Molecular characterization of a novel polymycovirus identified from phytopathogenic fungus *Colletotrichum gloeosporioides*

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Abstract

A novel polymycovirus isolating from plant-pathogenic fungus *Colletotrichum gloeosporioides* was identified. The virus were composed nine RNA segments, ranging from 699 bp to 2,444 bp, and except that RNA5 contains two open reading frames (ORF5-1 and ORF5-2), other RNA segement contain only one ORF. In addition,the proteins encoded by ORFs 1-8 were homologous to the protein encoded by ORFs 1-8 in *Colletotrichum camelliae* filamentous virus 1(CcFV-1) by BLASTp. The amino acid sequence between the RNA-dependent RNA polymerase (RdRp) encoded by ORF1 and the viral methyltransferase encoded by ORF3 shared 87.6% and 83.3% identity with CcFV-1. The proline-alanine-serine-rich protein (PASrp) encoded by ORF4 shows 86.6% sequence homology to that of CcFV-1. The sequence homology of proteins encoded by ORFs2, 5-1, 6, 7 and 8 with the corresponding proteins of CcFV-1 was 86.6%, 82.5%, 89.0%, 45.7% and 95.5%, respectively. However, RNA9 is a defective RNA of RNA2, missing the 1556 bp (nt 519 to nt 2074) intermediate fragment of RNA2. Phylogenetic analysis based on RdRp protein indicated that the virus, which is the phylogenetically closest relative of CcFV-1, clustered with members of the family *Polymycoviridae*, so we tentatively named it "*Colletotrichum gloeosporioides* polymycovirus virus 1" (CgPmV1). To our knowledge, this is the first report of a defective polymycovirus in *C. gloeosporioides.*

Introduction

Since 1962, when mycoviruses were first discovered in *Agaricus bisporus* [1], an increasing number of mycoviruses have been reported in diverse phytopathogenic fungi [2]. International Committee on Taxonomy of Viruses (ICTV) (https://ictv.global/taxonomy) established polymycovirdae in order to receive growing fungal RNA viruses with multi-segmented genome in 2020[3, 4]. The number of genomic segments of the members in the genus polymycovirus in the family *polymycoviridae* was mainly 4, 5 and 8 [3, 5–9]. Recently, BbPmV-4 (reported in 2021), whose RNA6 is the defective RNA of RNA4, consist of six genomic segments [4]. Nonetheless, all polymycoviruses contain four identical conserved genomic segments that encode three proteins with similarly function and unknown function putative protein, one of the proline-alanine-serine-rich protein (PASrp) encoded by conserved genome is closely related to the capsid that envelops dsRNA [3–10]. On the one hand, PASrp of most polymyviruses only covers dsRNA and does not constitute a true capsid [3–9]. On the other hand, PASrp of CcFV-1 can wrap dsRNA to form non-isometric filamentous virus particles [10]. This demonstrates that PASrp of polymycoviruses might undertake a vital role in the evolution of capsid and capsid-free viruses.

Colletotrichum spp., the eighth largest plant pathogenic fungus in the world, can cause anthracnose disease in ocean of plant species worldwide [11]. *Colletotrichum gloeosporioides* is commonly exist on angiosperms, causes leaf discoloration, stem end withering and fruit falling off when the anthracnose disease is serious [12]. Currently, a plenty of mycoviruses have been isolated and characterized in *Colletotrichum*. Among these, a novel chrysovirus [13], an unclassified mycovirus [14] and a novel ourmia-like mycovirus were isolated from *C. gloeosporioides* [15]. A dsRNA with non-isometric filamentous virus particles was identified in *C. camelliae* [10], and a few mycoviruses were found in *C. fructicola* [16] and *C.*

acutatum [17]. Moreover, mycoviruses have been reported in endophytic or phytopathogenic strains of the genus *Colletotrichum* [18, 19].

