Complete Genome Sequence of a Novel Umbra-like Mycovirus From the Plant Pathogenic Fungus *Phoma Mattheucciicola*

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Research Article

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Abstract

Here, a novel umbra-like mycovirus, 'Phoma matteucciicola RNA virus 2' (PmRV2), isolated from *Phoma matteucciicola* strain HNQH1 in Hainan province of China, was sequenced and analyzed. The complete genomic sequence of PmRV2 is 3,460 nucleotides (nts) with a GC content of 56.71%. Sequence analysis of PmRV2 indicated that the presence of two noncontiguous open reading frames (ORFs) encoding a hypothetical protein and a RNA-dependent RNA polymerase (RdRp), respectively. PmRV2 contains a metal-binding 'GDN' triad in Motif C of RdRp while most + ssRNA mycoviruses contained a 'GDD' motif in the same region. Additionally, a BLASTp search showed that the RdRp amino acid sequence of PmRV2 was most closely related to the RdRp of *Macrophomina phaseolina* umbra-like virus 1 (50.72% identity) and *Erysiphe necator* umbra-like virus 2 (44.84% identity). Phylogenetic analysis indicated that PmRV2 grouped together with *Erysiphe necator* umbra-like virus 2 (EnUIV2) within the recently proposed family of '*Mycotombusviridae*'.

Introduction

Mycovirus (fungal viruses) are viruses that can infect and replicate in phytopathogenic fungi, yeasts, or oomycetes [1, 2]. In the past few decades, the number of recognized fungal viruses has rapidly increased with the development and wide usage of next-generation sequencing technologies [3, 4]. Recently, over 300 mycoviral sequences have been recorded in the National Center for Biotechnology Information (NCBI) database, which are divided into 19 families and one unclassified genus by NCBI [5, 6]. Mycoviruses are mainly classified according to the viral genome segments and replication mode. The genomes of mycoviruses are composed of double-stranded RNA (dsRNA), positive-sense single-stranded RNA (+ssRNA), negative-sense single-stranded RNA (-ssRNA), or single-stranded DNA (ssDNA) [7-9]. Mycoviruses with a +ssRNA genome are classified into nine families, including *Alphaflexiviridae*, *Barnaviridae*, *Botourmiaviridae*, *Deltaflexiviridae*, *Gammaflexiviridae*, *Hypoviridae*, *Endornaviridae*, *Narnaviridae*, and the proposed family '*Mycotombusviridae*' [10]. Although the vast majority of mycoviruses cause cryptic infections, the infection of some mycoviruses causes obvious phenotypic alterations in growth, sporulation, pigmentation, and virulence, which often result in hypovirulence and debilitation [11]. Mycovirus-mediated hypovirulence generally has the potential for biological control of plant pathogenic fungus diseases. For instance, *Cryphonectria hypovirus 1* (CHV1) was successfully used as a biological control agent to control chest blight disease in Europe in the last century [12].

Phoma matteucciicola is a destructive pathogenic fungus of *Curcuma wenyujin* causing leaf blight disease in Hainan, China [6, 10, 11]. With respect to *C. wenyujin*-infecting *P. matteucciicola*, only three viruses, *Phoma matteucciicola* ourmia-like virus 1 (PmOLV1) [6], *Phoma matteucciicola* RNA virus 1 (PmRV1) [10], and *Phoma matteucciicola* partitivirus 1 (PmPV1) [13], have been reported. In the present study, we describe a novel umbra-like mycovirus being isolated from *P. matteucciicola* strain HNQH1. The virus is tentatively named *Phoma matteucciicola* RNA virus 2 (PmRV2), and proposed to be a new member within the recently proposed family of '*Mycotombusviridae*'.

Provenance of the virus material

P. mattheucciicola strain HNQH1 was originally isolated from *C. wenyujin*, showing symptoms of leaf blight disease in Hainan, China, in 2018, and identified as *P. mattheucciicola* based on morphological characteristics and molecular phylogeny [14]. The mycelia of HNQH1 were cultured for 7 days on cellophane membranes placed on top of potato dextrose agar (PDA) plates for dsRNA extraction. The viral dsRNA of HNQH1 was extracted from approximately 2.0 g fresh mycelia, using phenol-chloroform-ethanol method [15]. After extraction, the crude dsRNAs were purified with DNase I and S1 nuclease (Takara) digestions, respectively.

