Complete nucleotide sequence of a novel botourmiavirus from the rice blast fungus *Magnaporthe oryzae* isolate SH05

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Abstract

A novel mycovirus with the proposed name “*Magnaporthe oryzae* botourmiavirus 9” (MoBV9) was found in the rice blast fungus *Magnaporthe oryzae* isolate SH05. The virus has a positive single-stranded RNA genome of 2,812 nucleotides and contains a single open reading frame predicted to encode an RNA-dependent RNA polymerase that is closely related to those of some unclassified viruses of the family *Botourmiaviridae*, including Plasmopara viticola lesion associated ourmiavirus 44, Plasmopara viticola lesion associated ourmia-like virus 47, and Cladosporium uredinicola ourmiavirus 1. Genome sequence comparisons and phylogenetic analysis supported the notion that MoBV9 is a new member of the family *Botourmiaviridae*.

*Botourmiaviridae* is a newly established virus family related to *Narnaviridae*, *Mitoviridae*, and *Leviviridae*, according to current taxonomic information from the International Committee on Taxonomy of Viruses (ICTV, Virus Taxonomy: 2019 Release). The family *Botourmiaviridae* includes four approved genera, *Botoulivirus*, *Magoulivirus*, *Scleroulivirus* and *Ourmiavirus*. Members of the genus *Ourmiavirus* are plant viruses with a bacilliform virion structure whose genome usually contains three (+) ssRNA segments, encoding an RNA-dependent RNA polymerase (RdRp), a coat protein (CP), and a movement protein (MP), respectively [6]. Since the MP and CP of ourmiaviruses show significant similarity to those of other plant viruses, including tombusviruses and sobemoviruses, it has been proposed that ourmiavirus might have evolved by reassortment of genomic segments from viruses infecting fungi and plants [15]. Members of the genera *Botoulivirus*, *Magoulivirus*, and *Scleroulivirus* are mycoviruses. Similar to narnaviruses and mitoviruses, only one (+) ssRNA segment, encoding the RdRp, has been identified in all of these ourmia-like mycoviruses, and this segment has been demonstrated to be sufficient for replication, infection, and transmission [20]. Ourmia-like mycovirus-associated satellite-like RNAs have also been identified in *Magnaporthe oryzae*, but these are not essential for virus replication [14].

The filamentous fungus *M. oryzae* (teleomorph) (Hebert) Barr (anamorph: *Pyricularia oryzae*) [2] causes rice blast disease, which is one of the most serious diseases of cultivated rice worldwide, resulting in approximately 30% annual yield loss [16]. Recently, a number of mycoviruses from different families have been identified in *M. oryzae*. *Magnaporthe oryzae* viruses 1, 2 and 3 (MoV1, MoV2, and MoV3) are dsRNA viruses belonging to the family *Totiviridae* [12, 17, 21], *Magnaporthe oryzae* chrysovirus 1 (MoCV1) is a dsRNA virus belonging to the family *Chrysoviridae* [4, 18, 19], *Magnaporthe oryzae* partitivirus 1 (MoPV1) is a dsRNA virus belonging to the family *Partitiviridae* [3], *Magnaporthe oryzae* narnavirus virus 1 (MoNV1) is a (+) ssRNA virus belonging to the family *Narnaviridae* [10], (+) ssRNA viruses belonging to the family *Botourmiaviridae* also have been identified in *M. oryzae*, including *Magnaporthe oryzae* ourmia-like viruses 1 and 4 (MOLV1 and MOLV4), *Pyricularia oryzae* ourmia-like viruses 1, 2, and 3 (PoOLV1, PoOLV2

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and PoOLV3), and Magnaporthe oryzae botourmiaviruses 5, 6, and 7 (MoBV5, MoBV6 and MoBV7) [5, 9, 11, 14].

In this study, we report a novel mycovirus of the family Botourmiaviridae in M. oryzae strain SH05. Since eight viruses of the family Botourmiaviridae have been reported in M. oryzae, this virus was tentatively named “Magnaporthe oryzae botourmiavirus 9” (MoBV9).

M. oryzae strain SH05 was isolated from a lesion of a rice neck-panicle collected in Fujian province, China, in 2014, stored on rice stem nodes at –20 °C, and cultured on potato dextrose agar at 28 °C. dsRNA was extracted by the CF-11 cellulose chromatography method [13], and the 2.8-kb viral genomic dsRNA of MoBV9 was extracted from an agarose gel after electrophoresis, purified using an Agarose Gel DNA Purification Kit 2.0 (Takara), and treated with DNase I and S1 nuclease to eliminate contaminating DNA and ssRNA. cDNA was synthesized using a tagged random primer (5′-CGATCG ATCATGATGCAATGCNNNNN-3′) and amplified using a primer recognizing the tag sequence (5′-CGATCGATC ATGATGCAATGC-3′). The amplified cDNA products were cloned into the vector pMD19-T (Takara) and introduced by transformation into Escherichia coli strain Top10 for sequencing. To fill the gaps, the sequence data that were obtained were used to design dsRNA-specific primers, which were then used for RT-PCR. In order to determine the terminal sequences of the dsRNA, cDNA amplification of the 5′ and 3′ ends was performed using a ligase-mediated terminal amplification method as described previously [3, 10]. In both orientations, every base was determined by sequencing at least three independent overlapping clones. The complete nucleotide sequence of the MoBV9 genome has been deposited in the GenBank database with accession number MT995746.1. The amino acid (aa) sequence of the putative RdRp of MoBV9 was aligned with other virus RdRp sequences using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). RNA secondary structures of the termini of MoBV9 were predicted using the MFOLD web server (http://www.unafold.org/RNA_form. php) [22]. Conserved domains were identified using the NCBI Conserved Domain Database (CDD) (https://www.ncbi.nlm.nih.gov/cdd). On the basis of the aligned sequences, a phylogenetic tree was constructed by the neighbor-joining method using MEGA version 6.0 [8].

