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ANNOTATED SEQUENCE RECORD

Full genome sequence of a new polymycovirus infecting *Fusarium redolens*

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Abstract

By screening a collection of *Fusarium* spp. for the presence of dsRNA, the *Fusarium redolens* strain A63-1 was found harboring a pattern of multiple dsRNA bands when analyzed by agarose gel electrophoresis. Using NextSeq Illumina sequencing, the full sequences of eight dsRNA molecules were determined, compared to databases, and gathered into a new viral genome. This novel virus shares similarities with mycoviruses that were recently grouped in the proposed family "*Polymycoviridae*". Hence, the name "Fusarium redolens polymycovirus 1" is proposed for this virus. Each viral dsRNA contains only one ORF, except dsRNA 7, which has an additional one. Based on amino acid sequence similarities, the functions of the proteins encoded by dsRNA 1–4 can be hypothesized. On the other hand, the putative proteins encoded by dsRNA 5–8 exhibit no relevant homology to known proteins. In this report, the full genome sequence of this new virus is presented along with a primary bioinformatics analysis.

Introduction

Even though the discovery of the first mycovirus was made in 1962 in *Agaricus bisporus* [1], the known virome of fungi has long remained relatively small in comparison with those of bacteria, humans, animals and plants [2]. Recently, though, it has rapidly expanded thanks to large screenings of phytopathogenic, entomopathogenic and human-pathogenic fungi with the help of high-throughput sequencing technologies [3]. One of the main reasons to look for new mycoviruses is that some confer hypovirulence, *i.e.*, reduced virulence of their hosts, and could therefore become biocontrol tools, following the example of Cryphonectria hypovirus 1, which is used to control chestnut blight [4].

Potentially relevant hypovirulent mycoviruses are those associated with *Fusarium* species [5]. This genus includes phytopathogenic fungi [6] as well as fungal pathogens of immunocompromised humans [7]. Although numerous viruses have been discovered in *Fusarium* spp., only a limited number have been associated with hypovirulence [4, 8]. Therefore, there is a need for further virus screening in order to find new candidates, with emphasis on dsRNA, since it constitutes many of the known mycoviral genomes [4].

Via such dsRNA screening, we discovered a new segmented mycovirus in the plant pathogen *F. redolens*. Since this new virus shares similarities with members of the proposed family "*Polymycoviridae*", the name "Fusarium redolens polymycovirus 1" (FrPmV-1) is proposed.

Provenance of virus material

F. redolens strain A63-1 was isolated from maize in 2007 [9] and stored on synthetic nutrient-poor agar (SNA) slants under sterile mineral oil [10]. Species identification was achieved by partial sequencing of the gene encoding elongation factor 1-alpha and comparison with entries in the NCBI database. In order to produce large amount of mycelium, the strain was first revitalized on solid potato dextrose agar (PDA) medium and subsequently subcultured in 250 ml of liquid medium containing 2% (w/v) malt extract at 22 °C, with shaking at 150 rpm in the dark. After 10 days, the mycelium was collected by filtering through a nylon web and dried overnight under laminar flow. Total dsRNA was extracted from the dried mycelium according to a recently

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published protocol [11]. The dsRNA was then treated with S1 nuclease and DNase I (Thermo Fisher, United States) and analyzed by 1.2% agarose gel electrophoresis (Fig. 1B). Nine nanograms of treated dsRNA was sent to Fasteris (Switzerland) for NextSeq Illumina sequencing.

The sequencing data were uploaded to the Galaxy web platform [12] for further analysis. Read quality was first assessed using FastQC, and adaptor sequences were removed and quality trimming was done using Trimmomatic v0.36.5 [13]. Trimmed reads were then filtered against four complete *Fusarium* genome sequences: *F. culmorum* (LT598659.1), *F. oxysporum* (LT906350.1 and NC_030986.1), and *F. graminearum* (NC_026474.1) using HiSAT 2 v2.1.0 [14]. The remaining reads were assembled *de novo* using Trinity v2.8.4 [15] and used for a similarity search using BLASTn v2.7.1 [16]. Contigs were curated manually and visualized using UGENE [17]. Phylogenetic analysis was performed using MEGA X [18].