Here, we discribe the molecular characterization of a novel defective polymycovirus, which was named "Colletotrichum gloeosporioides polymycovirus virus 1" (CgPmV1), from *C. gloeosporioides.*

Provenance Of The Virus Material

C. gloeosporioides strain GJT5-3 was isolated from a discolored and misshapen citrus leaf infected with anthracnose in Hunan Province, China. It was cultured on potato dextrose agar (PDA) plates at 27℃. For dsRNA extraction, mycelial plugs were inoculated into the potato dextrose broth and shaken for 4–5 days in an orbital shaker at 27℃ with 180 rpm. DsRNA was extracted from fungal mycelium using the methods described by Morris and Dodds with minor modification [20]. Then, dsRNA was treated by DNase I and S1 nuclease (TaKaRa) and electrophoresis on 1% agarose gel, the observed band pattern shows that there are multiple dsRNA elements between size of 0.5 and 3 kb (Fig. 1A). Each section were excised from the agarose gel and purified. Construction of the cDNA library using random hexadeoxynucleotide primers (TaKaRa). The complete sequence of dsRNA1-9 was obtained by ligase-mediated terminal amplification method as described previously [21]. All of the amplified DNA fragments were purified, cloned and Sanger sequenced. Each base is confirmed by sequencing at least three separate clones. The assembled cDNA sequence was deposited in the GenBank database with the accession numbers OM812989-OM812997.

The BLASTp of the National Center for Biotechnology Information (NCBI) was used for homology search for amino acid (aa) sequences [22]. ORF finder and Conserved Domain Search Service (CD Search) in NCBI were used to predict the ORFs and identify the putative conserved domains, respectively [23]. Multiple sequence alignment was performed with DNAMAN. Phylogenetic analysis was carried out by the method of maximum-likelihood in MEGA 7 [24], with bootstrap values calculated after 1000 bootstrap.

Sequence Properties

The complete genome of CgPmV1 consist of nine segments, with full-length sequences of RNA1-9 are 2,444, 2,255, 2,012, 1,297, 1,082, 1,055, 1,004, 991 and 699 bp, respectively with GC content are 58%, 59%, 59%, 57%, 53%, 54%, 61%, 54% and 58%. RNA9 consists of 518 bp at the 5'-termini and 181 bp at the 3'-termini of the RNA2, losing the intermediate fragment of 1556 bp (nt 519 to nt 2,074).Except RNA5, which encodes two ORFs, the other eight sequences encodes only one ORF (Fig. 1B).

Multiple alignment of RNA 1–8 sequences, a conserved sequence (CGATAATAA) was found at the 5’-termini,whereas RNA 9 differed by only one nucleotide (Fig. 2A). The 3’-termini of dsRNAs 1–9 cDNA sequences has no strict sequence conservation, the tetranucleotide CCCC only exist in dsRNA2 and dsRNAs 7–9, the sequence $(CN)_4CGNC_2GCGNG_2CGNC_2NC$ is shared by dsRNAs 1 and 3, while $CTN_3GN_2TN_2CTN_3GNG_2N_2T$ only by dsRNAs 4 and 6 (Fig. 2B).

The 5'-termini untranslated regions (UTR) in front of ORFs 1–9 contained 33, 71, 59, 102, 183, 159, 90, 165 and 71 nt, the 3'-termini untranslated regions in back of ORFs 1–9 contain 95, 105, 96, 319, 98, 266, 179, 217 and 157 nt, respectively. Moreover, the interval between ORF5-1 and ORF5-2 is 84 nt. Proteins translated by ORFs 1–9 are termed P1-9 and their molecular masses are 84.8, 74.2, 66.1, 31.2, 21.6 (P5-1), 4.8(P5-2), 22.9, 25.8, 22.1 and 16.6 kDa, respectively.

