Phylogenetic Analysis of a Mitovirus in Fusarium oxysporum from Phytolacca dioica in the Canary Islands

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Abstract

We characterized a novel mitovirus from Fusarium oxysporum isolated from Phytolacca dioica (ombú) in the Canary Islands, Spain. Using transcriptome sequencing, we assembled the full-length genome of the novel Fusarium oxysporum mitovirus 2-like (FoMV2-like), which consists of 2,363 nucleotides and has a GC content of 30.3%. The genome harbors a single open reading frame (ORF) encoding a protein of 713 amino acids. Phylogenetic analysis of the RdRp amino acid sequence showed that FoMV2-like clusters closely with Fusarium oxysporum mitovirus 2, suggesting a close evolutionary relationship. DNADiff analysis revealed that the genome of FoMV2-like shares 100% alignment with Fusarium oxysporum mitovirus 2, with an average identity of 87.33%, supporting its classification as a distinct species within the Mitoviridae family. This study expands our understanding of mycoviral diversity in Fusarium oxysporum and contributes to the genomic characterization of mitoviruses.

Introduction

Mycoviruses infecting plant-pathogenic fungi exhibit great genomic diversity. According to the International Committee on Virus Taxonomy (ICTV) (https://talk.ictvonline.org/taxonomy/), thirty viral families include representatives that infect fungal species [1–3]. The majority of mycoviruses have RNA genomes, which can be positive (+) or negative (-) sense single-stranded RNA (ssRNA), or double-stranded RNA (dsRNA) [4]. Furthermore, in many cases, mycoviruses do not exhibit significant phenotypic effects on their fungal hosts [5].

Among the various mycoviruses, those infecting *Fusarium oxysporum* are of particular interest due to their potential impact on this cosmopolitan plant pathogen that infects more than 120 species and causes *Fusarium* wilt in several crops worldwide [7, 8]. Mycoviruses from different viral families have been reported with hypovirulence activity over *F. oxysporum* [9–12]. Viruses belonging to *Mitoviridae* family are positive-sense (+) single-stranded (ss) RNA, with genomes ranging from 2.3 to 5 kb [14]. Their genomes contain a single open reading frame (ORF), which encodes an RNA-dependent RNA polymerase (RdRp) [15, 16].

Mitoviruses replicate in fungal mitochondria, do not form virions, and are unencapsidated, generally causing mitochondrial alterations that result in hypovirulence [17–19]. Several members of the genus *Mitovirus* have been identified infecting different *Fusarium* species worldwide [20–23]. Within *Fusarium oxysporum*, several mitoviruses have been described to date, including Fusarium oxysporum f. sp. Dianthi mitovirus 1 (FodMV1) [24], Fusarium oxysporum mitovirus 1 (FoMV1) [11], Fusarium oxysporum mitovirus 2 (FoMV2), isolated from the pathogenic *Fusarium oxysporum* f. sp. *ginseng* [25], and Fusarium oxysporum mitovirus 3 (FoMV3), identified in *Fusarium oxysporum* f. sp. *Melonis* causal agent of Fusarium wilt in melons [26]. This study aims to contribute to the genomic understanding of mycoviruses in *Fusarium oxysporum* by investigating a novel mycovirus in a *Fusarium* isolate and its genomic characteristics.

Genome Assembly and Phylogeny of a Mitovirus from Fusarium oxysporum

The *Fusarium* strain was obtained of from *Phytolacca dioica* (ombú) in the Canary Islands, Spain, and stored as a conidial suspension. *Fusarium oxysporum* samples were cultured on potato dextrose agar (PDA) plates at 27 °C. To detect the presence of RNA viruses, a cellulose chromatography method was used to enrich the samples of dsRNA molecules, wich are the replication intermediates of most RNA mycoviruses [27]. For dsRNA extraction, *Fusarium oxysporum* samples were processed following the protocol described by Cañizares et al. (2015).

