Molecular Characterization of a Novel Single-stranded RNA Virus, ChRV1, Isolated From the Plant Pathogenic Fungus *Colletotrichum Higginsianum*

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Research Article

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Abstract

In this study, a novel single-stranded RNA virus was isolated from the plant pathogenic fungus, *Colletotrichum higginsianum* strain HTC-5, named “Colletotrichum higginsianum ssRNA virus 1” (ChRV1). The complete genome of ChRV1 is 3850 bp in length with a GC content of 52 % and encodes two in-frame open reading frames (ORFs): ORF1 (smaller) and ORF2 (larger). ORF1 encodes a protein with highest similarity to proteins encoded by Phoma matteucciicola RNA virus 1 (PmRV1, 47.99% identity) and Periconia macrospinosa ambiguivirus 1 (PmAV1, 50.73% identity). ORF2 encodes a protein with a conserved RNA-dependent RNA polymerase (RdRp) domain with similarity to RdRps of PmRV1 (61.41% identity) and PmAV1 (60.61% identity), which are unclassified (+)ssRNA mycoviruses reported recently. Phylogenetic analysis of the RdRp domain suggested that ChRV1 grouped together with PmRV1, PmAV1 and other unclassified (+)ssRNA mycoviruses, and had a distant relationship to invertebrate viruses and plant viruses of the family *Tombusviridae*. This is the first report of a novel (+)ssRNA virus infecting the phytopathogenic fungus *C. higginsianum*.

Introduction

Mycoviruses (also called fungal virus) have been identified in all major groups of fungal groups [1, 2]. The majority of mycoviruses are mainly include four groups: double-stranded RNA (dsRNA), positive-sense single-stranded RNA (+ssRNA), negative-sense single-stranded RNA (-ssRNA) and single-stranded DNA (ssDNA) genomes [3]. Due to the rapidly development of high-throughput sequencing technologies, a growing number of +ssRNA mycoviruses have been discovered recently, among which have not been assigned to formal taxa. For example: Trichoderma harzianum ssRNA virus 1 (ThAV1), Setosphaeria turcica ssRNA virus 1 (StAV1), Verticillium longisporum ssRNA virus 1 (VlAV1) and Periconia macrospinosa ssRNA virus 1 (PmAV1) [4-6], which were unclassified (+)ssRNA mycoviruses. Gilbert KB et al. [6] recently proposed the establishment of the family *Ambiguiviridae* that contains the above viruses and other discovered related viruses. Most mycoviruses are inconspicuous, causing little phenotypic effects in their fungal hosts. However, a few mycoviruses can induce phenotypic alterations, causing hypovirulence of their fungal host, which have potential as biological control agents of fungal diseases. The best exemplified example was the successful use of Cryphonectria hypovirus 1 (CHV1) to control chestnut blight disease in Europe [7].

Colletotrichum higginsianum is a hemibiotrophic pathogen and responsible for anthracnose disease on cruciferous plants, such as species of *Brassica* and *Raphanus* [8], also including *Arabidopsis*. The *Arabidopsis*/*C. higginsianum* pathosystem has emerged as an attractive model to study plant–pathogen interactions [9]. The discovery and identification of mycoviruses in *C. higginsianum* is an effective way to search potential biological control agents against anthracnose disease. To date, only two mycoviruses have been characterized from this fungus. Colletotrichum higginsianum non-segmented dsRNA virus 1 (ChNRV1), identified in a *C. higginsianum* strain IMI349063A in United States of America [10], and Colletotrichum higginsianum mitovirus 1 (ChMV1), which we recently reported from a *C. higginsianum* strain HTC-5.

In this study, we reported the characterization of a first novel (+) ssRNA mycovirus from *C. higginsianum* strain HTC-5, which we have provisionally named Colletotrichum higginsianum ssRNA virus 1 (ChRV1).

Provenance Of The Virus Material

The *C. higginsianum* strain HTC-5 was isolated from a cabbage leaf with anthracnose disease in Changsha, Hunan Province, China, and identified as *C. higginsianum* according to our previous study. This fungus was cultured on potato dextrose broth at 28°C in the dark for extraction of dsRNA. The dsRNA was extracted as described by Morris and Dodds using the CF-11 cellulose chromatography method [11]. Then the extracted dsRNA was digested with DNase I and S1 nuclease (Takara, Dalian, China) to eliminate DNA and ssRNA prior to gel electrophoresis and visualization. A dsRNA segment of approximately 3.9 kbp in size was observed and purified (Fig. 1a) using a FastPure® Gel DNA Extraction Mini Kit (Vazyme Biotech Co.,Ltd, Nanjing, China). A cDNA library was constructed using reverse transcriptase and random hexanucleotide primers (5'-CGA TCG ATC ATG ATG CAA TGCNNNNNN-3'). Sequence gaps were filled by RT-PCR according to sequence-primers based on the cDNA sequences obtained. The 5'- and 3'-terminal sequences were obtained by adapter ligation and PCR amplification as described previously [12]. All PCR amplicons were cloned into the pMD19-T vector (Takara, Dalian, China), transformed into *Escherichia coli* DH5α cells. Positive clones were selected and sequenced, and each clone was sequenced independently at least three times. The full cDNA sequence was assembled and subjected to GenBank database with accession number of MW218984.

