B I O D I V E R S I T A S
Volume 10, Number 2, April 2009
Pages: 70-75

ISSN: 1412-033X (printed edition)
ISSN: 2085-4722 (electronic)
DOI: 10.13057/biodiv/d100203

Partial Characterization of a Novel Reovirus Isolated from a Hypovirulent Strain (9B21) of *Cryphonectria parasitica* (Murrill) Barr.

SUPYANI♥
Laboratory of Plant Protection, Faculty of Agriculture, Sebelas Maret University (UNS), Surakarta 57126.

Received: 10th November 2008. Accepted: 26th December 2008.

ABSTRACT

A filamentous fungus *Cryphonectria parasitica* (Murrill) Barr. is the causal agent of the chestnut blight disease. This fungus has some hypovirulent strains. These strains are known to be infected by viruses. To date, many species of viruses have been found to be associated with *C. parasitica* strains. The number genome segments, their relative size, titer and sequence homology as well as the effects on host fungal virulence and morphology were varied among the *C. parasitica* associating viruses. *Cryphonectria parasitica mycoreovirus-1* (CpMYRV1) is a newly characterized reovirus isolated from *C. parasitica*. The viral genome consists of 11 segments of dsRNA termed S1 to S11. The aims of this research were to determine the complete sequences of the three shortest segments (S9-S11) of CpMYRV1, and to analyze the relationship of the dsRNA segments to the segments within the reoviruses genome. Sequence analyses showed that segments S9, S10, and S11 are 1072 bp, 975 bp, and 732 bp in size with single ORFs coding for 297, 247, and 101 amino acids respectively. Genome segments S9 to S11 have common terminal sequences, 5'GAUCA--- GCAGUCA3'. The deduced amino acid sequence coded by segment 9 showed a similarity to P11 of evolutionarily related *Rosellinia anti-rot virus* (*Reoviridae*), while S10 or S11 exhibited they were no significant sequence similarities to the known sequences published.

Key words: *Cryphonectria parasitica*, CpMYRV1, molecular characterization.

INTRODUCTION

The hypovirulence phenomenon on fungi caused by their association with viruses have been known since the discovery of swollen, superficial cankers and chestnut trees recovering from chestnut blight in Europe (Biraghi, 1953) and Michigan (Elliston et al*.,* 1977; Day et al*.,* 1977; Dodds, 1980). Many different types of dsRNA viruses have been found to be associated with filamentous fungus *Cryphonectria parasitica* (Murrill) Barr, the causal agent of the devastating chestnut blight disease. The number of dsRNA segments, their relative size, titer and sequence homology as well as the effects on host fungal virulence and morphology vary significantly among the dsRNAs examined (Dodds, 1980; Fulbright et al*.,* 1983; Hiremath et al*.,* 1986; Paul and Fulbright, 1988).

At least four virus families (*Reoviridae*, *Partitiviridae*, *Chrysoviridae* and *Hypoviridae*) are known to infect *C. parasitica* and some confer hypovirulence (Hillman and Suzuki, 2004). Reoviruses are characterized by distinct properties: (i) they have 10-12 dsRNA genomic segments mostly with a monocistronic nature; (ii) virus particles are multi-shelled; (iii) particles serve as viral mRNA synthesis factory.

Cryphonectria parasitica mycoreovirus-1 (CpMYRV-1), *Cryphonectria parasitica mycoreovirus-2* (CpMYRV-2), and *Rosellinia necatrix mycoreovirus-3* (RnMYRV-3) are the members of family *Reoviridae* that infect fungi (Mertens et al., 2004). These viruses also have the ability to persistently attenuate virulence and stably alter complex biological processes upon infection of their fungal hosts (Enebak et al., 1994; Osaki et al., 2002; Wei et al., 2003).

