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Brief Report

The nucleotide sequence and genome organization of Magnaporthe oryzae virus 1

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Summary

Magnaporthe oryzae virus 1 (MoV1) found in *Magnaporthe oryzae*, the pathogenic fungus responsible for rice blast, is a small icosahedral virus with a nonsegmented double-stranded RNA genome. The viral genome has two open reading frames (ORF 1 and 2). The deduced amino acid sequences of both ORF 1 and ORF 2 show a significant similarity to those of capsid protein and RdRp, respectively, of members of the family *Totiviridae*. Both a comparison of genome organization and phylogenic analysis have indicated that MoV1 is closely related to some of the totiviruses that infect filamentous fungi. These results suggest that MoV1 belongs to the family *Totiviridae*.

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Note: Nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank databases under the accession No. AB176964.

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Since Hollings [11] reported the presence of three kinds of viruses associated with a die-back disease of cultivated mushroom (*Agaricus bisporus*), many other fungal viruses have been described in all major groups of fungi [3, 12]. Most of these mycoviruses are classified into seven families based on the nature of their genome and capsid structures, although a subset of these viruses remains unclassified. The isometric double-stranded (ds) RNA mycoviruses are classified into two families, *Totiviridae* and *Partitiviridae*. Totiviruses have a nonsegmented genome, while partitiviruses have segmented genomes. Although a large number of mycoviruses have been reported to be cryptic [9, 10], some of them may be associated with debilitation of their fungal hosts, for example, Cryphonectria hypovirus 1 (CHV-1) infecting *Cryphonectria parasitica* [17]. Virus-mediated attenuation of virulence is one of the bases of methods used for the biological control of plant pathogenic fungi.

Magnaporthe oryzae (formerly known as *Magnaporthe grisea*; anamorph: *Pylicularia oryzae*) [5] is a causal agent of rice blast, which is the most devastating rice disease throughout the world. Viruses have been reported to be present in mycelia of

M. oryzae, [7, 23, 24]. Yamashita et al. [24] purified polyhedral virus particles with a diameter of about 36 nm from infected mycelia. It was the first virus isolated from plant pathogenic fungus. They also reported that the viruses are located mainly in the vacuole of the mycelium [25]. Hunst et al. [14] showed the variation of viral dsRNA from isolates of *M. oryzae* and demonstrated that most of them were transmitted through conidia. They noted that there was no conclusive evidence for a relationship between the existence of dsRNA and the fungal pathogenicity.

In this study, the complete nucleotide sequence and genome organization of MoV1 RNA were determined and compared with a selection of other dsRNA viruses.

MoV1 virions were purified from the virus-infected *M. oryzae* isolate TH65-105 using the procedure of Yamashita et al. [24]. Nucleic acid was extracted from purified virions. The cDNA from MoV1 RNA was synthesized using the "TimeSaver cDNA Synthesis Kit" (Amersham Biosciences) and inserted into a plasmid vector, pUC118. Both terminal sequences of MoV1 RNA were determined by 3′-RACE of both strands using the procedure of Weng and Xiong [22]. The sequences of ten cDNA clones, including the terminal sequences of each, were confirmed.

Both strands of the inserts from selected cDNA clones were sequenced. Sequence analysis was performed with an Applied Biosystems Model 3100 automated sequencer, using a DNA sequencing kit (Applied Biosystems).

Multiple alignments for sequence comparison with other viruses were performed using CLUSTAL W [21] as incorporated into DNASIS version 3.6 for Macintosh (Hitachi Software). GenBank searches were carried out using the BLAST programs [1]. Phylogenic trees were constructed using the neighbor-joining method in MOLPHY 2.3. Phylogenic support for the branching in the genealogies was investigated by bootstrapping the data 1000 times.

Analysis of the determined sequence of MoV1 RNA (5359 nt) revealed the existence of two ORFs (Fig. 1). The molecular masses of the proteins encoded by ORF 1 and ORF 2 were calculated to be 77 and 91 kDa, respectively.

Homology search results showed that the predicted amino acid sequences of ORF 1 and 2 were very similar to those of capsid protein (CP) and RNA-directed RNA polymerase (RdRp), respectively, which are encoded by the viruses belonging to the family *Totiviridae*. Sequence identities of 11–36% were observed in both ORFs. The amino acid alignment of RdRp of MoV1 and other selected totiviruses showed that the eight RdRp motifs [2] were highly conserved (Fig. 2).