Sequence analysis, such as ORF finding, homology search, and conserved domains search were performed using the website of the National Center for Biotechnology Information (NCBI) database. Multiple alignments were conducted using Clustal X 2.0 program [13] and annotated using the GeneDoc program [14] Phylogenetic tree was constructed using the neighbor-joining method in MEGA 7 Programs [15]. The transmembrane domains were predicted using TMHMM Server v. 2.0.

Sequence Properties

The full genome sequence of ChRV1 is 3850 nt in length, which encodes two noncontiguous ORFs. The 5'- and 3'- untranslated regions (UTRs) of ChRV1 are 633 nt and 559 nt in length, respectively (Fig. 1b).

ORF1 encoded a 347-aa protein with a predicted molecular mass of 38.3 kDa. A BLASTP search showed that the aa sequence of ChRV1 has a maximum identity of 50.73% (E-value: 7e-69; query cover: 78 %) and 47.99% (E-value: 6e-83; query cover: 86 %) to the protein encoded by Periconia macrospinosa ambiguivirus 1 (PmAV1) and Phoma matteucciicola RNA virus 1 (PmRV1), followed by other unclassified (+)ssRNA mycoviruses (Table S1), whose function were unknown. In addition, ORF1 and ORF2 were found in the same frame, the amber stop codon 'UAG' was found at the end of the ORF1, suggesting that the two ORFs are likely to produce a fusion protein with the downstream RdRp via a readthrough strategy, as described previously[6, 16]. Moreover, four possible transmembrane helixs were predicted at the N-terminus of the ORF1 protein (Fig. S1), suggesting that the transmembrane domains are important for (+)

ssRNA viruses and play a role in anchoring viral protein to host membrane, as proposed for MoVA and VdRV [16, 17].

ORF2 was predicted to encode a 495-aa protein with a predicted molecular mass of 56.2 kDa. A BLASTP search showed that the aa sequence of ORF2 is most closely related to the RdRps of PmRV1 (Identity:61.41%, Coverage:98%; E value:0.0) and PmAV1 (Identity:60.61%, Coverage:92%; E value:0.0), followed by other unclassified (+)ssRNA mycoviruses, including Soybean leaf-associated ssRNA virus 2 (SlaRV2) (Identity:58.04%, Coverage:91%; E value:1e-78), Soybean leaf-associated ssRNA virus 3 (SlaRV3) (Identity:58.73%, Coverage:88%; E value:9e-173), MoVA (Identity:54.73%, Coverage:91%; E value:4e-161) and Setosphaeria turcica ambiguivirus 1 (StAV1) (Identity:51.14%, Coverage:95%; E value:1e-156) (Table S1). The RdRp sequence of ChRV1 also shared sequence similarity with RdRps of Hubei tombus-like virus 12, Changjiang tombus-like virus 8, Changjiang tombus-like virus 15 and Wenling tombus-like virus 1, which were invertebrate viruses sequenced recently [18]. Additionally, it was also found to be similar to the RdRps of members of the plant virus in family *Tombusviridae* (Table S1). A conserved domain database (CDD) search and multiple protein alignment indicated that the 56.2-kDa protein encoded by ORF2 contained a conserved viral RdRp domain in the subfamily RdRP_3 (pfam00998) with five conserved motifs (Fig. 1c).

To determine further the relationship between ChRV1 and other mycoviruses, a phylogenetic analysis was constructed using the RdRp aa sequences of ChRV1 and other related RNA viruses. The results indicated that ChRV1 cluster together with PmRV1, PmAV1 and other other unclassified (+)ssRNA mycoviruses, but distinct from the invertebrate viruses and plant viruses (Fig. 2). All these selected unclassified (+)ssRNA mycoviruses and ChRV1 contain two non-overlapping ORFs and the RdRp domain is encoded by ORF2. Moreover, these unclassified (+)ssRNA mycoviruses have more similar genome organization with invertebrate viruses, presenting two in-frame ORFs [17], and differ from members of the family *Tombusviridae*, suggesting that these unclassified (+)ssRNA mycoviruses are more related to invertebrate viruses than plant viruses of the family *Tombusviridae*. Additionally, the ChRV1 RdRp contains the amino acid triplet GDN in motif Ⅲ, which differ from the GDD motif in + ssRNA viruses (Fig. 1c). The same triplet was found in previous studies [16, 19, 20]. Taken together, we concluded that ChRV1 is a novel unclassified (+)ssRNA virus, with closer relationship to members of the recently proposed family "*Ambiguiviridae*".

