Molecular Characterization of a Novel Mycovirus Infecting the Entomopathogenic Fungus *Beauveria Bassiana*

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Research Article

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Abstract

The entomopathogenic fungus Beauveria bassiana is used worldwide for its biological control. Seven dsRNA segments were detected from a single Beauveria bassiana strain RCEF 1446. High-throughput sequencing indicated the presence of three mycoviruses in the infected sample. Two known mycoviruses were identified as Beauveria bassiana Victorivirus 1 and Beauveria bassiana polymycovirus 1, and the novel mycovirus was designated as Beauveria bassiana bipartite mycovirus 1 (BbBV1). The complete sequence of the BbBV1 is described here. The mycovirus contains two dsRNA segments. The first dsRNA is 2,026 bp in length and encoding a RNA-dependent RNA polymerase (RdRp) (68.54 kDa), while the second segment encoding a hypothetical protein (35.55 kDa) of unknown function, was 1,810 bp in length. Moreover, the RdRp protein sequences showed the highest identity of 62.89% to Corynespora cassiicola bipartite mycovirus 1. Phylogenetic analysis of the RdRp reveals that the virus represents a distinct lineage of unassigned dsRNA mycoviruses infecting fungi.

Main Text

Mycoviruses (fungal viruses) are pervasive in filamentous fungi, yeasts, and oomycetes [1]. The majority of known fungal viruses have double-stranded RNA (dsRNA) genomes, while the minority of mycovirus with positive-sense single-stranded RNA (+ssRNA), negative-sense single-stranded RNA (-ssRNA), and single-stranded circular DNA (ssDNA) have been reported [2-4]. To date, dsRNA mycoviruses are classified into nine families (*Amalgaviridae*, *Chrysoviridae*, *Hypoviridae*, *Megabirnaviridae*, *Partitiviridae*, *Quadriviridae*, *Reoviridae*, *Totiviridae*, and the proposed family *Botybirnaviridae*) by the International Committee on Taxonomy of Viruses (ICTV) [5-7]. However, many dsRNA mycoviruses are not classified into the proper family. Of these mycoviruses, some were group into the proposed family or genus, the others were recognized as the unassigned group. A series of bipartite mycoviruses, an unassigned group, have been identified from various fungi that consist of two double-stranded RNA (dsRNA) segments, where the RdRP sequences have more similarity to the RdRPs of proposed *Unirnaviruses* (mono-segment genomes) than partitiviruses (two-segment genomes). To data, 23 bipartite mycovirus have been reported including Cryphonectria parasitica bipartite mycovirus 1, Lactarius rufus RNA virus 1, and Penicillium aurantiogriseum bipartite virus 1et al [8-11].

Entomopathogenic fungi *Beauveria bassiana* has a wide host range and used as a biological control agent for agricultural and forest insect pests [12]. A lot of *B. bassiana* strains can be isolated and deposited from the natural *Beauveria* population, and previous studies showed that mycoviruses infecting this fungus in the world are widespread and highly diverse. A series of dsRNA mycoviruses in the mycelia of *B. bassiana* was previously reported including viruses in families of *Partitiviridae*, *Totiviridae*, *Amalgaviridae*, *Polymycoviridae*, and proposed family "*Unirnaviridae*".

Recently, Beauveria bassiana partitivirus 3 (BbPV-3), a member of the proposed genus *Epsilonpartitivirus*, was reported in our lab. In this study, we found another novel mycovirus infecting *B. bassiana* deposited in the Research Center for Entomogenous Fungi of Anhui Agricultural University (RCEF), and described

the complete sequence of the bipartite dsRNA virus and analyzed its genome organization, which designated this virus as “Beauveria bassiana bipartite mycovirus 1” (BbBV1).

