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

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Abstract

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

Introduction

Botourmiaviridae is a newly established virus family which relates to *Narnaviridae*, *Mitoviridae* and *Leviviridae*, according to current taxonomic information from 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 contain three (+) ssRNA segments, encoding RNA-dependent RNA polymerase (RdRp), coat protein (CP), and movement protein (MP), respectively [6]. Since the MP and CP of ourmiaviruses showed significant similarity to other plant viruses, including tombusviruses and sobemovirus, it was 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 RdRp has been identified in all of these ourmia-like mycoviruses, and one (+) ssRNA segment was demonstrated to be sufficient for replication, infection and transmission [20]. Ourmia-like mycovirus associated satellite-like RNAs also have been recognized in *Magnaporthe oryzae*, which are not essential for virus replication [14].

The filamentous fungus *M. oryzae* (teleomorph) (Herbert) Barr (anamorph: *Pyricularia oryzae*) [2] causes the rice blast disease, which is one of the most serious diseases of cultivated rice worldwide, resulting an approximately 30% annual yield loss [16]. Recently, a number of mycoviruses from different families have been identified in *M. oryzae*. *Magnaporthe oryzae* virus 1, 2 and 3 (MoV1, MoV2 and MoV3) are dsRNA viruses which belong to the family *Totiviridae* [12, 17, 21]. *Magnaporthe oryzae* chrysovirus 1 (MoCV1) is a dsRNA virus belong to the family *Chrysoviridae* [4, 18, 19], *Magnaporthe oryzae* partitivirus 1 (MoPV1) is a dsRNA virus which belong to the family *Partitiviridae* [3]. *Magnaporthe oryzae* virus A (MoVA) is an unclassified (+) ssRNA virus that is related to members of the family *Tombusviridae* [1]. *Magnaporthe oryzae* narnavirus virus 1 (MoNV1) is a (+)ssRNA virus which belong to the family *Narnaviridae* [10]. (+) ssRNA viruses belong to the family *Botourmiaviridae* also have been recognized in *M. oryzae*, including *Magnaporthe oryzae* ourmia-like virus 1 and 4 (MOLV1 and MOLV4), *Pyricularia oryzae* ourmia-like virus 1, 2 and 3 (PoOLV1, PoOLV2 and PoOLV3) *Magnaporthe oryzae* botourmiavirus 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 8 viruses within the family *Botourmiaviridae* from *M. oryzae* have been reported, this virus was temporarily named as Magnaporthe oryzae botourmiavirus 9 (MoBV9).

Materials And Methods

M. oryzae strain SH05 was isolated from a lesion of a rice neck-panicle collected in Fujian province, China, in 2014, the strain was stored on rice stem nodes at -20 °C and cultured on potato dextrose agar at 28 °C. The viral genomic dsRNAs of MoBV9 were extracted by the CF-11 cellulose chromatography method [13], and purified by treated with DNase I and S1 nuclease to eliminate contaminating DNA and ssRNA. cDNAs were synthesized using tagged random primer (5'- CGATCGATCATGATGCAATGCNNNNNN -3') and amplified using primer (5'- CGATCGATCATGATGCAATGC -3'). The amplified cDNA products were cloned into pMD19-T Vector (Takara) and introduced by transformation into *Escherichia coli* strain Top10 for sequencing. To fill the gaps, according to the sequences obtained, dsRNA-specific primers were designed and 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 previously described [3, 10]. In both orientations, every base was determined by sequencing at least three independent overlapping clones. The complete nucleotide sequences of MoBV9 have been deposited in the GenBank databases with accession number MT995746.1. The amino acid (aa) sequence of the putative RdRp of MoBV9 were aligned with other virus RdRp sequences using the clustal omega programs (<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 searched using the NCBI Conserved Domain Database (CDD) (<https://www.ncbi.nlm.nih.gov/cdd>). On the basis of the aligned sequences, phylogenetic tree were constructed by the neighbor-joining method using the MEGA program version 6.0 [8].

Sequence Properties

dsRNA extraction and electrophoresis analysis shows that, only one 2.8-kb dsRNA, which representing the genome of MoBV9, could be detected in *M. oryzae* strain SH05 (Fig. 1A). The full-length nucleotide sequence of MoBV9 was determined, which is 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 658 -amino-acid (aa) protein with a molecular mass of 73.2 kDa (Fig. 1). Sequence search with BLASTP suggested that this 73.2 kDa protein was closely related to the RdRps of some unclassified viruses in the family *Botourmiaviridae*, such as Plasmopara viticola lesion associated ourmia-like virus 44 (GenBank accession number QGY72574.1, with identity of 62.6%, query cover of 96% and e-value of zero), Plasmopara viticola lesion associated ourmia-like virus 47 (GenBank accession number QGY72577.1, with identity of 60.4%, query cover of 94 %

and e-value of zero), and *Cladosporium uredinicola* ourmiavirus 1 (GenBank accession number QDB75001.1, with identity of 50.9 %, query cover of 97 % and e-value of zero). A multiple aa sequence alignment showed that this 73.2 kDa protein contains eight typical conserved motifs of the RDRP of (+) ssRNA viruses, including the highly conserved catalytic related GDD motif (motif VI) (Fig. 2) [7].

The potential terminal secondary structures of the 5' and 3'-terminal regions of MoBV9 were predicted via 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 stem-loop structures are very common in *Narnaviridae*, *Mitoviridae* and ourmiavirus, which might have important functions for virus replication and translation.

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 in 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).

Declarations

Compliance with Ethical Standards:

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Conflict of Interest: All authors declare that they have no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors. Informed consent: Informed consent was obtained from all individual participants included in the study. All authors have seen the manuscript and approved to submit to "Archives of Virology".

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Figures

Figure 1

Agarose gel electrophoresis analysis of the dsRNA extracted from *M. oryzae* strain SH05 and the genome organization of MoBV9. (A) dsRNAs electrophoresis pattern of strain SH05 was observed on 1% agarose gel, with the size estimated by the Lambda Hind III digest DNA marker. (B) Schematic representation of the genomic organization of MoBV9, with the open reading frame (ORF) and the untranslated regions (UTRs) are shown by an open bar and a single line, respectively

Figure 3

Potential secondary RNA structures of the 5' and 3' terminal sequences of MoBV9. Stem-loop structures could be 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 colours indicate hydrogen bonds between different base pairs (red: G-C pairs; purple: A-U pairs; green: G-U pairs)

Figure 4

Phylogenetic analysis of the RdRp gene of MoPV1 and related viruses in the four families Botourmiaridae, Four viruses in the families Narnavidae, Mitovividae 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 genetic distance. The sequences of RdRp were obtained from the GenBank database¹ accession numbers are given before the taxon names. The position of MoBV9 is indicated by a red star and the other 8 viruses from *M.oryzae* in the family Botourmiaridae are indicated by blue stars

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