Subsequently, RNA extraction was performed for sequencing and transcriptome analysis by RNA-seq. Mycelial mass was recovered from the culture medium and macerated in liquid nitrogen and immediately suspended in Trizol TM Reagent to stabilize the genetic material and subsequently processed for the extraction of total RNA, following the standard RNA extraction protocol described by the manufacturer (Trizol TM Reagent, Invitrogen; total RNA extraction protocol). RNA-seq was performed using the Illumina NovaSeq 6000 platform, generating paired-end reads of approximately 100 bp from libraries prepared with the TruSeq Stranded Total RNA kit with Ribo-Zero H/M/R Gold. Reads were quality filtered using Cutadapt v3.5 [28], retaining sequences with a minimum quality score of Q30 and a length of at least 70 bases. De novo assembly was performed using Trinity 2.13.2 with default parameters [29]. Putative viral contigs were identified through BLASTN searches against a custom database containing nucleotide and amino acid sequences from fungal mitovirus genomes and fungal virus genomes retrieved from RefSeq and NCBI Virus. This approach allowed the detection of viral sequences corresponding to a mitovirus, which were subsequently extracted using the RdRp amino acid sequence for further phylogenetic analysis.

For the phylogenetic analysis of the mitovirus, the amino acid sequence of its RNA-dependent RNA polymerase (RdRp) was aligned with RdRp sequences of representative members of the family *Mitoviridae* retrieved from RefSeq, as well as with mitovirus sequences previously described in *Fusarium* hosts. Narnavirus sequences were included as the outgroup. Multiple sequence alignment was performed using the MAFFT program [30, 31]. The phylogenetic tree based on the RdRp protein alignment was carried out using IQ-TREE 3 [32] under the Q.PFAM+F+I+R5 substitution model, and branch support was evaluated using the ultrafast bootstrap method with 5,000 pseudoreplicates [33] to infer evolutionary relationships among mitovirus taxa. Phylogenetic analysis based on RdRp amino acid sequences showed that the Fusarium oxysporum mitovirus 2-like clusters strongly with *Fusarium oxysporum mitovirus 2* (FoMV2), supported by high branch support values (UFB = 100). This clustering indicates a close evolutionary relationship between the two viruses, despite their detection in fungal hosts from geographically distinct locations (Figure 4).

DNADiff analysis revealed that the genome of FoMV2-like shares 100% alignment with Fusarium oxysporum mitovirus 2, with an average identity of 87.33%, and 93.93% alignment with Fusarium andiyazi mitovirus 2, with an average identity of 82.28%. Based on the species demarcation criteria for

mitoviruses outlined by the ICTV, mitovirus isolates with less than 90% amino acid sequence identity in their RdRp are classified as different species. Consequently, FoMV2-like should be regarded as a member of a new species within the family *Mitoviridae*.

The full-length genome sequence of Fusarium oxysporum mitovirus 2-like (FoMV2-like), accession number (ID 3036262) was obtained through transcriptome assembly. The genome is 2,363 nucleotides in length and has a GC content of 30.3%. A single open reading frame (ORF), starting at position 164 and ending at position 2,304 (Figure 2A), is present within the genome. ORF prediction was carried out using ORF Finder. To assess potential secondary structures, the 5'- and 3'-terminal regions of the mitovirus genome (positive strand) were predicted using the RNAfold WebServer. This ORF is translated into a protein of 713 amino acids, with a molecular weight of 83.136 kDa and an isoelectric point of 9.63. The 5'-terminal sequence is predicted to fold into a stable stem-loop structure with a ΔG value of -3.00 kcal/mol, while the 3'-terminal sequence folds into three stem-loop structures with a ΔG value of -7.00 kcal/mol (Figure 2B). The predicted RNA secondary structures of FoMV2-like resemble those reported for other mitoviruses and are characteristic of members of the Mitoviridae family [13].

Declarations

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Conflict of interest:

The authors declare 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 participants in the study.

