Molecular characterization of a novel victorivirus infecting *Corynespora cassiicola*

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**Abstract**

A novel victorivirus was detected in an isolate of *Corynespora cassiicola* strain 20180909-03 and was named "Corynespora cassiicola victorivirus 1" (CcVV1). The complete genome sequence of this virus is 5140 bp in length and contains 57% GC with two large open reading frames (ORFs) overlapping at the tetranucleotide AUGA. The ORFs were predicted to encode a coat protein (CP) and an RNA-dependent RNA polymerase (RdRp), respectively, which are conserved in dsRNA fungal viruses of the family *Totiviridae*. Comparison and phylogenetic analysis of the deduced amino acid sequences of RdRp and CP showed that CcVV1 is a new member of the genus *Victorivirus*. This is the first report of a genomic sequence of a victorivirus infecting *Corynespora cassiicola*.

**Keywords** Victorivirus · dsRNA · *Corynespora cassiicola*

*Corynespora cassiicola*, an ascomycete fungus, is a necrotrophic plant pathogen that causes a disease commonly known as target spot disease in many crops [1], including sesame (*Sesamum indicum*) [2], sweet pepper (*Capsicum annuum*) [3], cotton (*Gossypium hirsutum*) [4], soybean (*Glycine max*) [5], and various other crops, with a significant impact on production [6]. It has also been found in rare human infections [7].

Mycoviruses are ubiquitous and have been detected in the major phyla of fungi, including Ascomycota, Basidiomycota, and Glomeromycota [8]. Mycoviruses have diverse genome types, including ssDNA, +ssRNA, dsRNA, and -ssRNA [9]. Mycoviruses with dsRNA genomes belong to seven families: Partitiviridae, Reoviridae, Totiviridae, Megabirnaviridae, Amalgaviridae, Chrysoviridae, and Quadriviridae (https://talk.ictvonline.org). The family Totiviridae consists of five genera: Totivirus, Victorivirus, Giardiavirus, Trichomonasvirus, and Leishmaniavirus. Members of the genera Totivirus and Victorivirus infect fungi exclusively [10]. Members of the genus Victorivirus infect various phytopathogenic fungi, including *Ustilaginoidea virens* [11], *Nigrospora oryzae* [12], *Fusarium asiaticum* [13], *Aspergillus foetidus* [14], *Macrophomina phaseolina* [15], and *Rosellinia necatrix* [16]. The coding strands of victoriviruses contain two large open reading frames (ORFs) with the 5′-proximal ORF encoding the coat protein (CP) and the 3′-proximal ORF encoding the RNA-dependent RNA polymerase (RdRp) [17]. The stop codon of the upstream CP overlaps with the initiation codon of the RdRp in the tetranucleotide sequence AUGA, which is a common feature of members of the genus *Victorivirus* [10]. The region upstream of the AUGA motif usually has an H-type pseudoknot, which is required for reinitiation of viral RdRp translation [18]. A unique feature of the CPs of victoriviruses that distinguishes them from those of other members of the family *Totiviridae* is that they have an Ala/Gly/Pro-rich region near their C-terminus [15].

Fungal viruses that infect *C. cassiicola* have not been studied extensively. So far, only one unassigned dsRNA mycovirus infecting *C. cassiicola* has been reported [19].
Sequence properties

Sequence analysis revealed that *C. cassiicola* strain 20180909-03 contained a dsRNA virus belonging to the genus *Victorivirus*, which we named "Corynespora cassiicola victorivirus 1" (CcVV1).

The complete genome of CcVV1 (GenBank accession number OK317696) is 5140 bp long, and it has a GC content of 57% (Fig. 1A). Sequence analysis showed that CcVV1 has two large open reading frames (ORF). ORF1 (nt 303-2570) encodes the CP, and ORF2 (nt 2567-5050) encodes RdRp. In addition, the 5’ terminus of the genome contains two small ORFs (nt 95-184 and nt 197-301), but these have no similarity to other sequences in the NCBI database. The start codon of ORF2 overlaps with the stop codon of ORF1 at the tetranucleotide AUGA (nt 2567-2570). In addition, an H-type pseudoknot structure was found upstream of the AUGA motif, which is believed to be involved in the translation of the downstream ORF2 (Fig. 1B). The untranslated regions (UTRs) at the 5’ and 3’ ends are 94 and 90 bp long, respectively, and are predicted to have stable secondary structures (Supplementary Fig. S2).