The BLASTp comparison of P1-9 showed that P1-P9 had significant sequence similarity to the corresponding protein encoded by CcFV-1 P1, P3 and P4 have 87.6% (coverage 100%, E-value 0.0), 83.3% (coverage 100%, E-value 0.0) and 86.6% (coverage 100%, E-value 0.0) sequence similarity with RdRp, methyltransferase and PASrp encoded by CcFV-1, respectively. For P2 and P9, 86.6% (coverage 100%, E-value 0.0) and 84.3% (coverage 98%, E-value 6e-70) amino acid sequences were similar to the same hypothetical protein encoded by CcFV-1. P5-1 showed 82.5% (coverage 94%, E-value 9e-108) sequence similarity to a protein of CcFV-1, while P5-2 showed no similarity to any known protein. It is worth mentioning that the P6-P8 has only 89.0% (coverage 100%, E-value 9e-138), 45.7% (coverage 99%, E-value 4e-44) and 95.5% (coverage 99%, E-value 3e-138) sequence similarity to the corresponding hypothetical protein in CcFV-1, and no more comparison results have been obtained.

To examine the phylogenetic relationship between CgPmV1 and other polymycoviruses, a phylogenetic tree was constructed based on RdRp protein of CgPmV1 and other polymycoviruses, using MEGA 7.0 by maximum-likelihood method with1000 bootstrap replicated. The Hadaka virus 1 (HadV1), a positive single-stranded RNA (+ ssRNA) share the three conserved segments with determined polymycoviruses but lacked the segment of encoding PASrp [25],was elected to an outgroup. Phylogenetic tree showed that CgPmV1 was closest to CcFV-1 in phylogeny (Fig. 3A). The multiple sequences alignment of RdRp confirmed that CgPmV1 possessed three conserved motifs that ubiquitous in members of the *polymycoviridae* (Fig. 3B).

In conclusion, the novel polymycovirus with defective RNA is the first found in C. gloeosporioides. Although defective RNA is widespread in a variety of RNA viruses, it is not common in polymycoviruses [4]. Whether dsRNA9, as defective RNA, affect the proliferation of CgPmV1. In addition, CgPmV1 is the phylogenetically closest relative of CcFV-1, whether form unique virus particles and influence the virulence of the host. These are all things that need to be studied further.

Declarations

Compliance with ethical standards:

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Conflict of interest: The authors declare that they have no conflicts of interest.