RnMYRV-3 is a newly characterized virus from *Rosellinia necatrix* Prillieux, an ascomyceteous fungus. This virus consisted of 12 segments dsRNA, ranging from ca. 0.41 bp to 2.95 kbp in size. All 12 dsRNA genomic segments of this virus have conserved terminal sequences at both 5'- and 3'-ends. Double-shelled spherical particles approximately 80 nm in diameter are observed in a preparation from the mycelial tissue of the host.

CpMYRV-1 and CpMYRV-2 are reoviruses recently isolated from *C. parasitica*. CpMYRV-2 was recovered from a canker in West Virginia in September 1989, whereas CpMYRV-1 was recovered in September 1982 from a virulent canker approximately 20 miles from where the CpMYRV-2

♥ **Correcponding address:**
Jl. Ir. Sutami 36 A, Kentingan Surakarta, 57126.
Tel. & Fax.: +62-271-637457
e-mail: supyani@mail.uns.ac.id

was recovered, in the same district (MacDonald and Double, unpublished). Both viruses contain 11 segments of dsRNA. CpMYRV-2 contains 11 segments of dsRNA in equal molar amounts ranging from 1 to 5 kb in size, whereas isolate CpMYRV-1 is 0.7 to 5 kb (Enebak et al., 1994).

The three largest dsRNAs of CpMYRV-1 were determined by Hillman et al. (2004). Genome segment 1 (S1) is 4127 bp in size with single ORF codes for 1354 amino acids. The size of S2segment was 3846 bp with single ORF codes for 1238 aa, whereas S3 was 3254 bp in size with single ORF codes for 1065 aa. S1 to S3 of the virus were found to have common terminal sequences (5'GAUCA --- CGCAGUCA3'). Icosahedral particles with a double-layered structure of approximately 80 nm in diameter were purified from the mycelial tissue of the host (Hillman et al., 2004). Regarding CpMYRV2, icosahedral particles approximately 60 nm in diameter were purified from the mycelial tissue of the host (Enebak et al., 1994). Only limited sequence data is available for this virus.

The aims of this research were to determine complete sequences of the three shortest segments (S9-S11) of CpMYRV1, and to analyze the relationship of the dsRNA segments to the segments of other closely related reoviruses.

MATERIALS AND METHODS

Fungal strain and maintenance

Strain 9B21 (hypovirulent, CpMYRV1 infected) of *C. parasitica* was a generous gift from Dr. William MacDonald (West Virginia University, Morgantown). A single conidial, virus-free isolate was obtained from the 9B21 strain as described by Hillman et al. (2004). Cultures were maintained on regeneration media at 4^{o}C in a refrigerator.

CpMYRV1 dsRNA Extraction

Extraction of CpMYRV1 dsRNA was done as described by Hillman et al. (1990) and Morris and Dodds (1979) with several modification. Fungal tissue (7.5 g) harvested from a 10-day-old culture of *C. parasitica* strain 9B21 in 300 mL potato dextrose broth was used as a material source. The dsRNA purification was performed using CC-41 cellulose according to the method of Morris and Dodds (1979). Double stranded RNA was analyzed by electrophoresis through 0.7 % agarose gel cast in TAE buffer. Gels were stained with ethidium bromide and photographed.

CpMYRV1 dsRNA Segments Isolation

For gene cloning purpose, each dsRNA segment was isolated. Total of 8, 2 μg dsRNA (in 200 μL) was run in 10% SDS-PAGE followed by slicing each band using a razor. The dsRNA segments were purified from the gel by electrophoresis using purification buffer in autoclaved cellophane tube for 2 hours at 200 volts. The samples then subjected to phenol chloroform extraction followed by ethanol precipitation, and the pellets were diluted in 20 μL purified H_2O.

cDNA synthesis

Double strands complementary DNA (cDNA) were synthesized as described by Gubler and Hoffman (1983) using a commercial kit (TimeSaver cDNA Synthesis Kit, Pharmacia). The procedure of cDNA synthesis is outlined schematically in Figure 1. Two micro grams dsRNA of each segment (of the three shortest segments) were used as templates. The product was subjected to phenol/chloroform extraction and purified using GeneClean II Kit (BIO 101) according to kit protocol. The cDNA was eluted in 15 μL purified H_2O.