Sequence properties

The genome of FrPmV-1 is composed of eight segments (dsRNAs 1-8), the nucleotide sequences of which have been deposited in the GenBank database under accession numbers MK609920-MK609927. These segments are 2470,

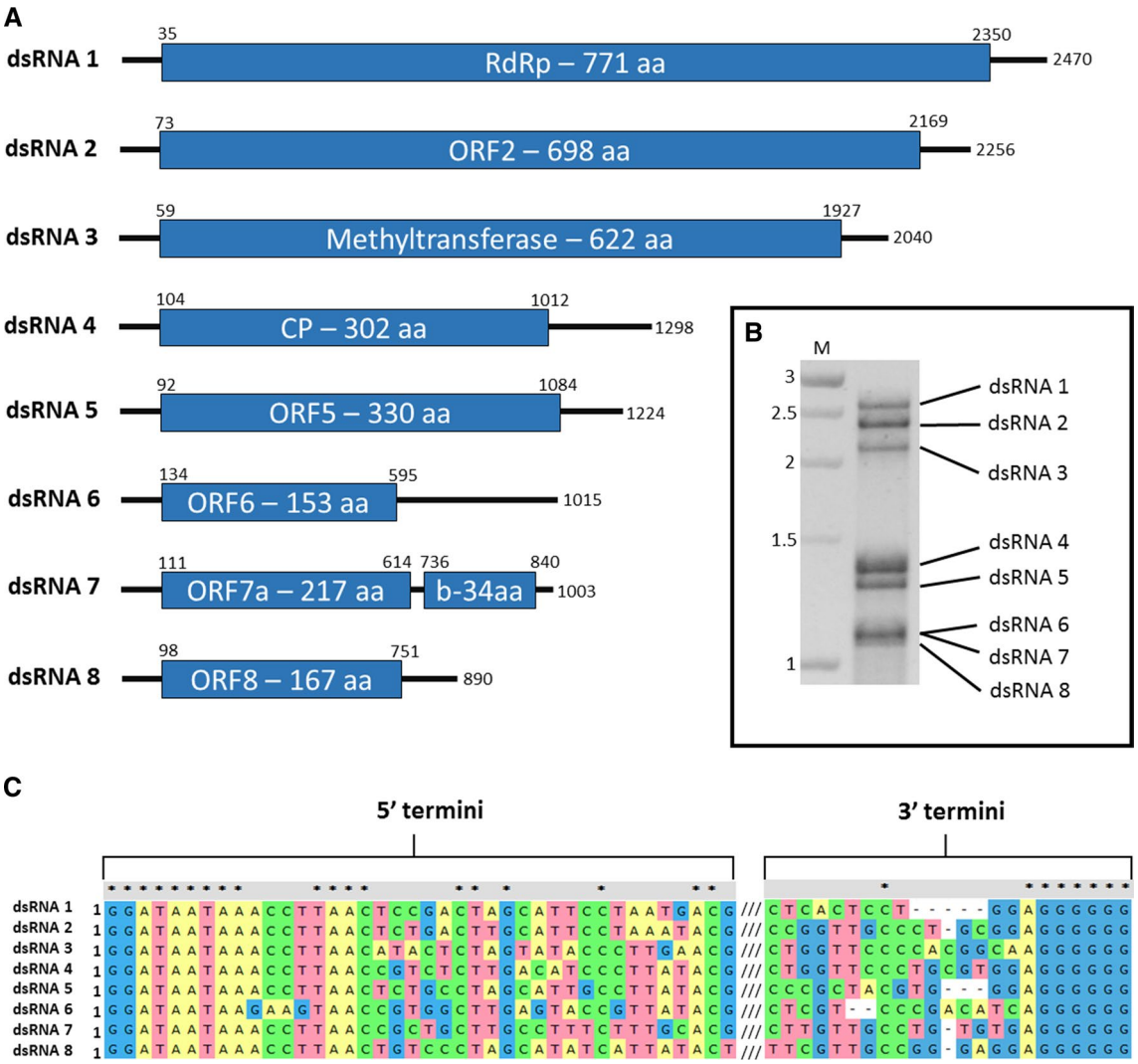

Fig. 1 Genomic characterization of Fusarium redolens polymycovirus 1. (A) Schematic representation of the eight FrPmV-1 dsRNA genomic segments (not to scale). Boxes representing ORFs are shown with their nucleotide positions and the putative functions of the corresponding proteins. (B) 1.2% agarose gel showing FrPmV-1 dsRNA 1-8 treated with DNase I and S1 nuclease. M = Promega 1 kb DNA ladder. Numbers on the left refer to the sizes of DNA markers expressed in kb. The dsRNA segments detected by Illumina sequencing are indicated on the right. (C) Conserved sequences in the 5' and 3' termini of the cDNA obtained for the eight dsRNA segments of FrPmV-1. Conserved residues are indicated by asterisks

