Complete Genome Sequence of a Novel Mitovirus From the Phytopathogenic Fungus Fusarium Oxysporum

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Abstract

Fusarium oxysporum is a cosmopolitan plant pathogen causing Fusarium wilt and Fusarium root rot in many economically important crops. There is still limited information about mycoviruses that infect *F. oxysporum*. Here, a novel mitovirus tentatively named Fusarium oxysporum mitovirus 1 (FoMV1) was identified from *F. oxysporum* strain B2-10. The genome of FoMV1 is 2,453 nt in length with a predicted AU content of 71.6%, and contains one large open reading frame (ORF) using the fungal mitochondrial genetic code. The ORF encodes RNA-dependent RNA polymerases (RdRp) of 723 amino acids with a molecular mass of 84.98 kDa. The RdRp domain of FoMV1 shares 29.01–68.43% sequence similarity to the members of the family *Mitoviridae*. Phylogenetic analysis further suggested that FoMV1 is a new member of a distinct species in the genus *Mitovirus*.

Introduction

Fusarium oxysporum is one of the most destructive pathogen in agricultural production that can infect more than 120 plant species including some economically important crops such as cotton, tomato, banana, and tobacco [1, 2]. This species complex not only causes vascular disease in a large number of economically important crops, but also causes Fusarium root rot in different *Solanaceae* species [3, 4]. The typical symptoms of Fusarium rot root in tobacco include the chlorosis of lower leaves, decaying of root and vascular discoloration. Generally, stem and taproot showed reddish to brown vascular discoloration, and the whole plant died in the end [5]. This pathogen was soil bored and could be detected both inside and exterior of the seed. Moreover, it can interact with nematodes of parasitic root knot and cyst nematodes to increase disease, and also survive in the soil for years in the absence of a host crop [6]. Although the fumigants were the most consistently effective management tactics against *F. oxysporum*, the application of methyl bromide and chloropicrin resulted in poor wrapper leaf quality and burn [6]. Hence, resistance varieties are also the most effective and economical means of reducing disease [7].

Mycoviruses are viruses that infect fungi, whose hosts include phytopathogenic and entomopathogenic fungi [8-12]. Most of the mycoviruses are cryptic infections and seldom produce symptoms [9]. Some mycoviruses belong to different viral families who possess variable effects on the morphological changes of their host, including reduced mycelia growth and increased pigmentation [13, 14]. Mycovirus-induced hypovirulence is very attractive that appeals to pathologists most. The successful case of applying hypovirulent strains of *Cryphonectria parasitica* to combat chestnut blight in Europe has provided impetus for exploiting mycoviruses as virocontrol agents [15]. The other mycoviruses with significant biocontrol effects include Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV1) and Rosellinia necatrix megabirnavirus 1 (RnMBV1) [16-19]. With the development of high-throughput sequencing technology, more and more novel mycoviruses could be identified. Hence, there will be more chances to screening for novel fungal viruses that could be used as biological control agents.

The knowledge of mycoviruses in phytopathogenic species *Fusarium oxysporum* is still very limited. Four dsRNA segments were first detected in the hypovirulent stain of *F. oxysporum* isolated from soybean seeding, but the mycoviruses corresponding to dsRNA have not been identified [20]. Two mycoviruses Fusarium oxysporum f. sp. dianthi mycovirus 1 (FodV1) and Fusarium oxysporum ourmia-like virus 1 (FoOuLV1) associated with hypovirulence were identified and characterized in *F. oxysporum* isolated from diseased carnation plant and bitter gourd, respectively [21, 22]. After that, some novel mycoviruses have been reported, such as Fusarium oxysporum f. sp. dianthi hypovirus 2 (FodHV2) and Fusarium oxysporum f. sp. dianthi mitovirus 1 (FodMV1). FodHV2 belongs to the family *Hypoviridae* but did not alter the vegetative growth or virulence of the host [23, 24]. A novel polymyco-related virus termed Hadaka virus 1 (HadV1) identified from *F. oxysporum*. HadV1 has 11(+) RNA genomic segments and lacks a typical structural protein that reveals a potential novel lifestyle of multi-segmented RNA viruses [25]. All these results indicated that the mycoviruses harbored by *F. oxysporum* have a rich diversity, and some of them have potential as biological control agents. Here, a novel mitovirus was identified in strain B2-10 of *F. oxysporum*, and described the characterization of the genome, and tentatively named as "Fusarium oxysporum mitovirus 1" (FoMV1).