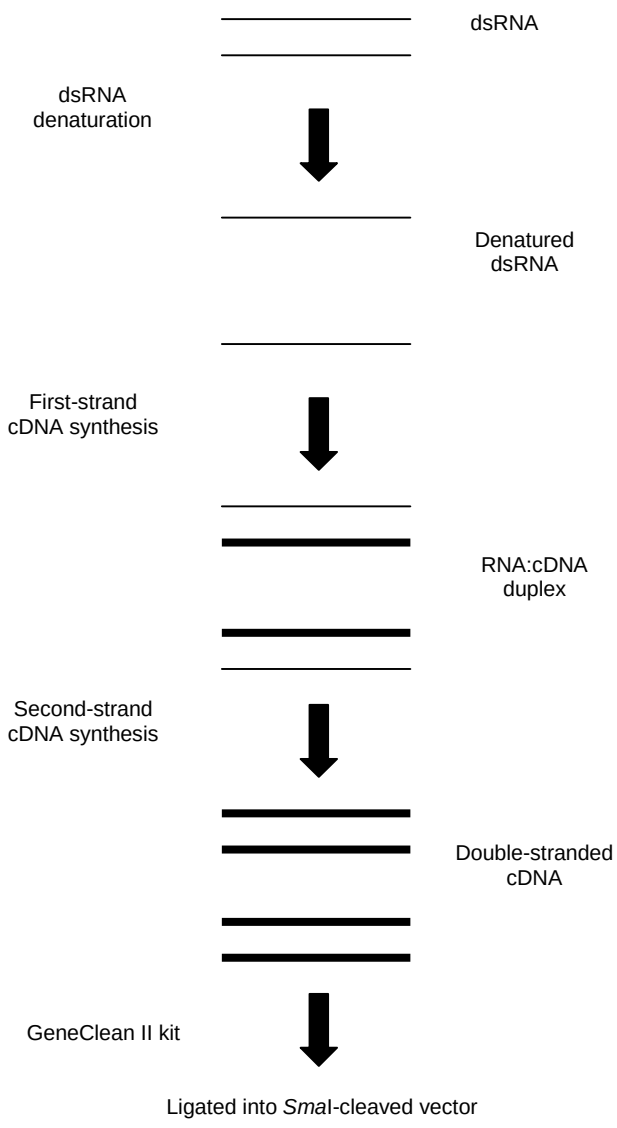

Figure 1. The procedure of double-stranded cDNA synthesis.

Ligation and transformation

The blunt-ended cDNA were ligated into *Sma*I-digested pBluescript II SK (+/-) (Stratagene). Recombinant plasmids were transformed into competent *E. coli* strain DH5α. The recombinant were grown on 500 μL LB medium and spread on LB plate containing 50 ug/mL ampicillin, 50 μg/mL 5-bromo-4-chloro-3-indolyl-B-D-galactoside (X-gal, Takara) and 0.1 mM isopropyl-B-D- thiogalactopyranoside (IPTG, MBI). Selected recombinant plasmids were over night single cultured in LB containing 50 ug/mL ampicillin. Recombinant plasmids then purified from *E. coli* using QIAprep Spin Miniprep Kit (QIAGEN) according to the kit protocol.