The cDNA library was constructed with a RevertAid First Strand cDNA Synthesis Kit (Thermo) using tagged oligonucleotide (5'-CGATCGATCATGATGCAATGC-3') [16]. The reverse transcription-polymerase chain reaction (RT-PCR) products were purified with a gel extraction kit (Omega) and cloned into pMD19-T vector (Takara) for Sanger sequencing. Designing specific primers based on the obtained cDNA sequences, the internal gaps between the clones were obtained. To obtain the termini sequences of PmRV2, rapid amplification of cDNA ends (RACE) were performed as previously described [10]. The resulting sequence of PmRV2 was deposited in the GenBank database under the accession no. MW970051.

The virus genome sequence analysis, including sequence assembling and ORF prediction, was carried out using DNAMAN software package (version 6.0). RNA structures of the terminal sequence were performed using the Mfold program (<http://mfold.rna.albany.edu/?q=mfold/RNAFolding-Form2.3>) [17]. Multiple sequence alignments of the RdRp sequences were performed with Clustal-X program [18]. Phylogenetic tree was constructed using the maximum-likelihood (ML) method in Molecular Evolutionary Genetics Analysis (MEGA)-X program with 1,000 bootstrap replicates [18, 19]. Motif searches were performed in conserved domain database (CDD) (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) from NCBI (<https://www.ncbi.nlm.nih.gov/>).

Sequence properties

The complete sequence of PmRV2 is 3460 nucleotides (nt) in length with a GC content of 56.71%. The 5'- and 3'- untranslated regions (UTRs) of the sequence were 549 nt and 421 nt in length (Figure 1A). Two potential stem-loop structures could be predicted in 5'- and 3'- UTRs with initial ΔG values of -9.90 kcal/mol and -15.40 kcal/mol, respectively (Fig. 1B). The PmRV2 genome contains two noncontiguous open reading frames (ORFs), which were referred to as ORF1 (position 550-1419) and ORF2 (nt 1543-3039) (Figure 1A).

ORF1 is 870 nt long and encodes a 289 aa putative hypothetical protein with a predicted molecular mass of 30.1 kDa (Figure 1A). A BLASTp search analysis showed that the aa sequence of PmRV2 shares 50.29%, 40.74%, 42.63% sequence identities with *Erysiphe necator umbra*-like virus 2, *Macrophomina phaseolina umbra*-like virus 1, and *Macrophomina phaseolina umbra*-like virus 3, respectively.

ORF2 is 1497 nt long and encodes a 498 aa protein with a predicted molecular mass of 56.1 kDa. Database searches showed that ORF2 was most closely related to the RdRps of Macrophomina phaseolina umbra-like virus 1 (50.72% identity) and Erysiphe necator umbra-like virus 2 (44.84% identity), respectively. In addition, the PmRV2 RdRp has a GDN triad in motif C, while the GDD motif was usually found in +ssRNA viruses (Figure 1C). Previous study showed that modification of GDD to GDN has an adverse impact on enzymatic activity in +ssRNA viruses [20].

To dissect the relationship between PmRV2 and other mycoviruses, a molecular phylogenetic tree was constructed based on the amino acid sequences of the RdRp regions of PmRV2, twelve reported tombusviruses, and other five reported hypoviruses and narnaviruses. The ML tree revealed that PmRV2 grouped together with Erysiphe necator umbra-like virus 2 within the recently proposed family of mycotombus-like viruses (Figure 2). Therefore, PmRV2 should be considered a new member within the recently proposed family '*Mycotombusviridae*'.

Declarations

Compliance with ethical standards

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Conflict of interest All authors declare no conflict of interest.

Ethical approval This article does not contain any studies with animals or human participants performed by any of the authors.

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Figures

Figure 1

(A) Genome organization of the novel mycovirus PmRV2. The ORFs and the UTRs are indicated as rectangular boxes and single lines, respectively. The initiation and termination codons of the ORFs are shown by the numbers above the solid lines. The molecular weights of the predicted proteins are indicated in the rectangular boxes. (B) Predicted secondary structures of the 5'- and 3'-UTRs of PmRV2. (C) Multiple alignment of amino acid sequence of viral RdRps. Shaded areas indicate identical aa residues. The conserved abnormal GDN triplets are highlighted in a red rectangular box.

Figure 2

Phylogenetic analysis of PmRV2 (marked with a red star) based on the RdRp aa sequences was constructed using the program MEGA-X. Bootstrap scores (1,000 replicates) are represented at nodes. The scale bar corresponds to a genetic distance of 0.10 aa substitutions per site.

Supplementary Files

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- [Sequenceinformation.txt](#)