Declarations

Fundings

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Compliance with ethical standards

Conflict of interest

All authors declare no conflicts of interest.

Ethical approval

This article does not contain any experiments with human participants or animals performed by any of the authors.

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Figures

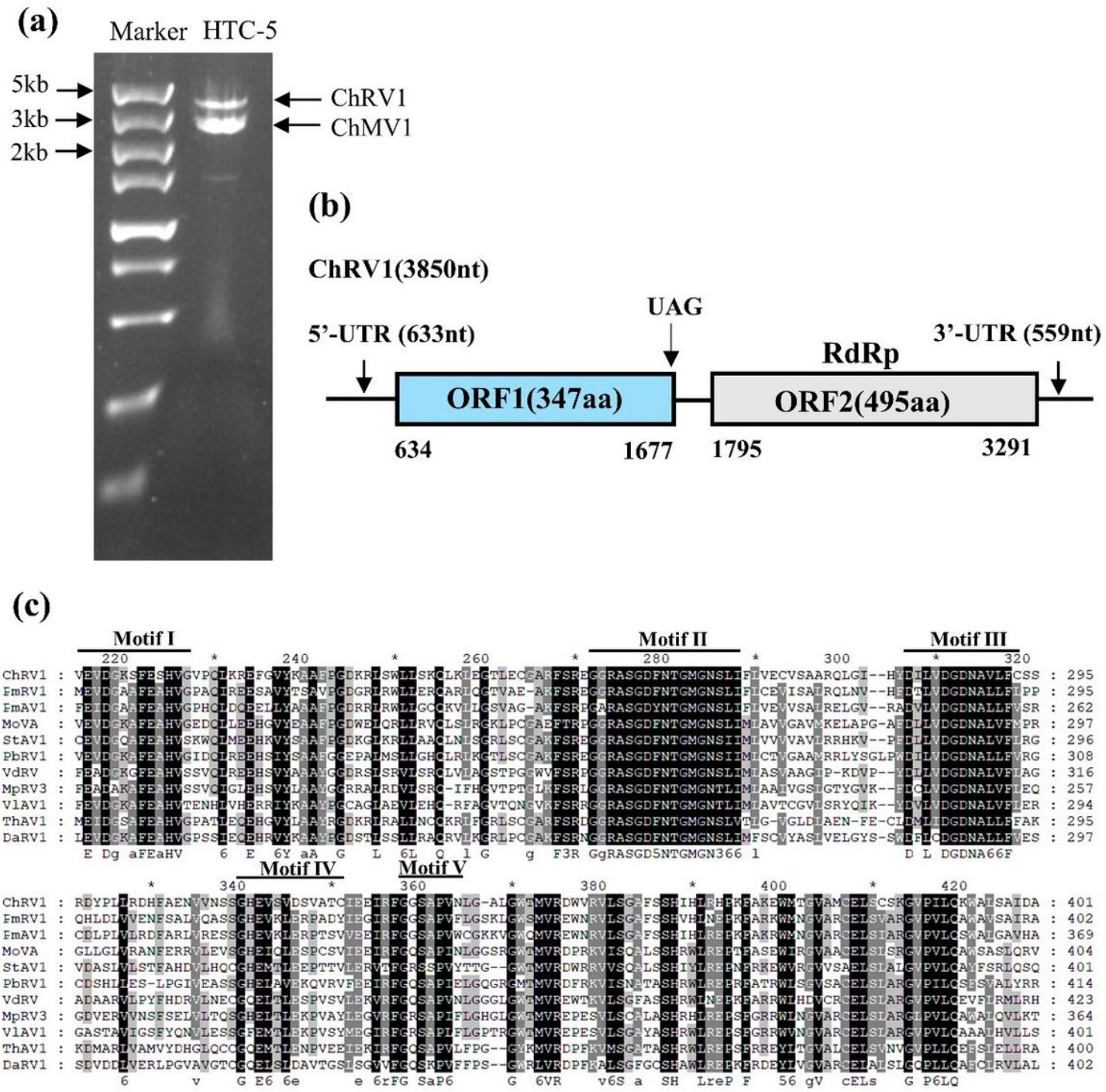

Figure 1

(a) dsRNA 1% agarose gel electrophoresis showing the dsRNA banding pattern of C. higginsianum strain HTC-5. (b) Organization of the genomes of ChRV1. The open reading frames (ORFs) and the untranslated regions (UTRs) are showed as open bars and single lines, respectively. (c) Multiple amino acid sequence alignment of RdRps of ChRV1 and other related mycoviruses. The conserved motifs are indicated by bold black lines and the Roman numerals I to V.