Sequencing and sequence analysis

Sequencing reaction was prepared using Dye Terminator sequencing kit supplied by Applied Biosystems Division. Plasmids (250 ng) containing the inserted genes were subjected to PCR reaction containing primer and premix supplied by kit and amplified. Primers used in this reaction were general primers M13 F (for 3' direction) and M13 R (for 5' direction). PCR products were precipitated with ethanol and resolved in sequence dye and run on 50% polyacrylamide gel (super reading DNA sequence solution, Toyobo) diluted in TBE buffer with ABI Prism 377 Genetic Analyzer Sequence (Applied Biosystems). Sequence fragment were assembled with AutoAssembler™ 2.0 ABI Prism (Perkin Elmer) and analyzed using Genetic-Mac 10.0 (Software Development). Homology search was performed with the BLAST suite of programs from National Center for Biotechnology Information (NCBI).

Terminal sequence determination

To cover until both termini of each segment, a 5'-rapid amplification of cDNA ends (5'-RACE) method as described by Polashock and Hillman (1994) and Hillman et al. (2004) was adopted. The cDNA segments were synthesized by RT reaction with virus specific primers (Table 1), tailed with oligo (dC), and amplified by PCR with nested virus-specific primers (Table 1), and abridged anchor primer. The PCR products were separated by agarose gel electrophoresis and purified by GeneElute™ Agarose Spin Columns (Sigma). The purified cDNA were ligated to pGEM®-T Easy Vector (Promega) and transformed to Competent High DH5α (Toyobo). The purified recombinant plasmids were sequenced according to the above method.

Synthesis of full-length cDNA

To construct a full-length cDNA clone of each segment, a set of primers pair from each terminal were designated and used in RT-PCR amplification. The full-length cDNAs were ligated to pGEM®-T Easy Vector (Promega), and transformed to Competent High DH5α (Toyobo).

RESULTS AND DISCUSSION

CpMYRV1 dsRNA examination

Several single conidial isolates derived from isolate 9B21 of *C. parasitica* were analyzed for the presence of dsRNA. Isolates that was similar in morphology to isolate 9B21 contained dsRNA, whereas those isolates that did not resemble isolate 9B21 were dsRNA-free. All 11 dsRNA segments found in isolate 9B21 were present in each of the dsRNA-containing single conidial isolates (Figure 2). Thus the dsRNA segments were transmitted to next generations in an all-or-none fashion. The genomic segments of CpMYRV1 were termed S1 to S11 with increasing order of mobilities in acrylamide gel as is for plant reoviruses. Order of migration of the segments was the same between agarose and acrylamide gels, although acrylamide gel gave better resolution.

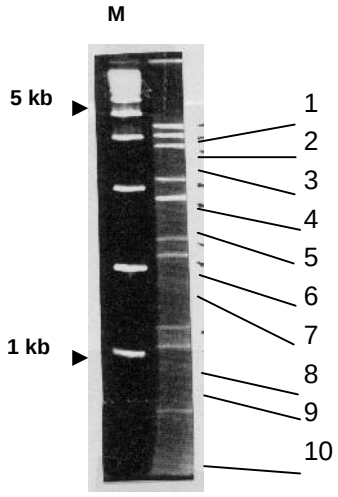

Figure 2. Polyacrylamide gel electrophoresis of the dsRNA extracted from mycelium of *C. parasitica* isolate 9B21. Segment 5 and 6 are similar in size and co-migrate on the gel. M, marker: 1 kb DNA Ladder.

Cloning of CpMYRV1 dsRNA

The three largest of the 11 genomic dsRNA segments (S1-S3) of CpMYRV1 were sequenced earlier by Hillman et al. (2004). Thus, in this study the rest of smaller segments were targeted. From cDNA libraries derived from dsRNA of the three shortest segments, cDNA clones with relatively large inserts of over 400 bp were randomly chosen. By assembling sequences of cDNA clones (approximately 50 clones to each gel-isolated segments), three contigs were obtained. Three cDNA clones representing each of the 3 contigs were chosen, and used for the next analysis.

