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 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 6,845 nucleotides containing three open reading frames (ORFs) and a poly(A) tail. The largest ORF, ORF1 encodes a large polypeptide 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, for which function is unknown now. Multiple sequence alignments and phylogenetic analysis revealed AsFV1 belonging to *Fusariviridae*. This is the first report of the full-length nucleotide sequence of a fusarivirus infected with *Alternaria solani*.

Introduction

Alternaria spp. is a ubiquitous genus of fungi that includes a complex group of filamentous fungi, some of which cause significant damage across a wide range of crops [1]. Early blight, caused by *Alternaria solani* is an important foliar disease of potato worldwide. At present, many fungal viruses isolated and identified from *Alternaria* including *Alternaria longipes* dsRNA virus 1 (AIRV1) [4], *Alternaria alternata* chrysovirus 1 (AaCV1) [5], *Alternaria alternata* partitivirus 1 (AtPV1) [6], *Alternaria dianthicola* dsRNA virus 1 (AdRV1) [7], *Alternaria arborescens* victorivirus 1 (AaVV1) [8], *Alternaria alternata* hypovirus 1 (AaHV1) [9], *Alternaria brassicicola* fusarivirus 1 (AbFV1) [10] etc. However, the isolation of fusarivirus from *Alternaria solani* has not 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 [11–13]. The majority of fungal viruses typically exist as latent infections, but several mycoviruses can cause hypovirulence of their hosts, which are potential biocontrol agents to combat plant fungal disease [14–16]. Most fusariviruses infect their hosts without causing any morphological changes. However, several fusariviruses have phenotypic changes on their hosts, such as a 7.5kbp dsRNA branching virus isolated from Gramineae strain DK21, which is associated with fungal morphological changes. These include reduced mycelium growth, increased pigmentation, reduced virulence to wheat, and reduced (60-fold) production of trichothecene mycotoxins [25].

The family *Fusariviridae* was proposed for encompassing the *Fusarium graminearum* virus-DK21 (FgV1) (the prototype of *Fusariviridae*) and other similar + ssRNA mycoviruses [17]. Up to date, the family *Fusariviridae* in NCBI database including *Auricularia heimuer* fusarivirus 1 (AhFV1), *Nigrospora oryzae* fusarivirus 1 (NoFV1), *Alternaria brassicicola* fusarivirus 1 (AbFV1), *Botrytis cinerea* fusarivirus 1 (BcFV1), *Botryosphaeria dothidea* fusarivirus 1 (BdFV1), *Neofusicoccum luteum* fusarivirus 1 (NIFV1), *Sodiomyces 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), *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 [18–19].

Here, we report the molecular characterization of a novel + ssRNA mycovirus *Alternaria solani* fusarivirus 1 (AsFV1). Sequence analysis showed that AsFV1 is closely related to other putative fusariviruses. We suggest AsFV1 to be placed into 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, the mycelium plugs were placed in potato dextrose broth for culture and shaken at 28°C 180 rpm for 5–7 days. Subsequently, dsRNA was extracted from mycelium using the CF-11 cellulose chromatography method as described by Morris and Dodds, with appropriate adjustments [20]. Then DNase I and S1 nuclease (Takara, Dalian, China) were used for digestion at 37°C for 40min to remove DNA and ssRNA of the extracted dsRNA,

then electrophoresis was performed on 1.5% (w/v) agarose gel, stained with Goldview and visualized under an UV trans-illuminator. A fragment of dsRNA banding with the size ranging from 5kbp to 7kbp was found and purified. Random hexanucleotide primers (5'-CGATCGATCATGATGCAATGCNNNNNN - 3') and reverse transcriptase were used to construct a cDNA library. Specific primers based on acquired cDNA sequence were used for RT-PCR amplification to fill the sequence gap, and the end sequences were acquired through a ligase-mediated terminal amplification [21]. All of the amplified cDNA products were cloned into pMD18-T vector (Takara, Dalian, China) and transferred into *E. coli* DH-5α for sequencing. Each product was sequenced by at least 3 independent clones to ensure the accuracy of the sequence. The complete genome sequence of AsFV1 was deposited in the GenBank database with the accession number (MW544173).

Using the national center for biotechnology information (NCBI) database (<http://www.ncbi.nlm.nih.gov/genomes>) analyze sequence, including the homology comparison, ORFs finder and conserved domain analysis. Multiple sequence alignments were conducted using ClustalX program [22] and annotated using the GeneDoc program [23]. Phylogenetic trees were constructed using the NJ method in MEGA 7.0, with a bootstrap test of 1000 replicates [24].