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Figures

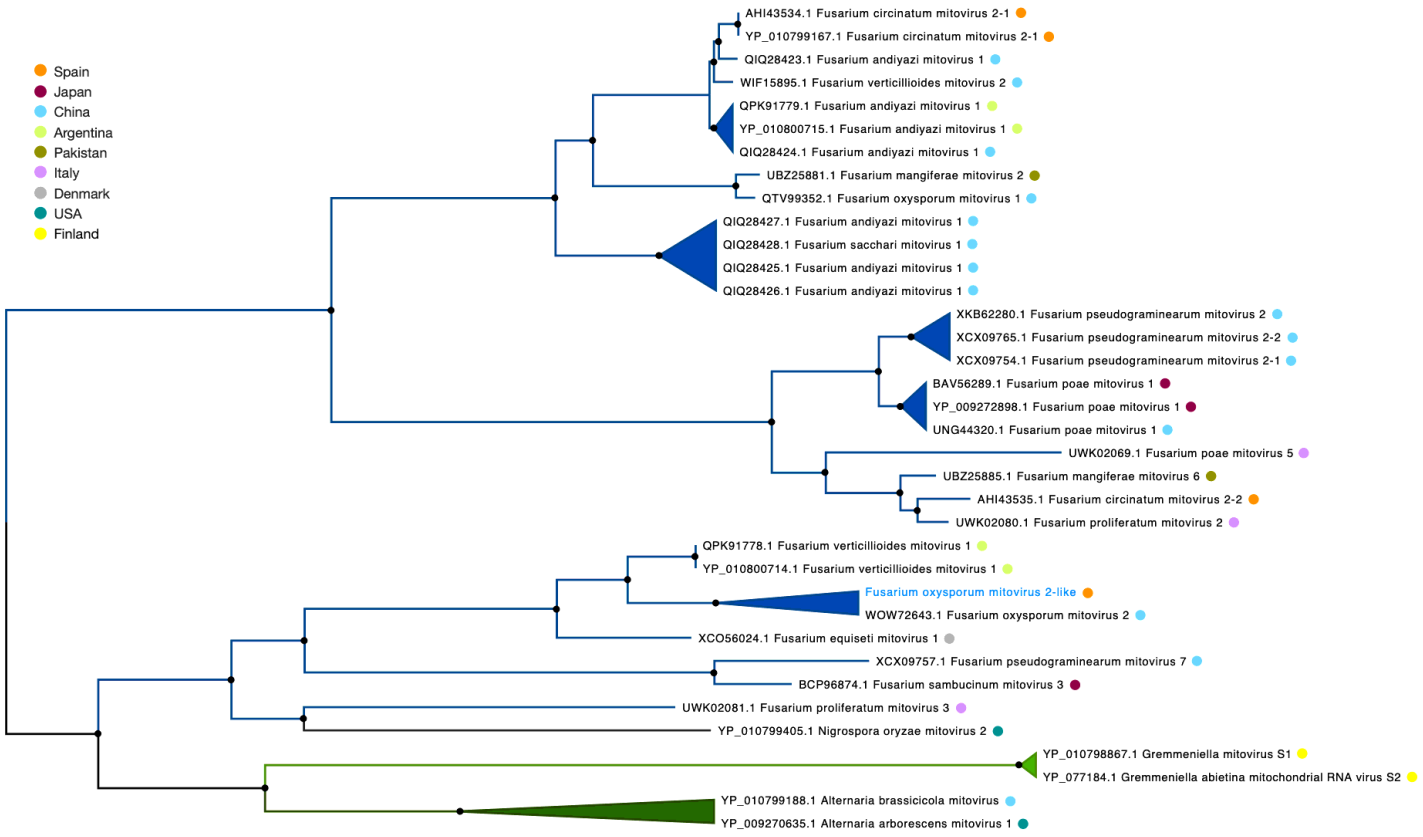

Figure 1

Maximum likelihood phylogenetic tree based on RdRp amino acid sequences of the studied mitovirus, representative members of the family *Mitoviridae*, and mitovirus sequences described in *Fusarium* hosts. Black circles indicate branches with strong support (UFB > 97).