Terminal structure

The classic 5'RACE was used to determine the terminal sequence of S9 to S11, as used for S1 and

S3 of CpMYRV1. Most 5' RACE clones of plus strands of genome segments were 5'-GATCA----, while some lacked several most 5' terminal nucleotides. The 5' sequence, GATCA was confirmed by sequencing RLM-RACE clones (data not shown). The minus strands of genome segments were also determined with the classic RACE analysis to be 5'-TGACTGC---. The 5' pentamer, 5'GAUCA--- and 3' octomer, ---CGCAGUCA3' are conserved among the three largest segments of CpMYRV1 (Hillman et al., 2004). However, the eighth residue C from the 3 end was not shared in S9 and S11 as summarized in Table 1. As reported by Hillman et al. (2004), the 3' end is similar to those of RnMYRV3 (---UGCAGAC3') and coltiviruses (--a/uUGyAGUg/c3'), while 5' terminus is similar to that of the genus *Oryzavirus* (5'GAU---) or coltiviruses (5' GA---).

Table 1. Primers couples used for 5' RACE and their deoxyoligo-nucleotides.

Segment	Primer name and position			
	5'- direction (→)		3'- direction (←)	
9	9B9-3 >	9B95	9B91 >	9B90
	(172-189 nt)	(90-107 nt)	(751-769 nt)	(807-826 nt)
			9B90 >	9B9-4
			(807-826 nt)	(926-944 nt)
10	9B10-3 > >	9B10-4	9B10-2 >	9B10-1
	(269-287 nt)	(191-208 nt)	(832-850 nt)	(871-890 nt)
11	9B83 > >	9B82	9B100 >	9B101
	(247-264 nt)	(181-199 nt)	(506-526 nt)	(571-589 nt)

Primer name	Deoxyoligonucleotides						
9B9-3	TCC	TGG	ACG	TGG	AAT	GAC	
9B95	CCA	ACT	ACG	GTG	AAA	GAC	
9B91	GCC	GAT	CAG	GAA	GCT	TGG	G
9B90	CAA	CGC	ACG	ACC	TTT	CAC	CG
9B9-4	CCC	AAT	CCG	TTT	CTG	GAT	C
9B10-3	GCA	GAA	TGA	CAC	CAA	CGC	G
9B10-4	AGC	GTC	AAG	GGG	CGG	GTC	
9B10-2	GAT	AAT	GCC	CCC	TGC	GGA	C
9B10-1	CGC	CTA	CAT	CCT	CAT	CAT	AG
9B83	CCC	CGA	GAA	ATT	GAG	TCG	C
9B82	GCG	ACT	CAA	TTT	CTC	GGG	G
9B100	CGA	TTA	CTG	CGC	CTG	ATG	AG
9B101	GCA	AGT	GCA	CCG	GCT	TTA	G

Sequences of CpMYRV1 S9-S11

The complete sequences of S9 to S11 were obtained from cDNA clones and RACE clones. As summarized in Table 2 the lengths of the CpMYRV1 genome segments range 1072 bp (S9) to 732 bp (S11), which are in accord with the agarose gel electrophoresis profile (Figure 2). S9 and S10 possess single large open reading frames (ORFs) spanning over 90% of their entire segment sizes. S11 is only an exception and has a relatively small ORF which corresponds to less than 50% of the entire segment sequence. Small ORFs comprising more than 300 nucleotides besides the large ORFs are found on the minus strand of S9, while it remains to be answered whether those small ORFs are expressed.

Table 2. Size of dsRNA segments S9, S10, and S11 of CpMYRV1 and their putative encoded proteins.

