Molecular Characterization of a Novel Alternavirus Infecting the Entomopathogenic Fungus Cordyceps Chanhua

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Short Report

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Abstract

In this study, a novel double-stranded RNA (dsRNA) mycovirus, named Cordyceps chanhua alternavirus 1 (CcAV1), was detected in the entomogenous fungus *Cordyceps chanhua* from China. The complete genome of CcAV1 contained three dsRNA genome segments, dsRNA 1 (3,512 bp), dsRNA 2 (2,655 bp), and dsRNA 3 (2,415 bp). All the three dsRNAs possess a single open reading frame (ORF). DsRNA 1 with 3,512 bp long encoded a putative RNA-dependent RNA polymerase (RdRp), while dsRNA 2 with 2,655 bp long and dsRNA 3 with 2,415 bp long encoded a hypothetical protein 1 (HP 1) and a hypothetical protein 2 (HP 2), respectively. The RdRp, HP 1 and HP 2 sequences had the highest identity of 66.99%, 49.30% and 56.91%, respectively, to those of Aspergillus foetidus dsRNA mycovirus. A maximum-likelihood phylogenetic tree from RdRp sequence revealed that CcAV1 was placed in the clade of the proposed family "*Alternaviridae*". Hence, we proposed that Cordyceps chanhua alternavirus 1 is a novel member of the proposed "*Alternaviridae*".

Introduction

Most of the mycoviruses have been found to be double-stranded (ds) RNA genomes (Wang et al. 2021). The International Committee on Taxonomy of Viruses (ICTV) (https://talk.ictvonline.org/) have classified dsRNA mycoviruses into eight families including *Amalgaviridae*, *Chrysoviridae*, *Hypoviride*, *Megabirnaviridae*, *Totiviridae*, *Reoviridae*, *Quadriviridae*, and *Partitiviridae*, and genus *Botybirnavirus*. However, the family "*Alternaviridae*" were proposed, but these have not yet been approved by ICTV. At least three genomic segments were contained in *"Alternaviridae"*, and the largest one encoded RNA-dependent RNA polymerase. In 2013, Aspergillus foetidus dsRNA mycovirus (Kozlakidis et al. 2013) was described as a species of the novel mycovirus genus ("*Alternavirus*") and family ("*Alternaviridae*"). To date, six mycoviruses were reported to be members of the proposed family "*Alternaviridae*". However, Fusarium graminearum alternavirus 1 (FgAV1) (He et al. 2018) should be regarded as the strain of the species Fusarium poae alternavirus 1 (FpAV1) (Osaki et al. 2016) due to the two strains with a co-genus host and a high similarity of RdRp, and similar situation was observed between Aspergillus foetidus dsRNA mycovirus (AfVF) and Aspergillus mycovirus 341 (AsV341) (Hammond et al. 2008). Therefore, the proposed family "*Alternaviridae*" have four definite mycovirus species, namely Alternaria alternata virus 1 (AaV1) (Aoki et al. 2009), AfVF, FpAV1, and Fusarium incarnatum alternavirus 1 (FiAV1) (Zhang et al. 2019) .

Cordyceps chanhua, as a traditional precious medicinal fungus in China, is an insecticidal fungus with both medicine and food. Taxonomically, due to its teleomorph was unknown, the species has long been regarded as the name *Isaria cicadae*. With recent discovery for the teleomorph of this taxon, the name *Cordyceps chanhua* (Li et al. 2021) was proposed and accepted for the species based on molecular data and the principle of one name for one fungus NE. Ref (Taylor 2011). Over the last three decades, more than 100 *Cordyceps chanhua* strains have been isolated and deposited at the Research Center for Entomogenous Fungi of Anhui Agricultural University (RCEF) from investigative studies of entomopathogenic fungi in China. Here, we report the complete genome sequence of a dsRNA virus

consisting of three dsRNA segments, derived from the *Cordyceps chanhua* isolate RCEF6000, and designated the mycovirus as Cordyceps chanhua alternavirus 1 (CcAV1). Further phylogenetic analysis of the RdRp confirmed that CcAV1 is a novel member of the proposed family "*Alternaviridae*".