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

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Figures

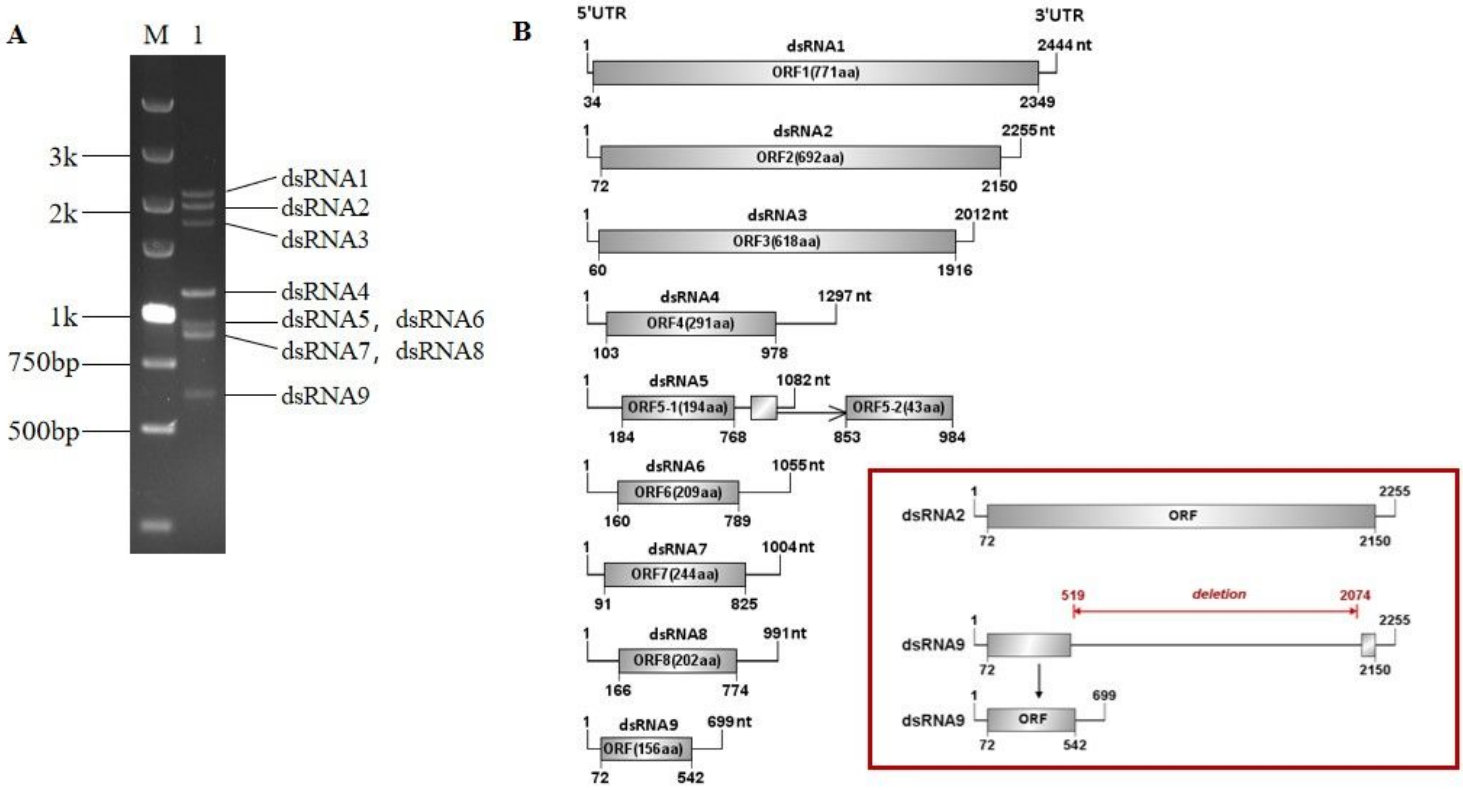

Figure 1

Electrophoretic analysis and genomic characterization of Colletotrichum gloeosporioides polymycovirus virus 1. (A) Electrophoresis of the dsRNAs from C. gloeosporioides strain GJT5-3-Y8 in 1.0% agarose gel. Lane M, DL 5000 DNA Marker; Lane 1, dsRNAs treated with DNase I and S1 nuclease. (B) The genome structure of CgPmV1. Each box in dsRNA represents a putative open reading frame (ORF), and the horizontal lines represent the untranslated regions (UTRs).

