Complete genome sequence of the first chrysovirus from the phytopathogenic fungus *Alternaria solani* on potato in China

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Received: 15 July 2021 / Accepted: 25 August 2021 / Published online: 8 October 2021
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Abstract

The complete genome sequence of a double-stranded RNA (dsRNA) mycovirus that was isolated from *Alternaria solani* strain DT-10 causing potato foliar disease was determined. The virus, designated as "*Alternaria solani* chrysovirus 1" (AsCV1), has four dsRNA segments (dsRNA 1-4) with a length of 3600 bp, 3128 bp, 2996 bp, and 2714 bp, respectively. The RNA-dependent RNA polymerase (RdRp, 1084 amino acids [aa]), putative capsid protein (905 aa), alphachryso-P3 (835 aa), and alphachryso-P4 (729 aa) were encoded by dsRNA1, dsRNA2, dsRNA3, and dsRNA4, respectively, which had the highest sequence identity of 41.77%-72.38% to their counterparts in *Helminthosporium victoriae* virus 145S (HvV145S) of the genus *Alphachrysovirus*, family *Chrysoviridae*. Moreover, the 5′-untranslated regions (UTRs) of AsCV1 dsRNA 1-4, which contained several unique inserts (3-37 bp) and deletions (5-64 bp), shared 51.65%-68.01% identity with those of HvV145S. Phylogenetic analysis based on RdRp sequences showed that AsCV1 clustered the most closely with HvV145S. Considering its distinct host specificity, the low sequence similarity of its encoded proteins to those of other viruses, the unusual features of the 5′-UTRs of its dsRNA 1-4, and the phylogenetic position of its RdRp gene, AsCV1 should be considered a member of a new species in the genus *Alphachrysovirus*. To the best of our knowledge, this is the first alphachrysovirus identified from phytopathogenic *A. solani*.

Mycoviruses infecting fungi and oomycetes can have various genome types, including double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), and DNA [1]. dsRNA mycoviruses belong to eight families, including *Chrysoviridae*, *Endornaviridae*, *Megabirnaviridae*, *Partitiviridae*, *Quadriviridae*, *Reoviridae*, *Totiviridae*, and *Polymycoviridae*, as well as the newly established genus *Botybirnavirus* [2–4]. The family *Chrysoviridae* includes two genera, namely, *Alphachrysovirus* (20 species) and *Betachrysovirus* (11 species). Members of the family *Chrysoviridae* possess 3-7 dsRNA segments, with each segment being encapsulated separately. Among these dsRNA segments, dsRNA1 and dsRNA2 encode the RNA-dependent RNA polymerase (RdRp) and capsid protein (CP), respectively. Hosts of *Chrysoviridae* family members include fungi, plants, and possibly insects [5].

In 1988, viral dsRNAs were detected in *Alternaria alternata* isolated from Japanese pear trees, representing the first mycovirus reported in *Alternaria* [6]. Since then, many mycoviruses have been identified in *A. alternata*, including *Alternaria alternata* virus 1 (AAV-1) [7], *Alternaria alternata* chrysovirus 1 (AaCV1) [8], *Alternaria alternata* parvitivirus 1 (AtPV1) [9], *Alternaria alternata* botybirnavirus 1 (AaBRV1) [3], *Alternaria alternata* hypovirus 1 [10], and *Alternaria alternata* victorivirus 1 [11]. In addition, several mycoviruses, including *Alternaria tenuissima* parvitivirus 1 (AtPV1) [12], *Alternaria brassicicola* endornavirus 1 (AbEv1) [13], *Alternaria longipes* dsRNA virus 1 [14], *Alternaria arborescens* victorivirus 1 [15], and *Alternaria dianthicola* dsRNA virus 1 (AdRV1) [16], had been reported in five other species of *Alternaria* (*A. tenuissima*, *A. brassicicola*, *A. longipes*, *A. arborescens*, and *A. dianthicola*). Recently, an ssRNA mycovirus named *Alternaria solani* fusarivirus 1 (AsFV1) was isolated from *A. solani* [17].

Handling Editor: Ioly Kotta-Loizou.
In our previous study, *A. solani* was documented to cause potato early blight in China [18, 19]. In this study, *A. solani* strain DT-10, which was recovered from diseased leaves of potato with the symptoms of early blight in the city of Datong, Shanxi province, China, was identified using methods described previously [18, 19]. A new alphachrysovirus was found to be associated with *A. solani* and was designated as "Alternaria solani chrysovirus 1" (AsCV1).

**Provenance of the virus material**

*Alternaria solani* strain DT-10 was cultured on potato dextrose agar (PDA) plates overlaid with cellophane membranes for 7 days in the dark at 25°C. Multiple dsRNAs were extracted from the mycelia by binding to CF-11 cellulose powder in 15% ethanol as described by Morris and Dodds [20]. After treatment with S1 nuclease and DNase I (TaKaRa, Dalian, China), the dsRNAs were analyzed by 1.0% agarose gel electrophoresis (Fig. 1A) and purified using a gel extraction kit (Aidlab Biotechnologies, Beijing, China). Partial complementary DNA (cDNA) sequences of the dsRNA segments were obtained and assembled as described previously [21]. Using specific primers designed based on the cDNA sequences (Supplementary Table S1), the terminal sequences were determined by the RNA-ligase-mediated rapid amplification of cDNA ends (RLM-RACE) method as described previously [22, 23]. The full genome sequence of AsCV1 was obtained by assembling all of the partial cDNA and terminal sequences using DNAMAN 7.0 (Lynnon Biosoft, USA) and was deposited in the GenBank database with the accession numbers MW656210, MW656211, MW656212, and MW656213 for dsRNA1, dsRNA2, dsRNA3, and dsRNA4, respectively.