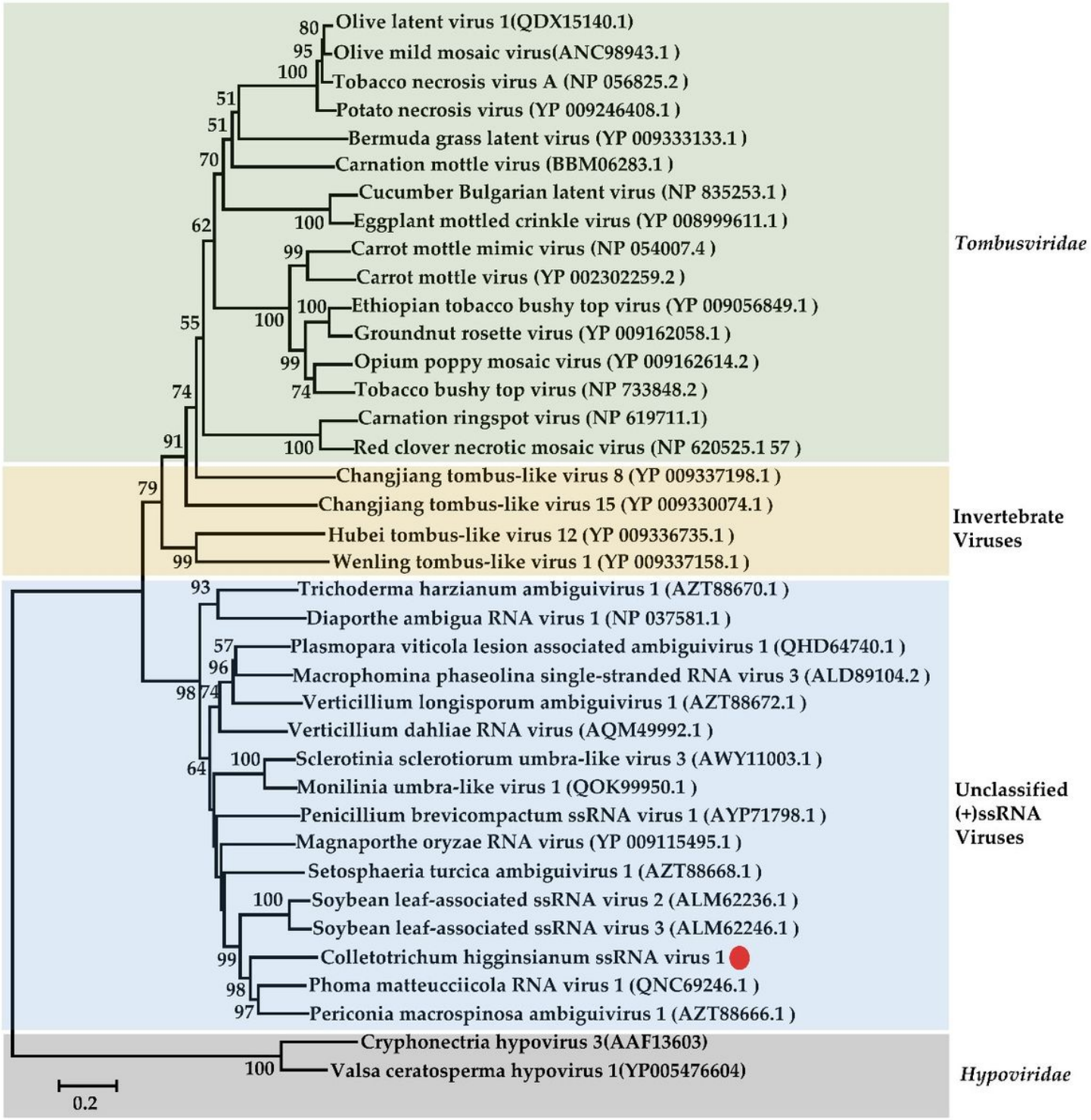

Figure 2

Phylogenetic analysis of ChRV1. The phylogenic tree was constructed based on RdRp aa sequences using MEGA 7 by the neighbor-joining (NJ) method with 1000 bootstrap replications. The scale bar represents a genetic distance of 0.2 amino acid substitutions per site. ChRV1 is indicated in the phylogram by a red dot. Bootstrap values lower than 50% are not shown.

Supplementary Files

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