Materials And Methods

Sampling of *Cordyceps chanhua*

The strain RCEF6000 isolated from cicada in Anhui Province of China was identified as *Cordyceps chanhua* based on its morphological features and molecular data (sequence of the ITS region and the translation elongation factor 1-α gene). The strain was incubated on SDAY medium (1% w/v peptone, 4% w/v dextrose, 0.2% w/v yeast, 1.5% w/v agar) at 25 °C for 5 days (Shi et al. 2019).

dsRNA enrichment, metagenomic sequencing, RT-PCR, and RACE cloning

The dsRNA from harvested mycelia was extracted, purified and electrophoresed using a method described previously. *C. chanhua* strain RCEF6000 was found to harbor five distinct dsRNA bands of approximately 3.5, 2.6, 2.4, 1.8, and 1.6 kb in length, which we named dsRNA 1-5, respectively (**Fig. 1A**). All dsRNAs were sequenced on an Illumina HiSeq 2500 platform at BGI (Shenzhen, China), producing approximately 9,500 contigs, which (>200 nt) were used to search for similar sequences in the GenBank database using BLASTx. The blast and RT-PCR results confirmed that the strain was infected by two different mycoviruses including a partitivirus with (dsRNA 4, dsRNA 5) and a alternavirus (dsRNA 1, dsRNA 2 and dsRNA 3). Further analyses indicated that contig 8618 (3,465 bp) corresponding to dsRNA 1 had the highest sequence identity (66.99%) to Aspergillus foetidus dsRNA mycovirus, while contig 3757 (2,607 bp) corresponding to dsRNA 2 and contig 3831 (2,359 bp) corresponding to dsRNA 3 had 48.61% and 56.91% amino acid sequence identity to Aspergillus foetidus dsRNA mycovirus, respectively. Thus, dsRNA 1, dsRNA 2 and dsRNA 3 were segments of a potential novel mycovirus, designated as Cordyceps chanhua alternavirus 1 (CcAV1). The terminal sequences of CcAV1 were determined previous protocols described by Coutts and Livieratos (Coutts and Livieratos 2003), and amplified PCR products were then cloned into pMD18-T (TAKARA) and sequenced separately three times.

Bioinformatic and phylogenetic analyses of the sequence data

The complete genome sequence of CcAV1 was deposited in the GenBank database (accession numbers OK481552, OK481553 and OK481554). The putative ORFs of three dsRNAs were predicted using ORFfinder (https:// www.ncbi. nlm. nih. gov/ orffinder/) (**Fig. 1B**), and the amino acid sequence of the putative RdRp of CcAV1 was aligned with those of other dsRNA viruses using the Multiple Alignment using Fast Fourier Transform (MAFFT) program (Katoh et al. 2018). A phylogenetic tree was constructed by the maximum-likelihood (ML) method with the LG+G+I+F model and 1000 bootstrap replicates, using MEGA X (Kumar et al. 2019). The resulting phylogenetic tree was exported to Figtree 1.4.4 (http://tree.bio.ed.ac.uk/softw are/figtree/).

Results And Discussion

The complete genome of CcAV1 is composed of three dsRNA segments, dsRNA 1 (3512 nt), dsRNA 2 (2,655 nt), and dsRNA 3 (2,415 nt), and the genetic organization of CcAV1 is shown in (**Fig.1B**). The G+C content of dsRNA 1, dsRNA 2 and dsRNA 3 was 55.4%, 57.1% and 59.7%, respectively. Each dsRNA encodes a single ORF. The largest segment encodes a 126.41-kDa protein of 1,127 amino acids (aa), which is a putative RNA-dependent RNA polymerase (RdRp), while the two smaller segments, dsRNA 2 and dsRNA 3, encode 90.73-kDa protein of 831 aa (HP 1) and 78.68-kDa protein of 731 aa (HP 2), respectively. A conserved domain database (CDD; NCBI) search for the RdRp protein sequences from CcAV1 reveals the presence of the expected eight conserved domains found within viral RdRp proteins. Further analyses showed that the triad has an alanine (ADD) instead of the nearly universally conserved glycine (GDD) within domain VI of RdRp.

The 5' untranslated regions (5' -UTRs) of dsRNA 1, 2 and 3 are 48, 51 and 53 bp in length, respectively, and alignment of the 5' UTR sequences of the three genomic segments of CcAV1 demonstrates sequence conservation(GGCTGACAGCCTGAGTGGTGNNCCTAATCNANTACNCACCAGCTGTGC) (**Fig. 1 C**). The presence of 3' -poly (A) tails in all three dsRNA segments range from 23 to 46 nt in length on the coding strand, and the nucleotide sequence identity between these 3' UTRs, excluding the poly(A) tails (**Fig.1C**), is 27.27%. A BLASTp search for homologues showed that the RdRp, HP 1 and HP 2 of CcAV1 had the highest sequence similarity to the corresponding proteins of the Alternaviruses AfVF (66.99%, 49.30% and 56.91%, respectively). In order to determine the taxonomic position of CcAV1, a ML phylogenetic tree of RdRp amino acid sequences was constructed with 21 mycoviruses, including ten totiviruses, five chrysoviruses and four alternaviruses, with two partitiviruses as outgroups. The phylogenetic tree indicated that CcAV1 is group with all "*Alternaviridae*" species as a cluster, and is closer AfVF than other members of the "*Alternaviridae*" (**Fig.2**). Therefore, we propose CcAV1 to be a new member of the proposed family "*Alternaviridae*" based on phylogenetic analysis, RdRp sequence comparisons, and the identification of conserved motifs. This is the first report of a mycovirus in the proposed family "*Alternaviridae*" that infects entomopathogenic fungus.