Sequence Properties

The full-length cDNA sequence of AsFV1 is 6,828 nt excluding a poly (A) tail and the base composition is 25% A, 22% C, 23% G, and 30% T. The lengths of the 5'-UTRs and 3'-UTRs were 12 and 200 bp, respectively. The genomic organization of AsFV1 is shown in Fig. 1A. The ORF1 encodes a large polypeptide of 1,556 amino acids (aa) with conserved RNA-dependent RNA polymerase (aa position 502

to 835) and helicase domains (aa position 1073 to 1356) and an approximate molecular mass of 174.96 kDa by using CDsearch program of NCBI website. The BLASTP research showed that the RdRp of AsFV1 was the most similar to the RdRp of these viruses: *Pleospora typhicola* 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), *Plasmopara viticola* lesion associated fusarivirus 1 (GenBank accession No. QHD64725.1, 63.97% identity, 96% query coverage), *Plasmopara viticola* lesion associated fusarivirus 3 (GenBank accession No. QHD64735.1, 64.82% identity, 93% query coverage). The amino acid sequence identity level of the RNA-dependent RNA polymerase and the corresponding proteins of *Fusariviridae* members was limited and the maximum identification level was 64.99% compared with the *Pleospora typhicola* fusarivirus 1.

The second and third ORF have overlapping regions, encoding a putative protein of 522 amino acids (aa) and a putative protein of 105 amino acids (aa) respectively, for which function is unknown now. The approximate molecular mass of ORF2 and ORF3 were 58.28 kDa and 12.43 kDa respectively. Amino acid sequence alignment results showed that the virus most similar to ORF2 was *Pleospora typhicola* fusarivirus 1 with a similarity of only 48.20%. 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 virus, including hypoviruses. All amino acid sequences were divided into *Fusariviridae* and *Hypoviridae*. AsFV1 was classified into *Fusariviridae* and closely related to *Pleospora typhicola* fusarivirus 1, *Plasmopara viticola* lesion associated fusarivirus 1 and *Plasmopara viticola* lesion associated fusarivirus 3, and distinct from the genus of *Hypovirus* (Fig. 1B). Multiple alignments and comparisons 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 infection in the lower eukaryotes (Fig. 2)

Through homology comparisons and phylogenetic analysis, AsFV1 was consistent with all typical characteristics of fusarivirus, therefore AsFV1 belonged to a new member of *Fusariviridae*.

Declarations

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Compliance with ethical standards

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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Figures

Figure 1

Genomic organization and phylogenetic relationships of *Alternaria solani* fusarivirus 1 (AsFV1). (A) Graphical representation of the genome organization of AsFV1. The AsFV1 genome is 6828 bp in length and contains three ORFs (ORF1–ORF2 and ORF3). Open reading frames (ORF) are represented by boxes. The sizes of the deduced proteins and of the 5' and 3' untranslated regions (UTR) are indicated in amino acids and nucleotides, respectively. Conserved RNA-dependent RNA polymerase (RdRp) and RNA helicase

(Hel) domains in the ORF1-encoded protein are represented by gray shading and blue shading respectively. (B) Phylogenetic analysis based on the RdRps domains of AsFV1 using the neighbor-joining (NJ) method with 1,000 bootstrap replicates by MEGA 7.0 software. Bootstrap values higher than 50% are shown. The scale bar represents a genetic distance of 0.2 amino acid substitutions per site. The AsFV1 was indicated by black asterisk. The virus names and their GenBank accession numbers are as follows: *Pleospora typhicola* fusarivirus 1 YP_009182158.1 Plasmopara viticola lesion associated fusarivirus 1 QHD64725.1 Plasmopara viticola lesion associated fusarivirus 3 QHD64735.1 Fusariviridae sp. QDH87553.1 *Neurospora discreta* fusarivirus 1 AZT88657.1 *Sodiomyces alkalinus* fusarivirus 1 YP_009551681.1 *Fusarium poae* fusarivirus 1 YP_009272906.1 *Sodiomyces alkalinus* fusarivirus 1 ATP75827.1 *Sodiomyces alkalinus* fusarivirus 1 ATP75829.1 *Rosellinia necatrix* fusarivirus 1 YP_009047147.1 *Penicillium roqueforti* ssRNA mycovirus 1 YP_009052456.1 *Macrophomina phaseolina* single-stranded RNA virus 1 ALD89094.1 *Fusarium graminearum* dsRNA mycovirus-1 QKX95871.1 *Fusarium graminearum* dsRNA mycovirus-1 YP_223920.2 *Monilinia barnavir* J QED43004.1 *Botryosphaeria dothidea* fusarivirus 1 QHI00151.1 *Rutstroemia firma* fusarivirus 1 AZT88659.1 *Phaseolina* single-stranded RNA virus QGZ00445.1 *Fusariviridae* sp. QDH88266.1 *Cryphonectria hypovirus* 4 YP_138519.1 *Cryphonectria hypovirus* 3 AAF13603.1 *Phomopsis longicolla* hypovirus YP_009051683.1 *Valsa ceratosperma* hypovirus 1 YP_005476604.1.

Figure 2

Multiple alignments of the aa sequences of viral RdRp domains that are characteristic of positive ssRNA viruses between AsFV1 and other similar viruses (PtFV1, SaFV1, RnFV1, PrRV1, MpRV1). The alignment was carried out using CLUSTAL X and highlighted with JAVAVIEW application. Number (I-VIII) represents the eight conserved motifs of RdRp in these reference viruses.

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