Molecular characterization of a novel fusarivirus infecting the plant-pathogenic fungus *Alternaria solani*

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Abstract

A novel mycovirus belonging to the proposed family “*Fusariviridae*” was discovered in *Alternaria solani* by sequencing a cDNA corresponding to double-stranded RNA extracted from this phytopathogenic fungus. The virus was tentatively named “*Alternaria solani* fusarivirus 1” (AsFV1). AsFV1 has a single-stranded positive-sense (+ssRNA) genome of 6845 nucleotides containing three open reading frames (ORFs) and a poly(A) tail. The largest ORF, ORF1, encodes a large poly-peptide of 1,556 amino acids (aa) with conserved RNA-dependent RNA polymerase and helicase domains. The ORF2 and ORF3 have overlapping regions, encoding a putative protein of 522 amino acids (aa) and a putative protein of 105 amino acids (aa), respectively, both of unknown function. A multiple sequence alignment and phylogenetic analysis revealed that AsFV1 could be a new member of the “*Fusariviridae*”. This is the first report of the full-length nucleotide sequence of a fusarivirus that infects *Alternaria solani*.

*Alternaria* is a genus that includes a complex group of filamentous fungi, some of which cause significant damage in a wide range of crops [1]. Early blight, caused by *Alternaria solani*, is an important foliar disease of potato worldwide. Although many fungal viruses have been isolated from *Alternaria* spp., including *Alternaria longipes* dsRNA virus 1 (AIRV1) [2], *Alternaria alternata* chrysovirus 1 (AaCV1) [3], *Alternaria alternata* partitivirus 1 (AtPV1) [4], *Alternaria dianthicola* dsRNA virus 1 (AdRV1) [5], *Alternaria arborescens* victorivirus 1 (AaVV1) [6], *Alternaria alternata* hypovirus 1 (AaHV1) [7], and *Alternaria brassicicola* fusarivirus 1 (AbFV1) [8], the isolation of a fusarivirus from *Alternaria solani* has not yet been reported.

Mycoviruses, also called fungal viruses, are widespread in many simple eukaryotic organisms, such as yeasts, mushrooms, oomycetes, and filamentous fungi, including phytopathogenic fungi [1, 9, 10]. The majority of fungal viruses typically cause latent infections, but several mycoviruses can cause hypovirulence in their hosts, making them potential biocontrol agents for combating plant fungal disease [11–13]. Most fusarviruses infect their hosts without causing any phenotypic changes. However, several fusarviruses, such as a 7.5-kbp mycovirus isolated from *Fusarium graminearum* strain DK21, are associated with reduced mycelium growth, increased pigmentation, reduced virulence to wheat, and reduced (60-fold) production of trichothecone mycotoxins [14].

The establishment of the family “*Fusariviridae*” was proposed to include *Fusarium graminearum* virus-dsRNA mycovirus 1 (FgV1) (the prototype of the “*Fusariviridae*”) and other similar positive single-stranded RNA (+ssRNA) mycoviruses [15]. Currently, the NCBI database includes the following members of the proposed family “*Fusariviridae*”: Auricularia heimer fusarivirus 1 (AhFV1), Nigrospora oryzae fusarivirus 1 (NoFV1), Alternaria brassicicola fusarivirus 1 (AbFV1), Botrytis cinerea fusarivirus 1 (BeFV1), Botryosphaeria dothidea fusarivirus 1 (BdFV1), Neofusisococum luteum fusarivirus 1 (NIFV1), Sordoiymes alkalinus fusarivirus 1 (SaFV1), Rosellinia necatrix fusarivirus 1 (RnFV1), Sclerotinia sclerotiorum fusarivirus 1 (SsFV1), Fusarium graminearum dsRNA mycovirus 1 (FgV1), Pleospora typhicola fusarivirus 1 (PtFV1), Penicillium aurantiogriseum fusarivirus 1 (PaFV1), Macrophomina phaseolina single-stranded RNA virus 1 (MpRV1), and Penicillium roqueforti ssRNA mycovirus 1 (PrRV1).
The genomes of viruses in this family are +ssRNA, with sizes of 6–10 kb and one to three ORFs. [15, 16].

Here, we report the molecular characterization of a novel +ssRNA mycovirus tentatively named “Alternaria solani fusarivirus 1” (AsFV1). Sequence analysis showed that AsFV1 is closely related to other putative fusariviruses. We think that AsFV1 could be included as a new member of the proposed family “Fusariviridae”.