Declarations

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Author contributions YZ and NS conceptualized, designed, and performed the experiments, analyzed the data, and wrote the manuscript. PW, QZ and GY analyzed the data. BH acquired the research grant for this study, collected and identified the fungus sample. YZ, NS, PW, QZ, GY and BH participated in revising the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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

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

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Figures

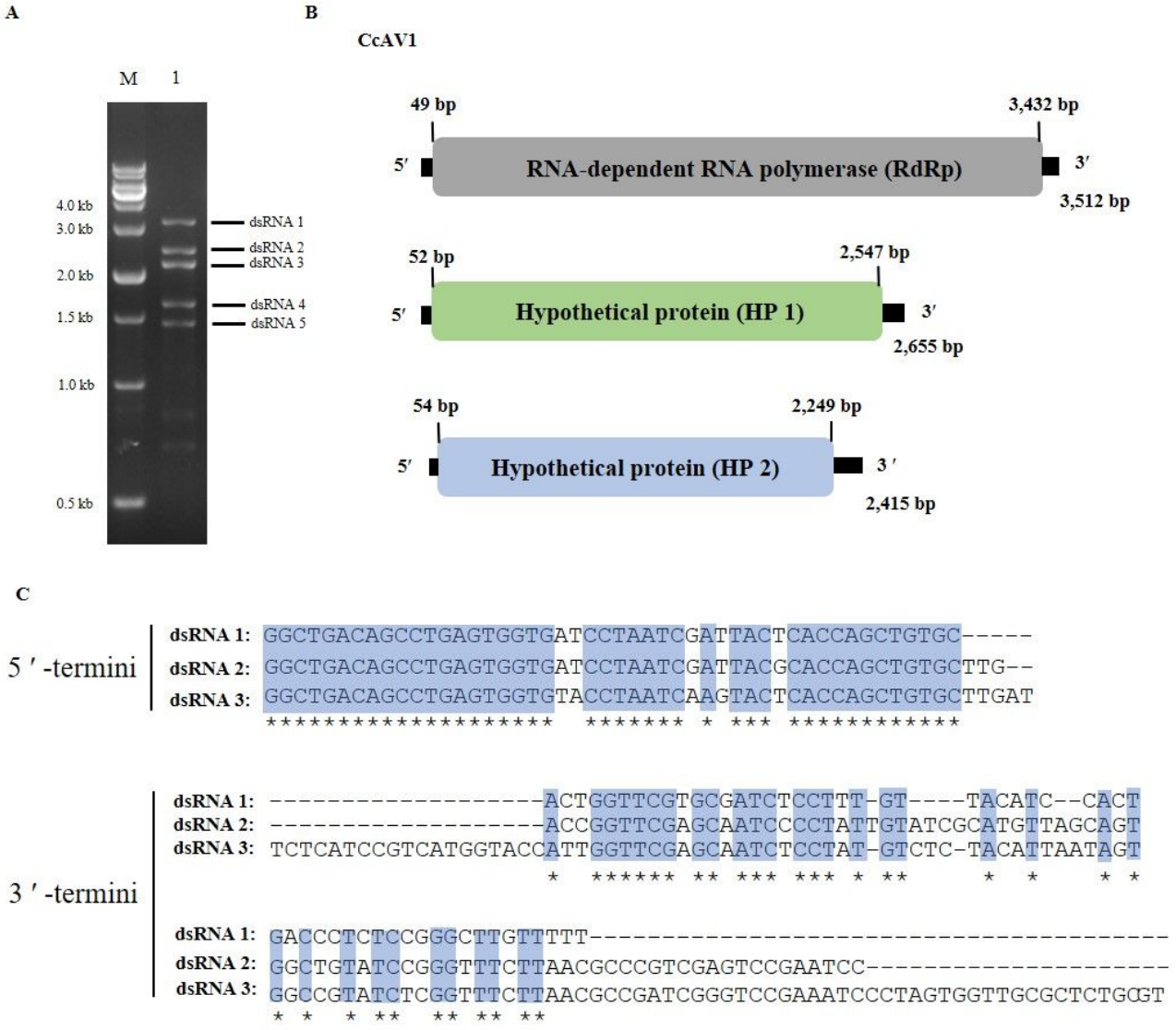

Figure 1

A Purified dsRNA extracted from C. cicadae strain RCEF6000 was electrophoresed in a 1.5% agarose gel. M, DNA marker; lane 1, dsRNAs. B Schematic representation of the CcAV1 genomic structure. C Comparison of the 5'- and 3'- terminal sequences of dsRNA 1, dsRNA 2 and dsRNA 3 of CcAV1. “*” indicates a conserved nucleotide acid.