2256, 2040, 1298, 1224, 1015, 1003 and 890 bp in length, respectively. A schematic representation of the FrPmV-1 genome is given in Fig. 1A. All dsRNAs contain a single ORF on the positive-sense strand, with the exception of dsRNA 7, which contains two ORFs. Notably, the 5' terminus of each segment starts with the sequence GGAUAA $UAAN_{(4)}UAACN_{(5)}CUA/UGN_{(5)}CN_{(5)}AC$, and the 3' terminus of each segment ends with the conserved motif $AG_{(6)}$ (Fig. 1C).

FrPmV-1 dsRNA 1 contains one large ORF (ORF1, positions 35-2350) that potentially encodes for a protein of 771 amino acids (aa) with an estimated molecular mass (M_r) of 86.3 kDa. This protein is likely to be the RNA-dependent RNA polymerase (RdRp). Indeed, a BLASTp search showed sequence similarity to the RdRp (ASV63092.1) of Colletotrichum camelliae filamentous virus 1 (CcFV-1) [19] (58.2% identity, 97% coverage, E-value = 0) and to the RdRp (YP_009052470.1) of the uncharacterized Cladosporium cladosporioides polymycovirus 1 (CcPmV-1) (54.1% identity, 96% coverage, E-value = 0). In addition, several other hits with lower scores were found for RdRps from other segmented dsRNA mycoviruses (data not shown).

Since RdRps are usually selected for phylogenetic analysis of viruses, amino acid sequences of the ORF1-encoded protein of FrPmV-1 and those of related viral RdRps were used to build a phylogenetic tree (Fig. 2A). As anticipated by their sequence similarity, this tree shows that CcFV-1 is the phylogenetically closest virus to FrPmV-1. Both viruses are grouped within the proposed family "*Polymycoviridae*" [20], in which they form a specific clade. The RdRps of members