At present, the family *Totiviridae* is divided into three genera, *Totivirus*, *Giardiavirus* and *Leishmaniavirus* [6]. Phylogenetic analysis has suggested that the genus *Totivirus* forms two separate clusters, the viruses that infect fungi (F group) and the viruses that infect yeast and smut fungi (YS group) (Fig. 3). Each of these virus groups has a different mechanism for the expression of RdRp. It is suggested that RdRp is translated by an internal initiation mechanism in HvV190S and other known totiviruses included in the F group [13]. In contrast, totiviruses included in the YS group, such as Saccharomyces cerevisiae virus L-A (ScV-L-A), express their RdRp as CP-RdRp fusion proteins by ribosomal frameshifting [8]. The Ustilago maydis virus H1 (UmV-H1) genome is translated as a polyprotein, which is afterward presumably processed autocatalytically by a viral protease [15]. It has been noted that those two groups should be classified into two new genera based on their phy-

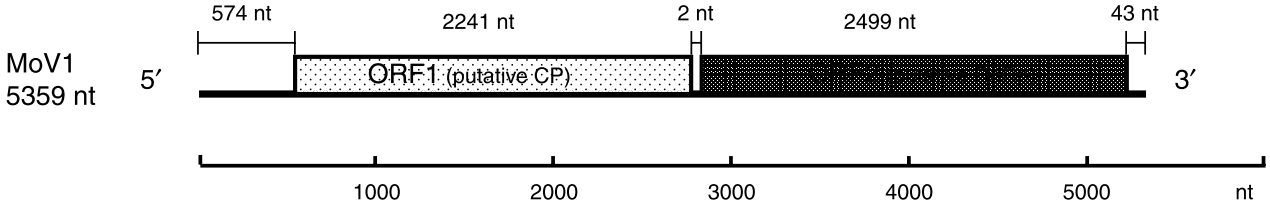

Fig. 1. The genome organization of Magnaporthe oryzae virus 1 (MoV1). The number of nucleotides is indicated by the scale at the bottom of the figure

