition. Therefore, it seems likely that monokaryotic strains of *H. mompa* carry either defects or mutations in mating-type factors and that the instability of self–nonself recognition may permit dsRNA transmission between different strains. In our results, the monokaryotic strains of *H. mompa* did not form interaction zones with adjoining colonies of different strains (see Table 2). This finding seems to support our speculation.

Although not all the strains of *H. mompa* have unstable cellular ploidy (Aimi et al. 2001), we expect unstable strains to be able to infect different individuals of *H. mompa* with dsRNA elements via hyphal anastomosis. Although combi-

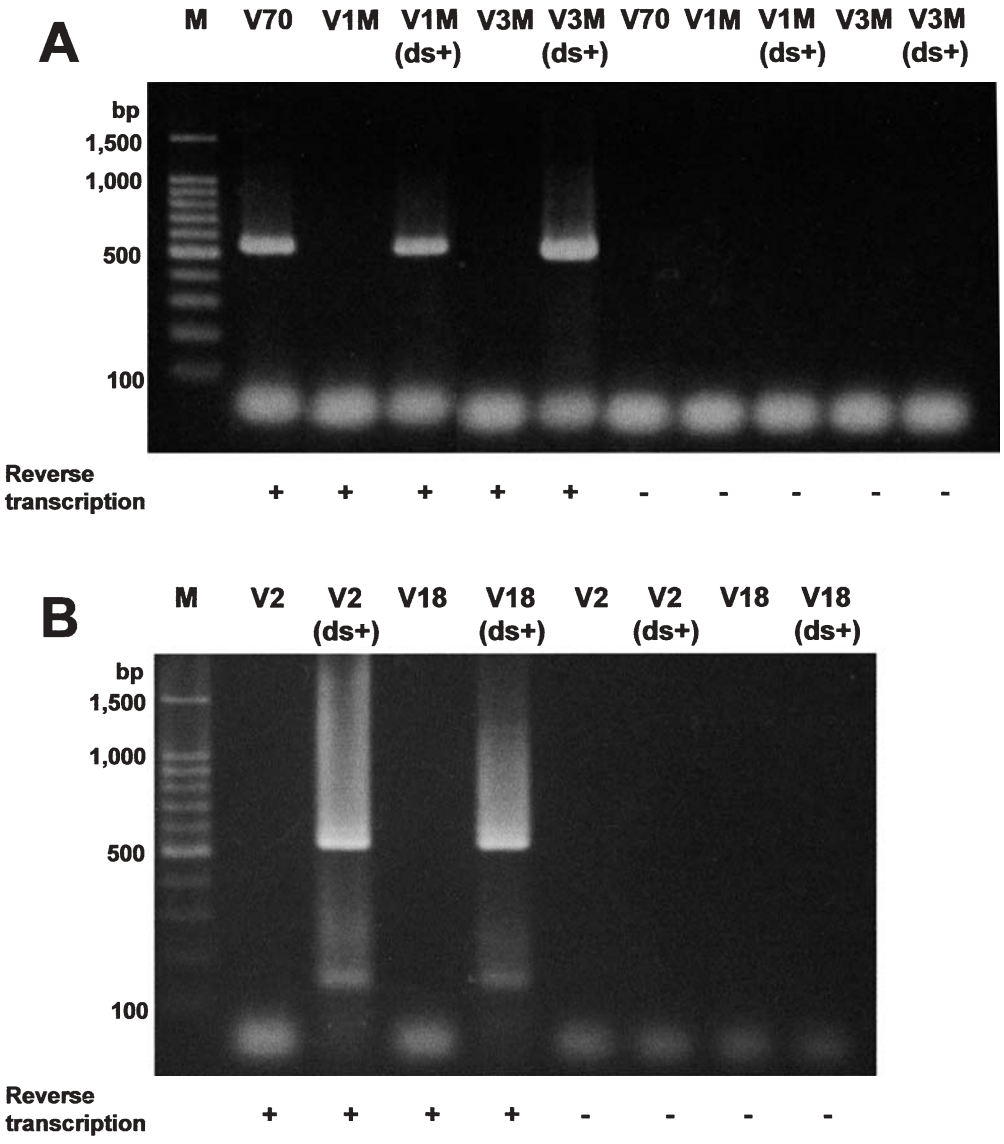

Fig. 5. Identification of dsRNA-1 by reverse transcription-polymerase chain reaction (RT-PCR). **A** Amplified DNA segments from strains V1M and V3M. **B** Amplified DNA segments from strains V2 and V18. *Strain numbers* are shown above the respective lanes. Postfixed (*ds+*) means that dsRNA-1 was detected in each strain after pairing. *Reverse transcription minus below* both parts of the figure means omission of reverse transcription in RT-PCR experiment. No DNA segments were amplified without reverse transcription; therefore, DNA segments of 550 bp were certainly amplified from dsRNA-1. *M*, 100-bp DNA ladder marker (Takara)