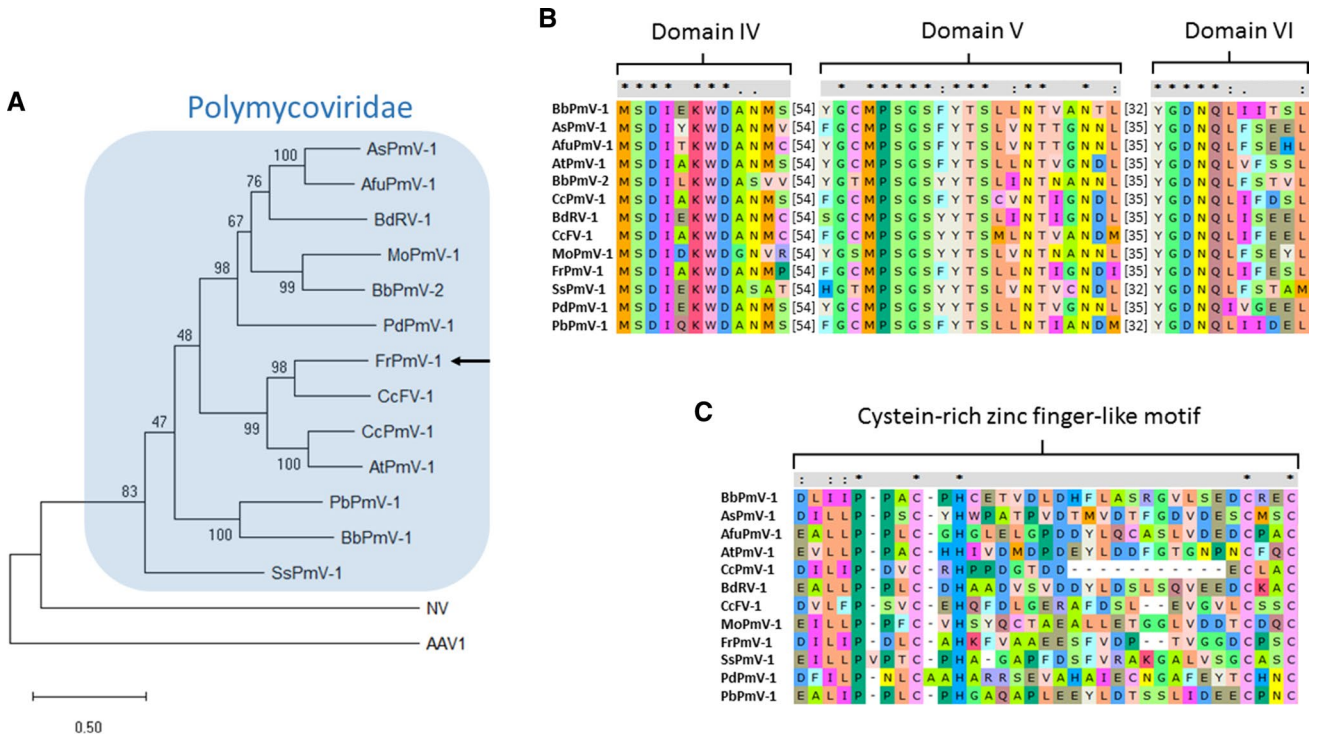

Fig. 2 (A) Maximum-likelihood phylogenetic tree based on an alignment of RdRp aa sequences of members of the proposed family "*Polymycoviridae*" and representative members of the families *Caliciviridae* (NV, Norwalk virus, NP_056820) and *Astroviridae* (AAV1, avastrovirus 1, CAB95006). The tree was constructed using 1000 bootstrap replicates. Numbers next to the branches refer to the percentage of bootstrap replicates that cluster the taxa together. The arrow shows the position of FrPmV-1 in the tree. (B) Alignment of the conserved domains IV, V and VI of the picorna-like RdRp family found in polymycovirus RdRp aa sequences. (C) Alignment of the conserved cysteine-rich zinc-finger-like motif in ORF2-encoded proteins of polymycoviruses. In the alignment, identical residues are indicated by asterisks, highly conserved residues by double dots, and less conserved but related residues by single dots. Abbreviations and accession numbers of the RdRps and of the ORF2-encoded proteins are as follows: CcFV-1, Colletotrichum camelliae filamentous virus 1 (ASV63092.1, ASV63093.1); CcPmV-1, Cladosporium cladosporioides polymycovirus 1 (YP_009052470.1, YP_009052471.1); AtPmV-1, Alternaria tenuissima polymycovirus 1 (AJP08049.1, ACL80752.1); AfuPmV-1, Aspergillus fumigatus polymycovirus 1 (AXE72937.1, AXE72938.1); AsPmV-1, Aspergillus spelaeus polymycovirus 1 (AYP71805.1, AYP71808.1); PdPmV-1, Penicillium digitatum polymycovirus 1 (AVZ65983.1, AVZ65984.1); BbPmV-1, Beauveria bassiana polymycovirus 1 (YP_009352879.1, YP_009352876.1); BbPmV-2, Beauveria bassiana polymycovirus 2 (CUS18599.1); BdRV-1, Botryosphaeria dothidea virus 1 (YP_009342446.1, YP_009342447.1); MoPmV-1, Magnaporthe oryzae polymycovirus 1 (QAU09249.1, QAU09250.1); SsPmV-1 Sclerotinia sclerotiorum polymycovirus 1 (AWY10945.1, AWY10946.1); PbPmV-1, Penicillium brevicompactum polymycovirus 1 (AYP71801.1, AYP71804.1)

of this family are related to those of positive, single-stranded RNA viruses belonging to the families *Caliciviridae* and *Astroviridae* [20]. The proposed family "*Polymycoviridae*" includes dsRNA mycoviruses with a variable number of segments. Interestingly, FrPmV-1 and CcFV-1 are the only members of this family with eight genomic dsRNA segments; the other polymycoviruses have fewer (4 to 7). All RdRps of polymycoviruses have the conserved domains IV, V and VI of the picorna-like RdRp family (RdRP_1, PF00680) with the striking difference that the GDD motif is replaced by GDNQ [20]. This is also the case for the RdRp of FrPmV-1 (Fig. 2B).