Provenance and sequencing of strains

The strain B2-10 of *F. oxysporum* was isolated from the diseased root of a tobacco plant with typical symptoms of Fusarium root rot, which was collected from Xuchang city, Henan province, China, in 2019. The strain was purified by single spore isolation technique. The specific primers of translation elongation factor 1-alpha (EF-1α) and *F. oxysprorum* were used for *Fusarium* species identification [26]. Mycelial agar plugs of strain B2-10 were transferred to a PDA plate (9 cm diam) covered with cellophane membrane and cultured at 25 °C in the dark for four to six days. Mycelial mats were harvested using a sterilized medicine spoon and stored at -70 °C until use. Total RNA was extracted from 1.0 g of mycelium using an RNAiso kit (TaKaRa, China). Total RNA of strains were sent to Shanghai Bohao Biotechnology Co., Ltd. for high-throughput sequencing on an Illumina HiSeq 2500 platform. The rRNA was depleted using a Ribo-ZeroTM rRNA Removal Kit (Illumina, CA, USA). Detailed parameters used in the bioinformatics pipeline according to the procedures described by Wang et al. (2020) [27]. Finally, the contigs that were identical or complementary to the viral genomic sequence were identified as putative viral sequences.

The cDNA of strain B2-10 was synthesized using the PrimerScript™1st Strand cDNA Synthesis Kit (TaKaRa, China) following the manufacturer's instruction. The specific primers (F1: 5'-AGGTCAACCAATGGGAAC-3'; R1: 5'-CCAACAAAAGTAGGATAG-3') were designed to verify the presence of newly discovered mitovirus FoMV1 with the PCR amplicon of 756 bp in length. The system and step of RT-PCR were conducted refer to the procedure described by Wang et al. (2020) [27]. A SMARTer^{R} RACE 5'/3' Kit (TakaRa, China) was used to complete the 5'- and 3'-terminal genomic sequence with the help of gene-specific primers (GSPs). GSP-763F1 (5'-GGAATACCAGTAAGAGGGATA-3') and GSP-763F2 (5'-GTAAAAGCCTAGAGGTTGGT-3') were used for 3'-RACE as inner and outer primers, respectively. Similarly, GSP-763R1 (5'-AAAGAGAAAAGGGTTTGAAC-3') and GSP-763R2 (5'-CTTGCAGTCCTATGGTCTAC-3') were

used for 5’-RACE as inner and outer primers, respectively. The procedure was performed following the kit manufacturer’s instruction. The target PCR product was gel-purified by MiniBEST Agarose Gel DNA Extraction Kit (TaKaRa, China), and cloned into the pMD19-T vector (Sangon Biotech, China), then introduced into *Escherichia coli* Trelief 5α (TSINGKE Biotech, China). The target clones were sent to Sangon Biotech for sequencing.

Putative open reading frames (ORFs) were predicted using ORF Finder in the NCBI database (https://www.ncbi.nlm.nih.gov/orffinder/). The potential secondary structures for the 5’-terminal and 3’-terminal nucleotide sequences of FoMV1 (positive strand) were predicted using RNAfold WebServer (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi). Multiple sequence alignment of the RdRp sequences encoded by FoMV1 and other mitoviruses were performed using DNAMAN software (Version 9) and CLUSTALX program (version 2.1). A phylogenetic tree was constructed using the neighbor-joining method with a bootstrap value of 1000 replicates through the MEGA-X program (Version 10.1.8).

Sequence properties

The complete genome sequence of FoMV1 obtained by assembling partial-length cDNAs that amplified from the total RNA, the 5’- and 3’-terminal sequences were determined Using RT-PCR with oligo (dT) primers and rapid amplification of cDNA ends. Then, the full-length sequence of FoMV1 has been submitted to the GenBank database with the accession number MW690927 (not yet released).