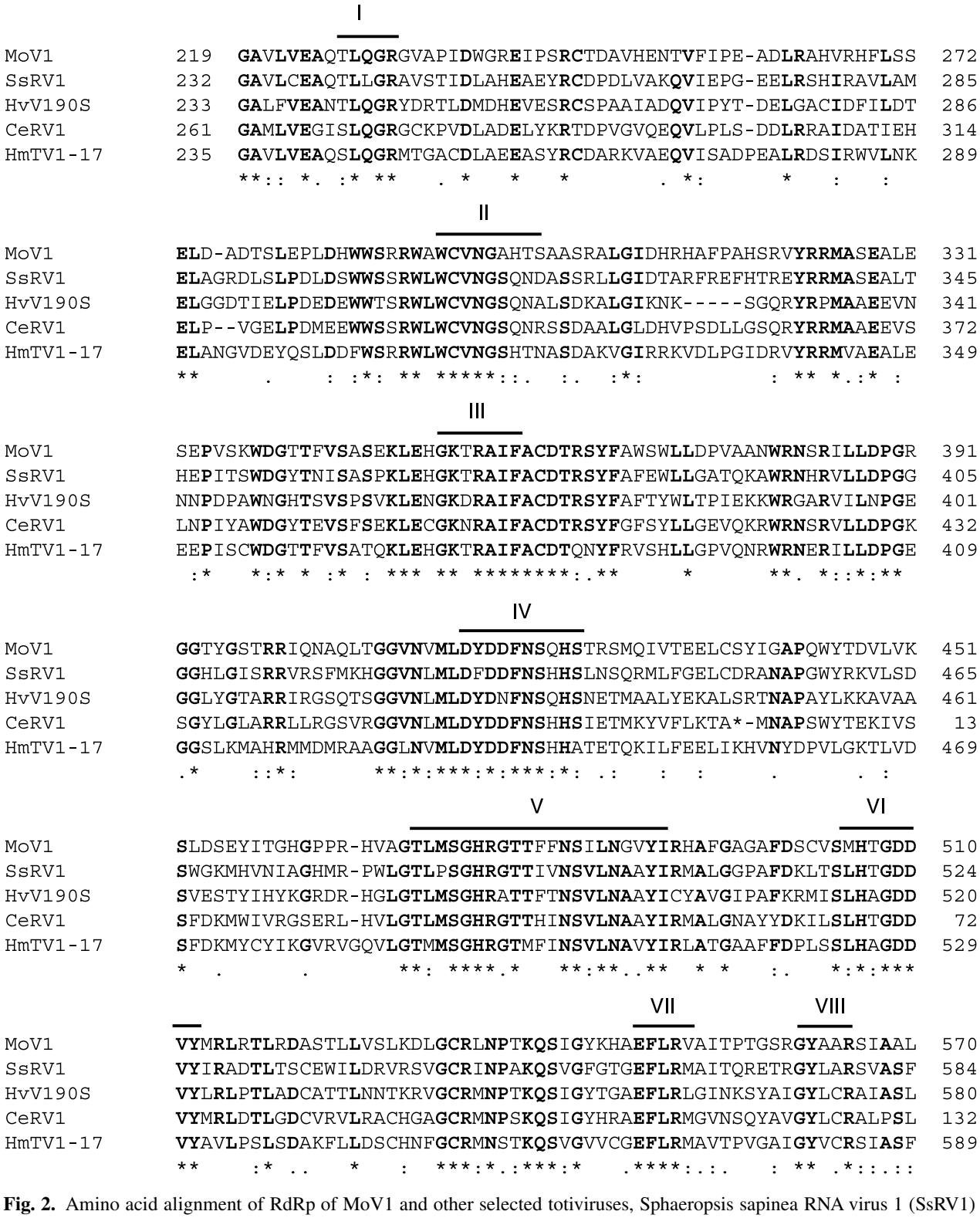

Fig. 2. Amino acid alignment of RdRp of MoV1 and other selected totiviruses, Sphaeropsis sapinea RNA virus 1 (SsRV1) [19], Chalara elegans RNA virus 1 (CeRV1) [18], Helminthosporium victoriae virus 190S (HvV190S) [13] and Helicobasidium mompa Totivirus 1–17 (HmTV1–17) [16]. The conserved motifs (I–VIII) in RdRp of totivirus according to Bruenn [2] are indicated. Identical residues are shown by asterisks, and similar residues by colons. Numbers on the top and bottom refer to the amino acid positions in the ORF and the distances from the protein termini, respectively. Amino acids that are conserved in at least in four sequences are marked in bold type