Segment	Total nucleotides (bp)	Putative encoded proteins
S9	1072	32,880 kDa (297 aa)
S10	975	27,758 kDa (247 aa)
S11	732	11,461 kDa (101 aa)

As is the case with other reoviruses, each segment commonly consists of 5' conserved penta-nucleotide, (5'GAUCA--), and 3' hepta-nucleotide (---GCAGUCA3') conserved sequence (Table 3). Nucleotides at positions 6 to 8 from the 5' are semi-conserved (a/u-a/u-a/u). As reported for CpMYRV1 S1 to S3 (Hillman et al., 2004), no typical inverted repeat structure, which is found in plant reoviruses (Xu et al., 1989; Kudo et al., 1991), is formed by the terminal sequences adjacent to or the conserved terminal residues. The conserved terminal sequences and the number of genome segments are among very important criterion of reovirus taxonomy (Mertens et al., 2000). The tentative *mycoreovirus* genus is different from other genera in that the genus contains species with different numbers of genome segments: 11 for CpMYRV1 and CpMYRV2, and 12 segments for RnMYRV3. A hint to the problem of the genome segment number may come from the evolutionary scenario proposed by Hillman et al. (2004) that mycoreoviruses and coltiviruses might have diverged from a progenitor virus infecting an Acari arthropod species and having 12 genome segments. During the course of evolution the *C. parasitica* mycoreoviruses may have lost one of the segments which were dispensable for virus life cycle in the fungal host.

Table 3. The 5'- and 3'-non-coding regions of dsRNA segments S9, S10, and S11 of CpMYRV1.

Seg-ment	5' Non-coding region		3' Non-coding region	
	Size (bp)	Terminal sequences	Terminal sequences	Size (bp)
S9	82	5'-GAUCAUUUGA…	…AUUGCAGUCA-3'	91
S10	74	5'-GAUCAAAAAT…	…UACGCAGUCA-3'	160
S11	300	5'-GAUCAUAAUA…	…AUUGCAGUCA-3'	126

Interviral amino acid sequence similarities and their sequence motifs

Osaki et al. (2002), Wei et al. (2003, 2004) and Hillman et al. (2004) noted the relatedness of four viruses: two fungus-infecting viruses, CpMYRV1 and RnMYRV3 and two members of the genus *Coltivirus* (CTFV and EyaV). The proteins encoded by the different dsRNA segments were designated P_N, where N refers to the number of the RNA segment

```
CpMYRV1: 5 VVGSNIYDTTLLMTRKGQN-GAPDEVIPRPGFLTLLLNDIDSLRTRVELHNLIDNLN-LA 62
 + +N T+++ R +N E+I +P FLTLL + E L NL+

RnMYRV3: 88 LTSNNFATATIVVERTNRNQNNRREIIAKPAFLTLLFSHKADALKNPEFRGL--NLDAFY 261


CpMYRV1: 63 TNEDYVKFAEYRTLFSQTTDMIRLAYTNGQPAVQTRATDSRTGS--VFYANTLTGDKAGN 120
 + +D + E RT + + Y+ G P + R G V + L+G

RnMYRV3: 262 STKDMMDTNEVRTRLGSGNGL-KFHYSKGVPTISQRGEAPVNGKMIVVWTPVLSGSSLHV 438


CpMYRV1: 121 LFRLLAPIAYRYLDVGLPRLFSYIHAQIGTTPAFRYNFDIQPIIKLAITNEPLDYGEWIG 180
 L R++ + Y+ G+P +Y FD + N DY G

RnMYRV3: 439 LNRVILSMYSEYVINGIPEFLAYAKTTYNAQTLNSVVFDSLTAARFIAMNVDFDYTNLFG 618


CpMYRV1: 181 ---QEG---------IHELERNVMIILSCSNITILAVLSIVGLGVGSHIMT 219
 +G I E ERN ++ ++ S TI + GLG+ +H+M+

RnMYRV3: 619 VAPADGEPDPTDNLTIEEAERNYLLAVAPSQGTIAVAIQCGGLGL-AHLMS 768
```

Figure 3. The result of homology searching for putative protein encoded by segment 9 using BLAST program.

based on its electrophoretic mobility. Sequence database searches were performed with the newly determined sequences of CpMRYV1 P9 to P11 using the BLAST and FASTA programs. In addition to the previously identified equivalent segments, a BLAST search identified CpMRV1 S9/RnMRV3 S11. Sequence similarities (23% identity, Figure 3), and optimal scores among the four viruses were summarized in Table 4. CpMRV1 S10 or S11 did not yield any significant hit in BLAST or FASTA3 searches. It remains unclear which segments of RnMYRV3 and coltiviruses are counterparts of CpMYRV S10 and S11, which show no significant similarities to known sequences.