The full-length cDNA sequence of FoMV1 is 2,453 nt long and is relatively high A+U content of 71.6%. The AU-rich (71.6%) of FoMV1 is higher than the AU-rich of Fusarium andiyazi mitovirus 1 and Fusarium oxysporum f. sp. dianthi mitovirus 1 with 70.5% and 58.8%, respectively [28, 24]. AU-richness is a common characteristic of fungal and plant mitochondrial mitovirus [29]. The nucleotide sequence of FoMV1 was examined by ORF Finder using the fungal mitochondrial codon usages. The positive strand was found to contain one major large ORF (nt 241-2,412) and one small ORF (nt 83-172). The untranslated regions (UTRs) at the 5’- and 3’- ends are 240 nt and 41 nt long, respectively (Fig. 1A). The large ORF encodes a 723-aa protein with a calculated molecular weight of 84.98 KDa and a predicted pI of 9.83. A total of 13 UGA-encoded tryptophans were found in this ORF [30]. A small ORF near the 5’-terminus encodes a protein of 29-aa with a calculated molecular weight of 3.31 KDa and a predicted pI of 8.94. The 5’- and 3’- UTRs of FoMV1 were predicted for potential secondary structure. The result indicated that the 5’-terminal sequence (nt 4-47) could be folded into three stem-loop structure with a ΔG value of -15.60 kcal/mol (Fig. 1B). The 3’-terminal sequence (nt 2,415-2,453) could be folded into a stable stem-loop structure with a ΔG value of -8.70 kcal/mol (Fig. 1B). Moreover, the 5’- and 3’- UTRs of the positive strand of FoMV1 had an inverted complementarity, which a potentially stable panhandle structure was predicted with a ΔG value of -11.00 kcal/mol (Fig. 1B).

A search of BLASTX in NCBI indicated that the internal 2,150 nt region (241-2,391) of the FoMV1 sequence is 74.41% identical (E-value 0.0) to the full-length genome sequence (229-2,386) of Fusarium andiyazi mitovirus 1 (GenBank Acc. No. QIQ28423). A homology search with BLASTP showed that this amino acid sequence of RdRp of FoMV1 is most closely related to the RdRp of Fusarium andiyazi

mitovirus 1, Fusarium circinatum mitovirus 2, Fusarium sacchari mitovirus 1, and Fusarium poae mitovirus 1 with the identity of 45.19%-68.43% (E-value 0.0). A consered domian database (CDD) search confirmed that position 925-2,136 nt of the RdRp of FoMV1 contained a conserved domain Mitovir_RNA_pol (pfam05919). Furthermore, a multiple alignment of amino acid sequences of RdRp between FoMV1 and other mitoviruses suggested that it contained six conserved motifs (Fig. 2). That is a characteristic of the RdRps of mitochondrial viruses [31, 32]. Therefore, according to the rules of species demarcation criteria about mitoviruses defined by the ICTV (https://talk.ictvonline.org/), suggesting that FoMV1 should be considered a novel mitovirus. It was a tentative member of a new species that belonged to the family Mitoviridae. However, there were no proteins or polypeptides homologous to the putative protein sequence of the small ORF. In addition, the presence of small ORF coding for polypeptides with unknown function near the 5'-terminal region has been previously reported in other mycovirus, including Botrytis cinerea mitovirus 1 [33], Helminthosporium victoriae 190S virus [34], Macrophomina phaseolina victorivirus 1 [35], and Rosellinia necatrix megabirnavirus 1 [18].

A phylogenetic tree based on the full length amino acid sequence of the viral RdRp of FoMV1 and 25 other selected mitoviruses were constructed using the Neighbor-Joining method (Fig. 3). FoMV1 clustered with sacchari mitovirus 1, Fusarium andiyazi mitovirus 1, and Fusarium circinatum mitovirus 2-1 to form a clade that clustered with Fusarium circinatum mitovirus 1, Fusarium globosum mitovirus 1, Fusarium coeruleum mitovirus 1, and Fusarium poae mitovirus 2 to form a large clade that were all mitochondrial viruses infecting *Fusarium* species. Fusarium oxysporum f. sp. dianthi mitovirus 1 (FodMV1) is the first mitovirus that identified in *F. oxysporum* [24]. However, FoMV1 didn't cluster with FodMV1, but with mitoviruses that infects Fusarium sp. This result of the phylogenetic analysis of RdRp sequences suggested that FoMV1 is a novel member of the family *Mitoviridae*.

Declarations

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Availability of date and material

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest/Competing interests

The authors declare no conflict of interest.

Author Contributions:

J.W., and S.J.L., conceived and designed the experiments; J.W. performed the experiments; R.Q., C.J.L., X.J.L., J.Z., J.K.B., and Y.G.C., collected the samples; J.W. wrote the paper; S.J.L., and R.Q., revised the paper.

Ethical approval

This article dose not contains any studies with human participants or animals performed by any of the authors.

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