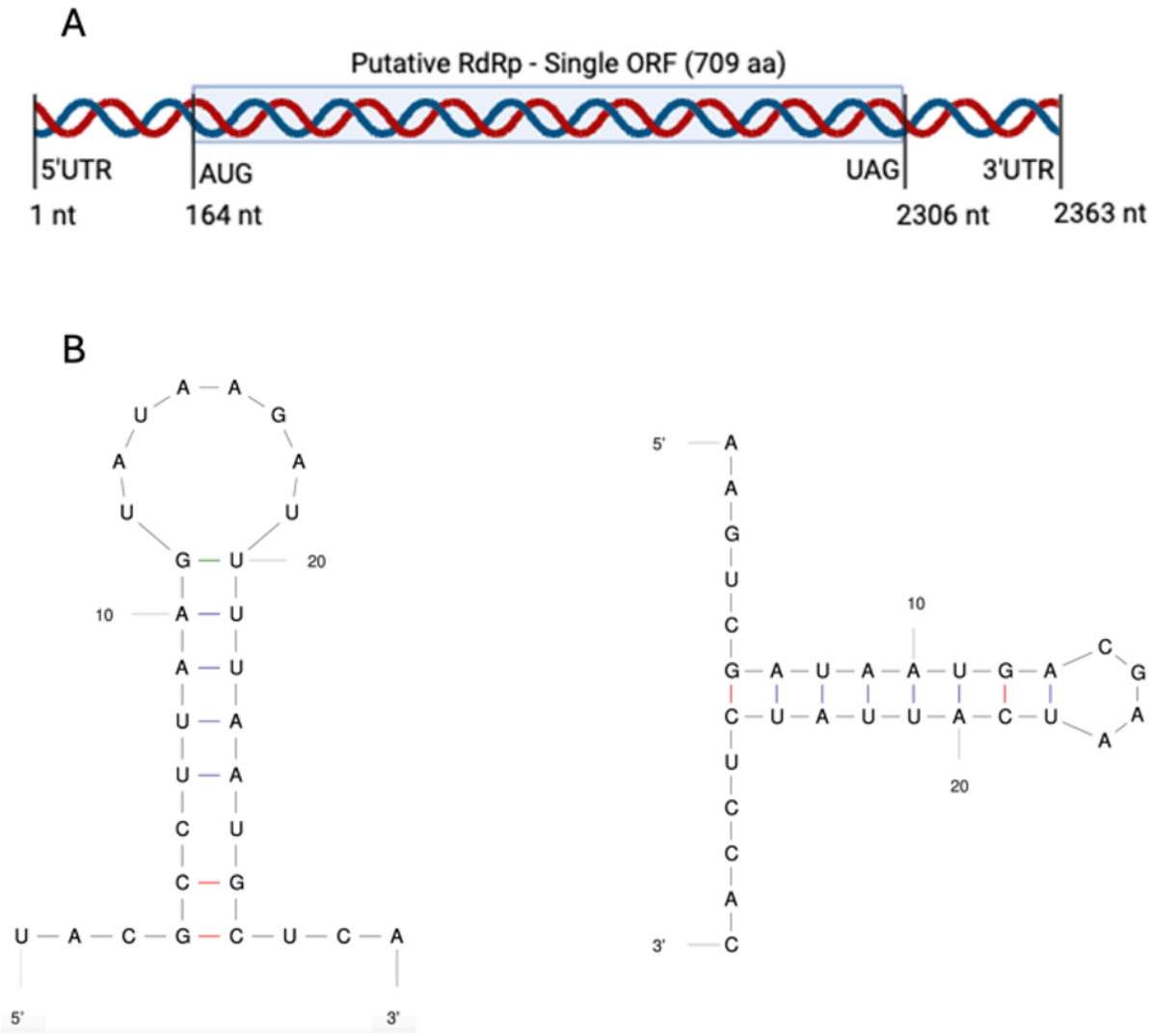

Figure 2

Genomic organization and terminal structure of *Fusarium oxysporum* mitovirus 2-like (FoMV2-like). (A) Schematic diagram of the genome organization of FoMV2-like. (B) Potential secondary structures of the 5’ and 3’ termini of FoMV2-like.