The CP encoded by ORF1 is 755 amino acids long, with a calculated mass of 79.36 kDa and a predicted isoelectric point of 5.88. A BLASTp search showed that the CcVV1 CP has the highest sequence similarity to the putative CP of *Beauveria bassiana* victorivirus NZL/1980 (BbVV_NZL/1980; YP_009032632.1, 63.89% identity, 99% coverage, E-value 0). A global pairwise alignment showed that the CPs of CcVV1 and BbVV_NZL/1980 were 64.1% identical. We also found an Ala/Gly/Pro-rich region in the C-terminal sequence of CcVV1 (Supplementary Fig. S3A).

To the best of our knowledge, this is the first report of a victorivirus infecting *C. cassiicola*.

Source of virus material

*C. cassiicola* strain 20180909-03 was isolated from a sesame spot leaf sample in Henan province, China, in 2018. dsRNA was extracted from 0.2 g of fungal mycelia, using CF-11 cellulose column chromatography [20]. The dsRNA sample was analyzed by 1.2% (w/v) agarose gel electrophoresis. After treating the crudely extracted dsRNA with DNase I and S1 nuclease (TaKaRa Dalian, China), a single band of approximately 5 kb was observed (Supplementary Fig. S1).

To further analyze this dsRNA virus, we performed high-throughput sequencing of total RNA from *C. cassiicola* extracted using RNAiso Plus (TaKaRa Dalian, China) on an Illumina HiSeq 2500 platform at Shanghai Bohao Biotechnology. The resulting contigs with a high degree of matching with virus sequences in the NCBI database using BLASTx (https://www.ncbi.nlm.nih.gov/) were identified as potential viral sequences. One of these contigs, contig1246, shared sequence similarity with members of the genus *Victorivirus*.

The complete sequence of contig1246 was confirmed by sequencing 11 virus-specific RT-PCR products generated using specific primers designed based on the contig1246 data (Supplementary Table S1). To complete the 5’- and 3’-terminal genomic sequences, rapid amplification of cDNA ends was performed using a SMARTer RACE 5'/3' Kit (TaKaRa, China). At least three PCR products were sent to Sangon Biotech for Sanger Sequencing to verify the sequences.

Putative ORFs in the CcVV1 genome were identified using ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder). The Conserved Domain Database was used to search for protein domains [21]. EMBOSS Needle was used to perform global pairwise alignments [22]. Multiple alignments of the protein sequences of different viruses were performed using DNAMAN software (version 9.0) and the Clustal W program in MEGA (version 7.0). Phylogenetic trees were constructed by the maximum-likelihood (ML) method using the Jones-Taylor-Thornton (JTT) model using the MEGA 7.0 program with 1000 bootstrap replicates [23]. The GC content was determined using OligoCalc [24]. The ProtParam from Expasy was used to calculate the protein molecular weight and isoelectric point [25, 26]. The Mfold was used to find potential secondary structures in the terminal sequences of the CcVV1 [27]. A genome diagram was made using Illustrator for Biological Sequences [28]. An H-type RNA pseudoknot was predicted using DotKnot [29].
which is a significant molecular feature of the members of the genus Victorivirus [15].

The CcVV1 RdRp contains 827 amino acids, and its molecular weight and isoelectric point were predicted to be 90.47 kDa and 8.82, respectively. A BLASTp search showed that the RdRp has the highest sequence similarity to the putative RdRp of Sclerotinia nivalis victorivirus 1 (SnVV1; YP_009259368.1, 56.85% identity, 99% coverage, E-value 0). A global pairwise alignment using EMBOSS Needle showed that the RdRps of CcVV1 and SnVV1 are 57.1% identical. Multiple sequence analysis showed that the RdRp sequence of CcVV1 contains eight conserved motifs [13] (Supplementary Fig. S3B).

A phylogenetic tree based on the CP sequences of CcVV1 and other dsRNA viruses of the family Totiviridae was constructed by the ML method (Supplementary Fig. S4). The CP of CcVV1 formed a branch with members of the genus Victorivirus. Similarly, phylogenetic analysis of the viral RdRp also showed a close relationship of CcVV1 to members of the genus Victorivirus (Fig. 1C).

According to the ICTV species demarcation criteria for the genus Victorivirus [30], which specify that the amino acid sequence identity in pairwise comparisons of either the CP or the RdRp gene product is no more than 60% and that the virus was isolated from a different filamentous fungus, CcVV1 should be considered a member of a new species of the genus Victorivirus. To the best of our knowledge, this is the first report of a victorivirus infecting the fungus C. cassiicola.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00705-022-05394-x.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflicts of interest.

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

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