**Sequence properties**

The full sequences of the four dsRNA segments are 3,600 bp, 3,128 bp, 2,996 bp, and 2,714 bp in length, respectively. The open reading frame (ORF) of each dsRNA sequence was analyzed using the ORFfinder online tool (https://www.ncbi.nlm.nih.gov/orffinder/). Each dsRNA contains a single ORF, which was named ORF1, ORF2, ORF3, and ORF4 for dsRNA1, dsRNA2, dsRNA3, and dsRNA4, respectively (Fig. 1B). BLASTp searches for the four ORFs showed that they were most similar to their counterparts in Helminthosporium victoriae virus 145S (HvV145S) of the genus *Alphachrysovirus*, family *Chrysoviridae*. ORF1 encodes a protein of 1084 amino acids (aa) (124.56 kDa) with 72.38% sequence identity to the RdRp of HvV145S-A9 (GenBank no. NC_005978). The predicted RdRp domain at aa 351-803 contains eight conserved motifs (Supplementary Fig. S1A). The protein encoded by ORF2 contains 905 aa (102.29 kDa) and shows 61.40% sequence identity to the putative CP of Bipolaris maydis chrysovirus 1 (BmCV1) (GenBank no. KY489955), an isolate from HvV145S [24]. BLASTp analysis also revealed that the ORF3-encoded protein (835 aa, 92.84 kDa) and the ORF4-encoded protein (729 aa, 82.31 kDa) had 69.84% sequence identity to alphachryso-P3 of HvV145S-A9 and 41.77% identity to alphachryso-P4 of BmCV1 (Supplementary Table S2). The functions of alphachryso-P3 and alphachryso-P4 are not clear at present [5]. ProDom database searches revealed that the N-terminal region in P3 of Penicillium chrysogenum virus (Pcv) had significant similarity to the corresponding N-terminal regions of the RdRps of Pcv and HvV145S-A9 [25]. Meanwhile, a phytoere S7 domain, which has been found in several phytoereovirus S7 proteins, has been reported in P3 of Pcv [25] as well as in the protein encoded by dsRNA4 of cherry chlorotic rusty spot associated chrysovirus, HvV145S-A9, and other members of the family *Chrysoviridae* [26, 27]. Results of searches for alphachryso-P3 and alphachryso-P4 of AsCV1 in the ProDom database revealed that a conserved domain (aa 104-260, E value, 6e-06) related to RdRp of Pcv is present in alphachryso-P4 of AsCV1. Furthermore, a conserved phytoere S7 domain has also been found in alphachryso-P4 of AsCV1 in a multiple sequence alignment of alphachryso-P4 of AsCV1 and P3 or P4 of other chrysoviruses made using ClustalX 2.0 [25–27].

The 5′ untranslated regions (UTRs) and 3′-UTRs of AsCV1 are 242 bp and 103 bp long in dsRNA1, 312 bp and 98 bp long in dsRNA2, 317 bp and 171 bp long in dsRNA3, and 315 bp and 209 bp long in dsRNA4, respectively (Supplementary Table S2). Multiple alignments of the 5′-UTR and 3′-UTR sequences of the four dsRNAs were performed using DNAMAN 7.0 (Lynnon Biosoft). The 5′-UTRs showed strong sequence similarity in their internal parts, sharing the strictly conserved terminal sequence CGATAAAACAAAAA. Meanwhile, the 3′-UTRs of the four dsRNA segments shared the conserved sequence GCT TTAAGTGT at their termini (Supplementary Fig. S1B). The 5′-UTRs and 3′-UTRs of AsCV1 dsRNA1-4 had 51.65%-68.01% and 30.04%-70.34% identity to those of HvV145S-A9 and BmCV1. Intriguingly, several unique inserts (3-37 bp) and deletions (5-64 bp) were found in the 5′-UTRs of AsCV1, which were different from those of HvV145S-A9 and BmCV1 (Fig. 2).

The RdRp sequences of AsCV1 and 24 members of the family *Chrysoviridae* were analyzed using ClustalX 2.0 and MEGA 6.0 [23]. A phylogenetic tree constructed by the neighbor-joining algorithm with 1000 replicates based on the RdRp sequences showed that AsCV1 formed a well-supported taxonomic cluster with the members of the genus
A new chrysovirus from *Alternaria solani* and was closely related to HvV145S-A9 and BmCV1 (Fig. 1C).

According to the ICTV, the host of isolation, nucleotide and deduced amino acid sequence data, the length of genome segments, and 5′-UTRs are useful species demarcation criteria for the genus *Alphachrysovirus* [5]. Based on the host fungus *A. solani*, the low sequence similarity of the encoded proteins, the unusual characteristics of the 5′-UTRs, and the phylogenetic relationships to known members of the genus *Alphachrysovirus*, AsCV1 should be considered a novel member of the genus *Alphachrysovirus*. This is the first report of the complete genome sequence of a new chrysovirus infecting *A. solani*.
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00705-021-05263-z.

Funding This work was financially supported by the National Key Research and Development Program of China (2018YFD0200803).

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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

**Fig. 2** Multiple sequence alignment of the 5′-untranslated regions (UTRs) of dsRNA1-4 of AsCV1, Helminthosporium victoriae virus 145S (HvV145S-A9), and Bipolaris maydis chrysovirus 1 (BmCV1). Dark blue and green represent 100% and 67% nucleotide sequence identity, respectively. The red stars indicate AsCV1.
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