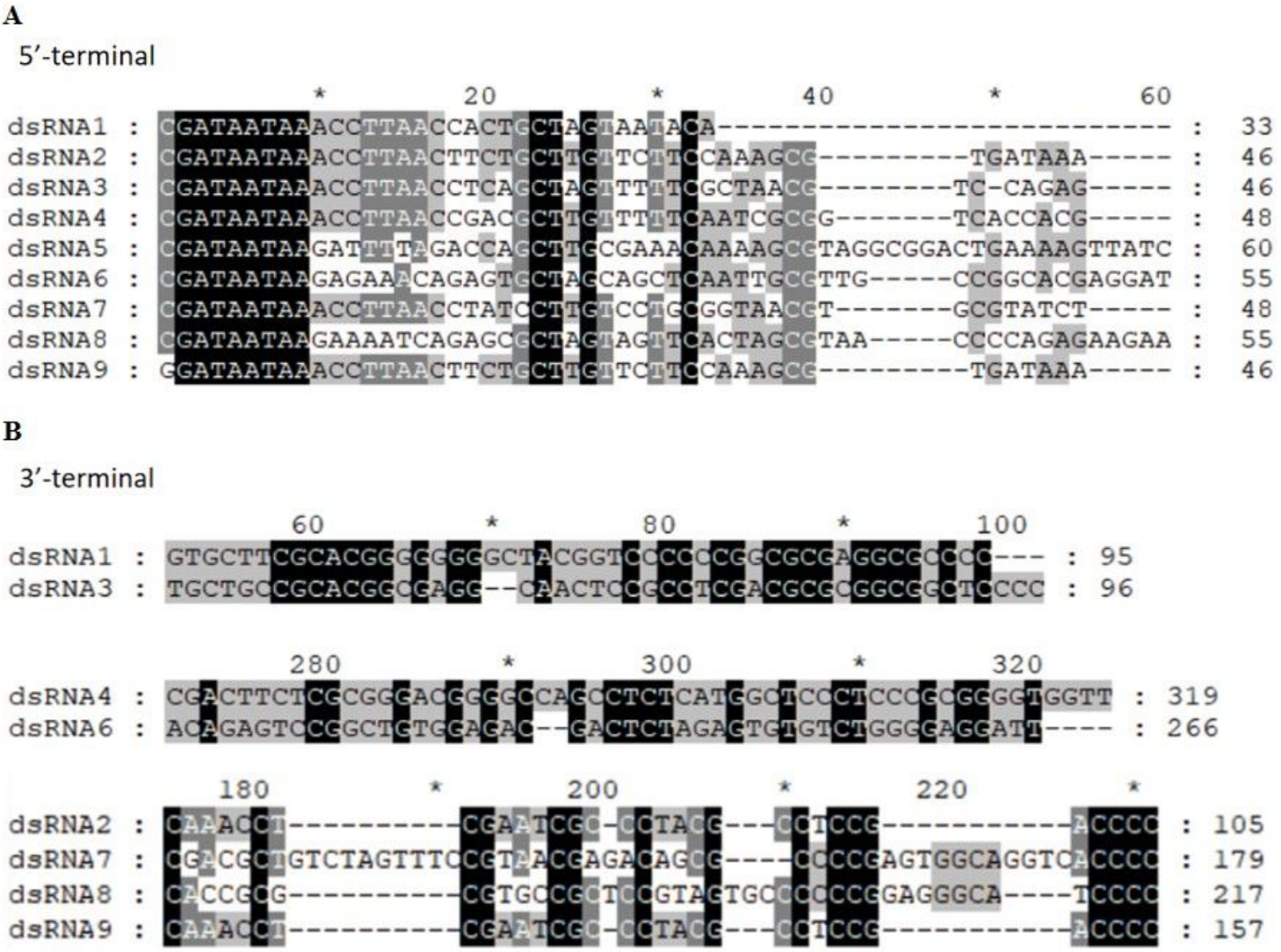

Figure 2

(A, B) Comparison of the 5'-terminl and 3'-terminl untranslated regions (UTRs) in dsRNAs 1-9. Black, Gray and light gray backgrounds show that the nucleotide sequence identity is not less than 100%, 80% and 60%, respectively.