Provenance of the virus material

*Alternaria solani* strain ZY-D was isolated from a potato leaf infected with early blight in Inner Mongolia Autonomous Region, China, in 2020. Strain ZY-D was cultured on potato dextrose agar (PDA) at 28 °C for 7 days, and the mycelium plugs were placed in potato dextrose broth for culture and shaken at 28 °C and 180 rpm for 5–7 days.
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Subsequently, dsRNA was extracted from mycelium using the CF-11 cellulose chromatography method as described by Morris and Dodds, with appropriate adjustments [17]. The sample was then treated with DNase I and S1 nuclease (Takara, Dalian, China) at 37 °C for 20 min to digest DNA and ssRNA, and electrophoresis was performed on a 1.5% agarose gel. Lane M, DNA marker (Trans 15K DNA Marker); lane 1, dsRNA sample after treatment with both RNase-free DNase I and S1 nuclease. The AsFV1 band is indicated by a red arrow. (B) Graphical representation of the genome organization of AsFV1. The AsFV1 genome is 6828 nt in length and contains three ORFs (ORF1, ORF2, and ORF3). Open reading frames (ORF) are represented by boxes. The sizes of the encoded proteins and of the 5′ and 3′ untranslated regions (UTR) are indicated in amino acids and nucleotides, respectively. The conserved RNA-dependent RNA polymerase (RdRp) and RNA helicase (Hel) domains in the ORF1-encoded protein are represented by gray and blue shading, respectively. (C) Phylogenetic tree based on the RdRp domains of AsFV1 and related viruses, constructed by the maximum-likelihood method with 1000 bootstrap replicates in MEGA 7.0 software. Bootstrap values higher than 50% are shown. AsFV1 is indicated by a black star. The virus names and their GenBank accession numbers are as follows: Pleospora tychipica fusarivirus 1, YP_009182158.1; Plasmodora viticola lesion associated fusarivirus 1, QHD64725.1; Plasmodora viticola lesion associated fusarivirus 3, QHD64735.1; "Fusariviridae sp.", QDH7553.1; Neurospera discreta fusarivirus 1, AZT88657.1; Sodomyces alkalinus fusarivirus 1, YP_009551681.1; Fusarium poae fusarivirus 1, YP_009279206.1; Sodomyces alkalinus fusarivirus 1, ATP75827.1; Sodomyces alkalinus fusarivirus 1, ATP75829.1; Rosellinia necatrix fusarivirus 1, YP_009047147.1; Penicillium roqueforti ssRNA mycovirus 1, YP_009051683.1; Macrophomina phaseolina single-stranded RNA virus 1, ALD89094.1; Fusarium graminearum dsRNA mycovirus-1, QKH095871.1; Fusarium graminearum dsRNA mycovirus-1, YP_223920.2; Monilinia barmarnavirüs 1, QED43004.1; Botryosphaeria dothidea fusarivirus 1, QKH00151.1; Rutsstromia firma fusarivirus 1, AZT88659.1; Phaseolina single-stranded RNA virus, QG003445.1; "Fusariviridae sp.", QDH88266.1; Cryphonectria hypovirus 4, YP_138519.1; Cryphonectria hypovirus 3, AAF13603.1; Phomopsis longicola hypovirus, YP_009051683.1; Valsa ceratospella hypovirus 1, YP_005476604.1

AsFV1 was deposited in the GenBank database with the accession number MW544173.

The National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/genomes) was used to perform sequence comparisons and identify ORFs and conserved domains. Multiple sequence alignments were made using the ClustalX program [19] and annotated using the GeneDoc program [20]. A phylogenetic tree was constructed using the NJ method in MEGA 7.0 with 1000 bootstrap replicates [21].

Sequence properties

AsFV1 has a single genome segment (Fig. 1A). The full-length genome sequence of AsFV1 is 6,828 nt in length, excluding the poly(A) tail, and the base composition is 25% A, 22% C, 23% G, and 30% U. The 5′-UTR and 3′-UTR are 12 and 200 nt in length, respectively. The genome organization of AsFV1 is shown in Fig. 1B. ORF1 encodes a large polypeptide of 1,556 amino acids (aa) with conserved RNA-dependent RNA polymerase (RdRp; aa 502 to 835) and helicase (aa 1073 to 1356) domains and an approximate molecular mass of 174.96 kDa, predicted using the CDsearch program on the NCBI website. BLASTp results showed that the RdRp of AsFV1 was the most similar to those of Pleospora tychipica fusarivirus 1 (GenBank accession no. YP_009182158, 64.99% identity, 97% query coverage), Erysiphe necator associated fusarivirus 1 (GenBank accession no. QIR30275.1, 63.97% identity, 96% query coverage), Plasmodora viticola lesion associated fusarivirus 1 (GenBank accession no. QHD64725.1, 63.97% identity, 96% query coverage), and Plasmodora viticola lesion associated fusarivirus 1 (GenBank accession no. QHD64735.1, 64.82% identity, 93% query coverage). The amino acid sequence similarity of the RdRp to the corresponding proteins of putative "Fusariviridae" members was limited, and the highest identity level was 64.99%, to the RdRp of Pleospora tychipica fusarivirus 1.

The second and third ORFs have overlapping regions, encoding a putative protein of 522 amino acids (aa) and a putative protein of 105 amino acids (aa) respectively, both of unknown function. The approximate molecular mass of ORF2 and ORF3 is 58.28 kDa and 12.43 kDa, respectively. Amino acid sequence alignment results showed that the virus with the most similarity in ORF2 was Pleospora tychipica fusarivirus 1, with only 48.20% sequence identity, and the virus with the most similarity in ORF3 was ORF-C of Erysiphe necator associated fusarivirus 1, with 32.14% identity. In order to determine the relationship between AsFV1 and other fungal viruses, a phylogenetic tree was constructed based on amino acid sequence of the RdRp domain of AsFV1 and other selected viruses.
including hypoviruses. All of the amino acid sequences clustered into groups corresponding to the proposed family “Fusariviridae” and the family Hypoviridae. AsFV1 was in the “Fusariviridae” cluster, and its closest neighbors in the tree were Pleospora tephicola fusarivirus 1, Plasmopara viticola lesion associated fusarivirus 1, and Plasmopara viticola lesion associated fusarivirus 3, all of which were separate from members of the genus Hypovirus (Fig. 1C) A multiple alignment and comparison of the RdRp domains of AsFV1 and other selected viruses showed that there were eight typical conserved motifs in the RdRp domains of fungal virus and viruses infecting lower eukaryotes (Fig. 2).

Sequence comparisons and phylogenetic analysis indicated that ASFV1 has all of the typical characteristics of a fusarivirus, suggesting that ASFV1 could be a new member of the proposed family “Fusariviridae”.

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Declarations

Conflict of interest All authors declare no conflicts of interest.

Ethical approval This study did not include experiments with human participants or animals performed by any of the authors.

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