The second genomic dsRNA contains a single ORF (ORF2, positions 73-2169) encoding a protein of 698 aa (M_r, 76.5 kDa) with an unknown function. A BLASTp search for this protein gives hits for proteins from the same viruses that were identified in the ORF1 search. The first two of these hits were again proteins from CcFV-1 (ASV63093.1, 48.7% identity, 99% coverage, E-value = 0) and CcPmV-1 (YP_009052471.1, 44.91% identity, 99% coverage, E-value = 0). Similarities were also found to a lesser extent to other hypothetical proteins from polymycoviruses (data not shown). Kotta-Loizou and Coutts [20] argued that proteins encoded by polymycovirus dsRNA 2 are scaffolds for the replication machinery and/or dsRNA chaperones. This hypothesis is supported by several predicted features, including a cysteine-rich zinc finger-like motif (Fig. 2C) and arginine repeats (R-R or R-X-R), which are also found in the ORF2-encoded protein of FrPmV-1.

FrPmV-1 dsRNA 3 contains one ORF (ORF3, positions 59-1927) encoding a single protein of 622 aa (M_r, 67.1 kDa) that is related to methyltransferases of polymycoviruses. Again, the most closely related proteins are proteins from CcFV-1 (ASV63094.1, 45.6% identity, 100% coverage, E-value = 2e-173) and CcPmV-1 (YP_009052472.1, 45.0% identity, 99% coverage, E-value = 5e-175). In addition, an S-adenosylmethionine-dependent methyltransferase domain (cd02440, E-value = 2.07e-08) was found in the ORF3-encoded protein at residues 131-238. As suggested for other polymycoviruses, this protein is probably involved in the capping of the 5' end of the viral RNA, which was proven experimentally for dsRNA 1 of Beauveria bassiana polymycovirus 1 (BbPmV-1) [20].

The fourth dsRNA exhibits a single ORF (ORF4, positions 104-1012) that encodes a potential protein (302 aa, 31.9 kDa) that is a proline-alanine-serine-rich protein (PASrp), where proline, alanine and serine represent 6.6%, 12.9% and 11.3%, respectively, of the total aa sequence. This PASrp is likely to be the capsid protein (CP), since it shares sequence similarity (45.7% identity, 99% coverage, E-value = 1e-74) with the CP of CcFV-1 (ASV63095.1). CcFV-1 particles have been shown to be filamentous [19]; hence, this could also be the case for FrPmV-1. However, no true virions have been observed for other polymycoviruses such as Aspergillus fumigatus polymycovirus 1 (AfuPmV-1). Instead, purified AfuPmV-1 exhibited chain-like molecules when examined by atomic force microscopy [21]. Those chain-like molecules correspond to an association of viral dsRNA with AfuPmV-1 PASrp (YP_009551544.1), which also shows similarity (36.7% identity, 97% coverage, E-value = 6e-42) to FrPmV-1 PASrp. Further characterization will be necessary to find out whether FrPmV-1 is conventionally or unconventionally encapsidated.

FrPmV-1 dsRNA 5 has one ORF (ORF5, positions 92-1084) potentially encoding a protein of 330 aa (M_r, 34.9 kDa). Only two hits with a low score (E-value = 1.9) were obtained from a BLASTp search, corresponding to hypothetical bacterial carboxyl-terminal proteases (data not shown). FrPmV-1 dsRNA 6 has one ORF (ORF6, positions 134-595) encoding a protein of 153 aa (M_r, 16.6 kDa). dsRNA 7 has two ORFs: ORF7a at positions 111-614 and ORF7b at positions 736-840. Hypothetically, ORF7a encodes a protein of 167 aa (M_r, 17.6 kDa), and ORF7b encodes a small protein of 34 aa (M_r, 3.6 kDa). dsRNA 8 has one ORF (ORF8, positions 98-751) encoding a protein of 217 aa (M_r, 22.8 kDa). None of the proteins encoded by dsRNA 6, 7 and 8 show homology to known proteins. In fact, proteins encoded by the smaller dsRNAs (5-8) of other polymycoviruses do not exhibit homology to known proteins [20], and their functions in the viral life cycle remain to be determined.

Concluding remarks

To our knowledge, this is the first description of a virus of *Fusarium redolens* and the first report of a polymycovirus in a member of the species of the genus *Fusarium*. Given that FrPmV-1 exhibits strong similarity to viruses that have been shown to confer hypovirulence on their respective host, such as CcFV-1 and Botryosphaeria dothidea RNA virus 1 [22], this aptitude is worth testing for this virus. Further research is under way to characterize potential viral particles and to investigate the host range and mode of transmission of this new mycovirus.

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