Molecular properties of CpMYRV1 are characteristic of reoviruses and strengthen a suggestion made by Enebak et al. (1998), Osaki et al. (2001), and Hillman et al. (2004) that the CpMYRV1-related mycoviruses make up a new genus within the family *Reoviridae*. These facts tempt to make a proposal to the International Committee of Virus Taxonomy (ICTV) that a new genus *mycoreovirus* is added to the family *Reoviridae* that contains CpMYRV1 as its type member, and two other members, *C. parasitica mycoreovirus* 2/C18 (CpMYRV2/C18) and *R. necatrix mycoreovirus* 3/W370 (RnMYRV3/W370). Recently, the proposal was approved, so the family *Reoviridae* now accommodates more than 11 genera with a large number of members infecting fungi, plant, invertebrates and vertebrates.

CONCLUSION

A novel mycovirus (CpMYRV1) was isolated from a hypovirulent strain (9B21) of the chestnut blight fungus, *Cryphonectria parasitica* (Murrill) Barr. The CpMYRV1 genome consists of 11 segments of dsRNA termed S1 to S11. Sequence analyses showed that segments S9, S10, and S11 are 1072 bp, 975 bp, and 732 bp in size with single ORFs coding for 297 aa, 247 aa, and 101 aa, respectively. Genome segments S9 to S11 have common terminal sequences, 5'GAUCA --- GCAGUCA3' which are identical to those of the previously sequenced segments. The deduced amino acid sequence coded by segment 9 showed a similarity to P11 of evolutionarily related *Rosellinia anti-rot virus* (*Reoviridae*), while S10 or S11 exhibited no significant sequence similarities to known sequences. These results support the previous suggestion that CpMYRV1 is a novel virus which belonging to the *Reoviridae*, acts as a hypovirulence factor on fungal host.

ACKNOWLEDGEMENTS

The author is grateful to Dr. William MacDonald (West Virginia University, Morgantown) for the generous gift of Strain 9B21 (hypovirulent, CpMYRV1 infected) of *C. parasitica*.

REFERENCES

Biraghi, A. 1953. Possible active resistance to **Endothia parasitica** in **Castanea sativa.** *Reports of the Congress of International Union of Forestry Research Organization.* Rome 11: 643-645.

Day, P.R., J.A. Dodds, J.E. Elliston, R.A. Jaynes, and S.L. Anagnostakis. 1977. Double-starnded RNA in **Endothia parasitica**. *Phytopathology* 67: 1393-1396.

Dodds, J.A. 1980. Association of type 1 viral-like dsRNA with club-shaped particles in hypovirulent strains of **Endothia parasitica**. *Virology* 197: 1-12.

Elliston, J.E., R.A. Jaynes, P.R. Day, and S.L. Anagnostakis. 1977. A native American hypovirulent strain of **Endothia parasitica**. *Abstracts of Proceedings of American Phytopathological Society*. 4: 83-84.

Enebak, S.A., B.I. Hillman, and W.L. MacDonald. 1994. A hypovirulent **Cryphonectria parasitica** isolate with multiple, genetically unique dsRNA segments. *Molecular Plant-Microbe Interactions* 7 (5): 590-595.

Fulbright, D.W., W.H. Weidlich, F.Z. Haufler, C.S. Thomas, and C.P. Paul. 1983. Chesnut blight and recovering American chestnut trees in Michigan. *Canadian Journal of Botany* 61: 3164-3171.