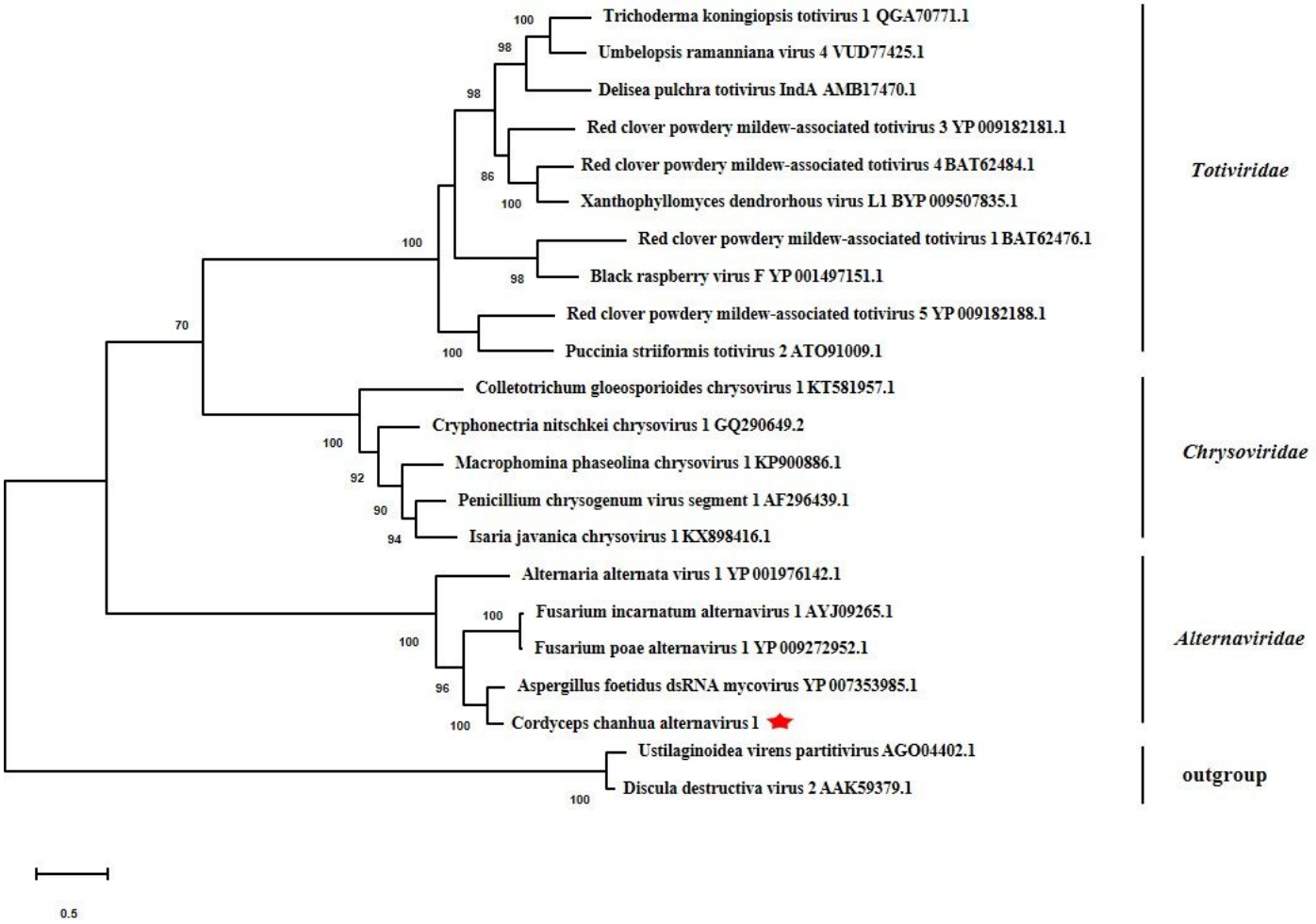

Figure 2

The phylogenetic tree was constructed with maximum-likelihood (ML), using the LG+G+I+F amino acid substitution model based on RdRp sequences. The scale bar on the phylogenetic tree represents 0.5 amino acid substitutions per site and numbers at the nodes indicate bootstrap support over 50% (1000 replicates).