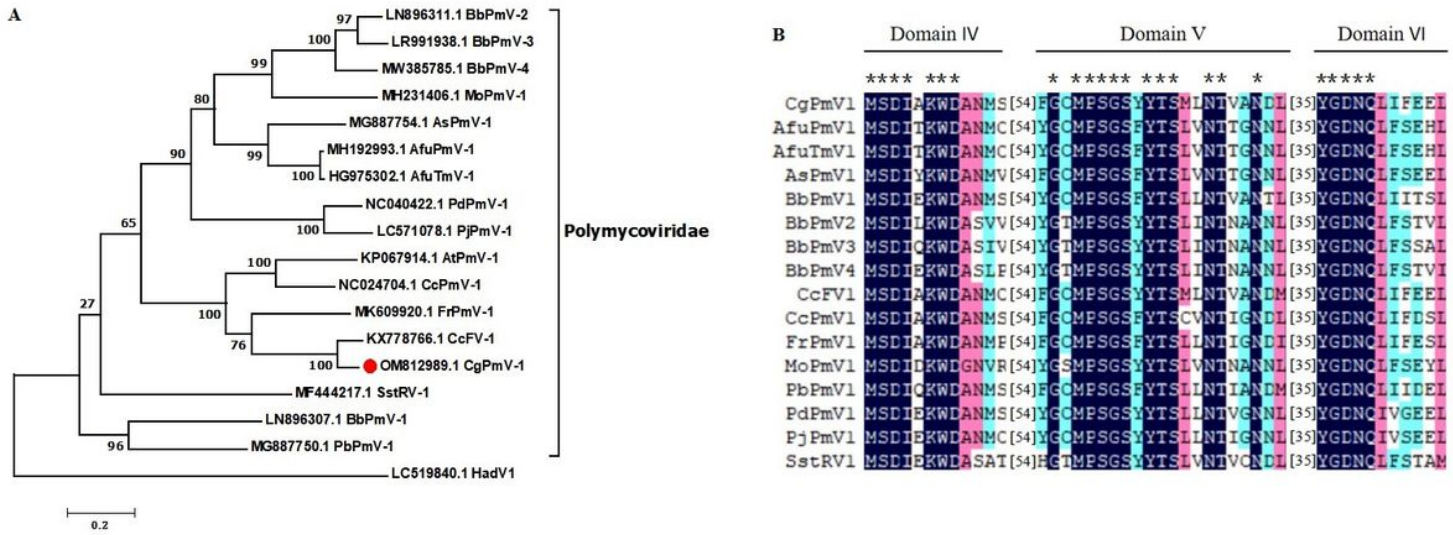

Figure 3

(A) Phylogenetic analysis was performed by the maximum-likelihood (ML) method in MEGA 7 with the Jones-Taylor-Thornton (JTT) model, based on the RdRp sequences of CgPmV1 and other viruses of the family *polymycoviridae*. The scale bar represents a genetic distance of 0.2 amino acid substitutions per site, and the red dot indicates the position of CgPmV1. The virus names and their abbreviations are as follows: Aspergillus spelaeus tetramycovirus 1 (AsPmV-1), Aspergillus fumigatus polymycovirus 1 (AfuPmV-1), Aspergillus fumigatus tetramycovirus 1 (AfuTmV-1), Alternaria tenuissima polymycovirus 1 (AtPmV-1), Beauveria bassiana polymycovirus 1 (BbPmV-1), Beauveria bassiana polymycovirus 2 (BbPmV-2), Beauveria bassiana polymycovirus 3 (BbPmV-3), Beauveria bassiana polymycovirus 4 (BbPmV-4), Colletotrichum camelliae filamentous virus 1 (CcFV-1), Cladosporium cladosporioides polymycovirus 1 (CcPmV-1), Colletotrichum gloeosporioides polymycovirus virus 1 (CgPmV1), Magnaporthe oryzae polymycovirus 1 (MoPmV-1), Penicillium digitatum polymycoviruses 1 (PdPmV-1), Penicillium janthinellum polymycovirus 1 (PjPmV-1), Penicillium brevicompactum tetramycovirus 1 (PbPmV-1), Fusarium redolens polymycovirus 1 (FrPmV-1), Sclerotinia sclerotiorum tetramycovirus 1 (SstRV-1), Hadaka Virus 1 (HadV1). (B) Multiple alignment of RdRp domains of CgPmV1 and other polymycoviruses indicating conserved motifs IV to VI, with dark blue, red, and light blue backgrounds denote amino acid identity of no less than 100%, 75%, and 50%, respectively.

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