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

Hillman, B. I. and N. Suzuki. 2004. Viruses in the chestnut blight fungus. *Advanced Virus Research* 63: 423-72.

Hillman, B.I., R. Shapira, and D.L. Nuss. 1990. Hypovirulence-associated suppresion of host functions in **Cryphonectria parasitica** can be partially relieved by high light intensity. *Phytopathology* 80: 950-956.

Hillman, B.I., S. Supyani, H. Kondo, and N. Suzuki. 2004. A Reovirus of the fungus **Cryphonectria parasitica** that is infectious as particles and related to the *Coltivirus* genus of animal pathogens. *Journal of Virology* 78 (2): 898-898.

Hiremath, S.T., B. L'Hostis, S.A. Ghabiral, and R.E. Rhoads. 1986. Terminal structure of hypovirulent-associated dsRNAs in the chestnut blight fungus **Endothia parasitica**. *Nucleic Acid Research* 14: 9877-9896.

Kudo, H., I. Uyeda, and E. Shikata, 1991. Viruses in the *Phytoreovirus* genus of the *Reoviridae* family have the same conserved terminal sequences. Journal of General Virology 72: 2857-2866.

Mertens, P. P. C., B. I. Hillman, and N. Suzuki. 2004. Genus *mycoreovirus*. In: Fauquet, C.M., M.A. Mayo., J. Maniloff., U. Desselbeger, and L.A. Ball. *Virus Taxonomy: Eighth Report of the International Committee for the Taxonomy of Viruses*. San Diego: Academic Press.

Mertens, P.P.C., M. Arella, H. Attoui and 41 other authors. 2000. *Reoviridae*. In: Van Regenmortel, M.H.V., C.M. Fauquet, D.H.L. Bishop, E.B. Carstens, M.K. Estes, S.M. Lemon, J. Maniloff, M.A. mayo, D.J. McGeoch, C.R. Pringle, and R.B. Wickner. (ed.) Virus Taxonomy: Seventh Report of the International Committee for the Taxonomy of Viruses. New York: Academic Press.

Morris, T.J., and J.A. Dodds. 1979. Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. *Phytopathology* 69: 854-858.

Osaki, H., C.Z. Wei, M. Arakawa, T. Iwanami, K. Nomura, N. Matsumoto, and Y. Ohtsu. 2002. Nucleotide sequences of double-stranded segments from hypovirulent strain of he white root rot fungus **Rosellinia necatrix**: Possibly of the first member of the *Reoviridae* from fungus. *Virus Genes* 25 (1): 101-107.

Paul, C.P., and D.W. Fulbright, 1988. Double-stranded RNA molecules from Michigan hypovirulent isolates of **Endothia parasitica** vary in size and sequence homology. *Phytopathology* 78: 751-755.

Polashock, J.J. and B.I. Hillman, 1994. A small motochondrial double-stranded (ds) RNA element associated with a hypovirulent strain of the chestnut blight fungus and ancestrally related to yeast cytoplasmic T and W dsRNAs. *Proceedings of National Academic Science USA*. 91: 8680-8684.

Wei, C. Z., H. Osaki, T. Iwanami, N. Matsumoto, and Y. Ohtsu. 2004. Complete nucleotide sequences of genome segments 1 and 3 of Rosellinia anti-rot virus in the family *Reoviridae*. *Archives of Virology* 149, 773-777.

Wei, C. Z., H. Osaki, T. Iwanami, N. Matsumoto, Y. Ohtsu. 2003. Molecular characterization of dsRNA segments 2 and 5 and electron microscopy of a novel reovirus from hypovirulent isolate, W370, of the plant pathogen **Rosellinia nectrix**. *Journal of General Virology* 84: 2431-2437.

Xu, Z., J.V. Anzola, and C.M. Nalin. 1989. The 3'-terminal sequence of a wound tumor virus transcript can influence conformational and functional properties associated with the 5'-terminus. *Virology* 170: 511-522.