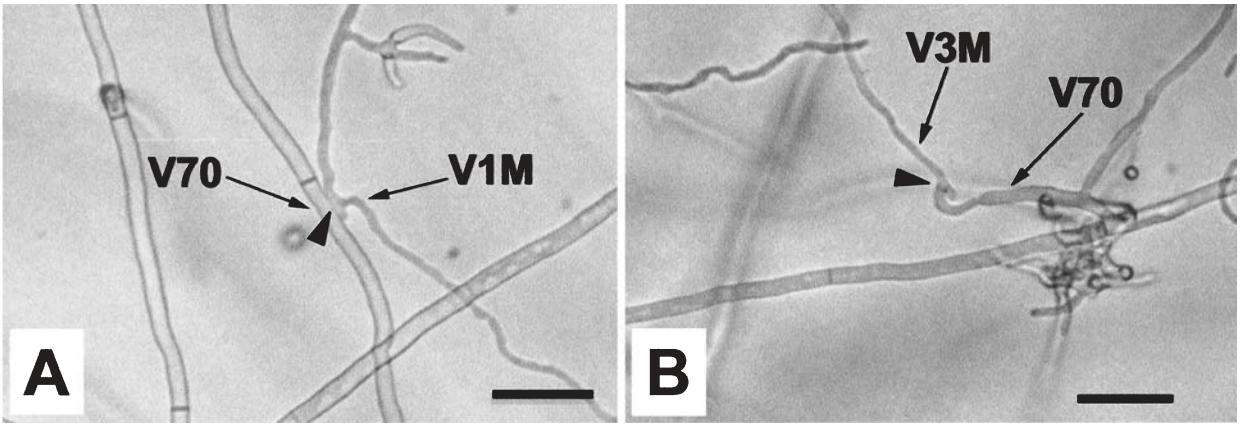

Fig. 6. Perfect fusions (*arrowheads*) between strain V70 and monokaryotic strains. **A** Strains V70 and V1M. **B** Strains V70 and V3M. *Arrows*, points at which hyphal anastomoses occurred. *Bars* 20 μm

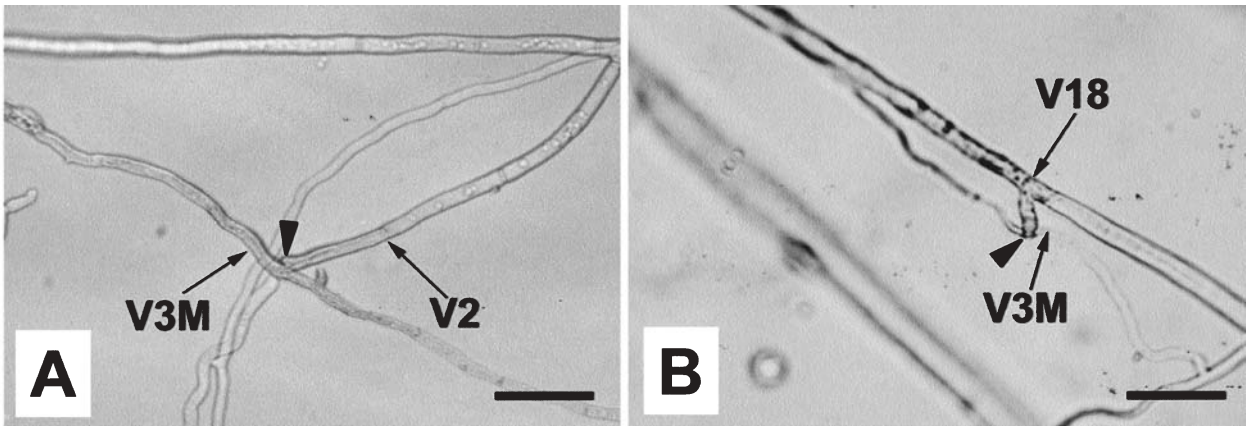

Fig. 7. Imperfect fusions (*arrowheads*) in pairings that permitted dsRNA transmission. **A** Strains V3M and V2. **B** Strains V3M and V18. *Arrows*, points at which hyphal anastomoses occurred. *Bars* 20 μm

nations of fungal strains may be limited, we consider that monokaryotic strains of *H. mompa* will be utilized as an effective tool for dsRNA infection. In the future, it will be necessary to ensure, by using several monokaryotic strains, whether monokaryotic strains of *H. mompa* are universally provided with instability in self–nonself recognition, and whether or not monokaryotic strains can permit dsRNA transmission between other different strains of *H. mompa*.

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