Sequence properties

dsRNA extraction and electrophoresis analysis showed that only one 2.8-kb dsRNA, representing the genome of MoBV9, could be detected in M. oryzae strain SH05 (Fig. 1A). The full-length nucleotide sequence of MoBV9 was determined and found to be 2,812 nucleotides (nt) long with a GC content of 54.1%. The 5′ untranslated region (UTR) is 179 nt long, and the 3′ UTR is 664 nt long, ending with a 9-nt poly (A) tail (Fig. 1B).

Using the standard genetic code, the MoBV9 genome was predicted to contain a single large open reading frame (ORF) on its positive strand, putatively encoding a 659-amino-acid (aa) protein with a molecular mass of 73.2 kDa (Fig. 1). A sequence search using BLASTp suggested that this 73.2-kDa protein is closely related to the RdRps of some unclassified viruses of the family Botourmiaviridae, including Plasmopara viticola lesion associated ourmia-like virus 44 (GenBank accession number QGY72574.1; identity, 62.6%; query coverage, 96%; e-value, 0), Plasmopara viticola lesion associated ourmia-like virus 47 (GenBank accession number QGY72577.1; identity, 60.4%; query coverage, 94%; e-value, 0), and Cladosporium uredinicola ourmiavirus 1 (GenBank accession number QDB75001.1; identity, 50.9%; query coverage, 97%; e-value, 0). A multiple aa sequence alignment showed that this 73.2-kDa protein contains eight typical
Fig. 2  Multiple alignment of the amino acid (aa) sequences of RdRPs encoded by MoBV9 and previously identified viruses of the family Botourmiaviridae from *M. oryzae* (MOLV1, MOLV4, PoOLV1, PoOLV2, PoOLV3, MoBV5, MoBV6, MoBV7). The eight conserved RdRP motifs are indicated by the Roman numerals I to VIII. The asterisks signify identical amino acid residues, colons signify highly conserved residues, and single dots signify less-conserved but related residues.

Fig. 3  Potential RNA secondary structures in the 5′- and 3′-terminal sequences of MoBV9. Stem-loop structures were predicted in the 5′ and 3′ termini of the MoBV9 genome, with ΔG values of −17.85 and −17.40 kcal/mol, respectively. Short lines in different colors indicate hydrogen bonds between different base pairs (red, G-C pairs; purple, A-U pairs; green, G-U pairs).

Conserved motifs of the RdRPs of (+) ssRNA viruses, including the highly conserved GDD motif (motif VI) associated with the catalytic site (Fig. 2) [7].

The potential terminal secondary structures of the 5′- and 3′-terminal regions of the MoBV9 genome were predicted using the MFold web server. The 5′-terminal sequence (nt positions 1–52) and the 3′-terminal sequence (nt positions 2756–2812) of MoBV9 could be folded into terminal stable stem-loop structures with ΔG values of −17.85 and −17.40 kcal/mol, respectively (Fig. 3). The typical terminal
The families Botourmiaviridae, Narnaviridae, and Mitoviridae were used as outgroups. The phylogenetic tree was constructed by the neighbor-joining method using the program MEGA 6.0. Bootstrap values (1000 replicates) are shown at the nodes, and the scale bar (0.1) corresponds to the genetic distance. The RdRp sequences were obtained from the GenBank database, and the accession numbers are shown before the taxon names. The position of MoBV9 is indicated by a red star, and the other eight viruses from M. oryzae belonging to the family Botourmiaviridae are indicated by blue stars.

To analyze the phylogenetic position of MoBV9, a molecular phylogenetic tree was constructed using aa sequences of the RdRp regions of MoBV9 and 73 other selected viruses of the families Botourmiaviridae, Narnaviridae, Mitoviridae, and Leviviridae. As shown in Fig. 4, the neighbor-joining tree strongly suggested that MoBV9 is a new member of the family Botourmiaviridae (Fig. 4).

Fig. 4 Phylogenetic analysis of the RdRp gene of MoBV9 and related viruses of the family Botourmiaviridae. Viruses of the families Narnaviridae, Mitoviridae, and Leviviridae were used as outgroups. The phylogenetic tree was constructed by the neighbor-joining method using the program MEGA 6.0. Bootstrap values (1000 replicates) are shown at the nodes, and the scale bar (0.1) corresponds to the genetic distance. The RdRp sequences were obtained from the GenBank database, and the accession numbers are shown before the taxon names. The position of MoBV9 is indicated by a red star, and the other eight viruses from M. oryzae belonging to the family Botourmiaviridae are indicated by blue stars.
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