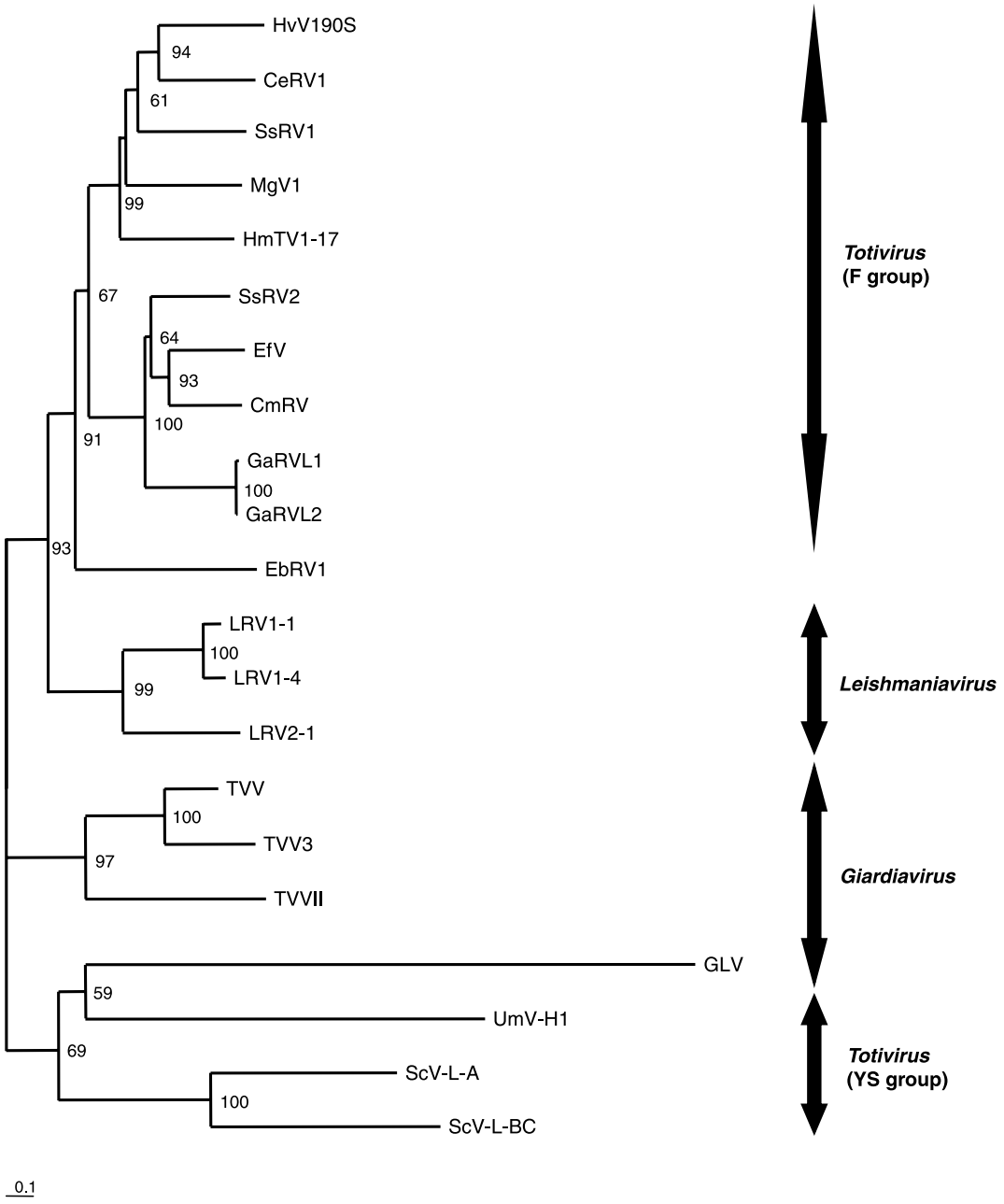

Fig. 3. Phylogenic tree of members in the family *Totiviridae* based upon a CLUSTAL W multiple alignment of the amino acid sequences of their conserved RdRp motifs. The scale bar indicates 0.1 amino acid substitutions per locus. HvV190S, Helminthosporium victoriae virus 190S [U41345]; CeRV1, Chalara elegans RNA virus 1 [AY561500]; SsRV1, Sphaeropsis sapinea RNA virus 1 [AF038665]; HmTV1–17, Helicobasidium mompa Totivirus 1–17 [AB085814]; SsRV2, Sphaeropsis sapinea RNA virus 2 [AF039080]; EfV, Epichloe festucae virus [AM261427]; CmRV, Coniothyrium minitans RNA virus [AF527633]; GaRVL1, Gremmeniella abietina RNA virus L1 [AF337175]; GaRVL2, Gremmeniella abietina RNA virus L2 [AY615210]; EbRV1, Eimeria brunetti RNA virus 1 [AF356189]; LRV1-1, Leishmania RNA virus 1-1 [M92355]; LRV1-4, Leishmania RNA virus 1-4 [U01899]; LRV2-1, Leishmania RNA virus 2-1 [U32108]; TVV, Trichomonas vaginalis virus [U08999]; TVV3, Trichomonas vaginalis virus 3 [AF325840]; TVVII, Trichomonas vaginalis virus II [AF127178]; GLV, Giardia lambria virus [L13218]; UmV-H1, Ustilago maydis virus H1 [V01059]; ScV-L-A, Saccharomyces cerevisiae virus L-A [U01060]; ScV-L-BC, Saccharomyces cerevisiae virus L-BC [J04692]

logenetic relationship, differences in genomic organization and their hosts [4, 18, 20].

The two ORFs of MoV1 are completely separate, which is a characteristic of this virus, while in other F group totiviruses, there are short overlaps between ORFs of CP and RdRp. The expression mechanism of RdRp of MoV1 has not yet been clarified, but it appears that the CP-RdRp fusion protein should not be made. In addition, MoV1 was shown to be closely related to the F group totiviruses by phylogenetic analysis. In conclusion, MoV1 should be classified as a new member of the genus *Totivirus* F group.

Further investigation is required to elucidate the function of MoV1 in rice blast disease. The construction of an infectious full-length cDNA clone of MoV1 will make it possible to characterize viral effects on the host as well as to investigate the possible utility of MoV1 as a biological control agent against rice blast disease.

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