The strain RCEF1446 isolated from *Formicidae* was identified as *Beauveria bassiana* by translation elongation factor 1-α gene sequence and morphological characters. To investigate the presence of mycovirus(es), in B. bassiana RCEF1446, the fungus was grown for 5 days in SDAY (1% w/v peptone, 4% w/v dextrose, 0.2% w/v yeast, 1.5% w/v agar) overlaid with a sterilized cellophane disc, and dsRNA was extracted from the fresh mycelia using CF-11 cellulose (Sigma) chromatography method described in a previous report [13]. The dsRNA was then digestion with DNase I and S1 nuclease (TaKaRa, Dalian, China). Finally, purified dsRNA was electrophoresed in 1.5% (w/v) agarose gel and separated into seven distinct bands of approximately 5.2, 2.4, 2.2, 2.0, 1.9, 1.8, and 1.3 kb in length (Fig. 1A). All dsRNAs were sequenced on an Illumina HiSeq 2500 platform at BGI (Shenzhen, China), and they were cleaned up for assembly and analysis. All contigs>200 nt were used to search for similar sequences using BLASTn and BLASTx against the GenBank database (http://www.ncbi.nlm.nih.gov/). The results indicated that the fungus was co-infected by three mycoviruses. Genomic analysis and RT-PCR confirmation showed that dsRNA1 (5.2-kb) was identified as Beauveria bassiana Victorivirus 1, and dsRNA2 (2.4-kb), dsRNA 3 (2.2-kb), dsRNA 5 (1.9-kb), and dsRNA 7 (1.3-kb) consisted of a mycovirus corresponding to Beauveria bassiana polymycovirus 1. However, contig 45 corresponding to dsRNA4 (2.0-kb) had the highest sequence identity (62.89%) to Corynespora cassiicola bipartite mycovirus 1 (QNC69629.1) (Fig. S1), while the contig 24 corresponding to dsRNA 6 (1.8-kb) have a sequence identity of 50.72% to Corynespora cassiicola bipartite mycovirus 1 (QNC69630.1). Thus, the two dsRNA segments including dsRNA4 and dsRNA6 were one potential new mycovirus genomes. To obtain the terminal sequences of each dsRNA4 and dsRNA6, the 5'- and 3'-terminal sequences of the mycovirus were determined following protocols described by Coutts and Livieratos [14], and amplified PCR products were then cloned into pMD18-T (TAKARA) and sequenced separately three times. The complete sequences of dsRNA4 and dsRNA6 were assembled using DNAMAN 7.0 (Lynnon Biosoft, USA) and deposited in the GenBank database under the accession numbers MW265927 and MW265928 (Supplementary Data 1), respectively. The putative ORFs of two dsRNAs were predicted using the ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/) (Fig. 1B), and the amino acid sequence of the putative RdRp gene of BbBV1 was aligned with those of other dsRNA viruses using the Multiple Alignment using Fast Fourier Transform (MAFFT) program [15]. A phylogenetic tree was constructed by the maximum-likelihood (ML) method with the LG+G+I+F model and 1000 bootstrap samples, using MEGAX [16].

The genome of BbBV1 is composed of two segments. The larger segment named dsRNA4 was 2,026 bp in length with a G+C content of 52.8%, while the smaller segment named dsRNA6 was 1,810 bp with a G+C content of 55.8%. Each of the segments only has a single ORF in the positive-sense strand. Further analysis indicated that ORF1 (nt125-1,915) of dsRNA4 encodes a putative RNA-dependent RNA polymerase (68.54 kDa), whereas ORF2 (nt111-1,079) in dsRNA6 encodes a unknown function protein (35.55 kDa). The 5’ UTR of dsRNA4 and dsRNA6 were 124 and 110 nt in size, respectively, and further sequence alignment indicated that the two 5’UTRs of dsRNA4 and dsRNA6 had a highly conserved element (CATAGAATTTAAGCCACTGTTCAGCAAACATT), which is necessary for virus replication. The

corresponding 3'UTRs were 111 and 731 nt in length, and they still have been identified as a possible conserved motif despite quite different in size (Fig. 1C). BLASTp searches demonstrated that the protein sequence of ORF1 shared the highest identity of 62.89% (E-value, 0.0; query cover, 97%) with the RdRp of Corynespora cassiicola bipartite mycovirus 1. The amino acid sequence of ORF2 shared the highest identity (50.72%) with a hypothetical unknown function protein of Corynespora cassiicola bipartite mycovirus 1.

Phylogenetic analysis was constructed based on the amino acid sequences of the RdRP from all unclassified viruses related to BbBV1, members of the proposed genus *Unirnavirus* and *Ustivirus*, and selected members of the families *Partitiviridae* and *Amalgaviridae*, as well as two species in *Totiviridae* was used as an outgroup [17-18] (Fig. 2). The phylogenetic tree demonstrated that the unassigned group is more closely related to the proposed genera "*Unirnavirus*" than other groups. The BbBV1 forms a sub-clade in the unassigned group with the Curvularia virus 2, Corynespora cassiicola bipartite mycovirus 1, and Podosphaera virus A, and represents a distinct lineage of unassigned dsRNA mycoviruses.

Based on its degree of sequence similarity and phylogenetic clustering with previously reported unassigned dsRNA mycoviruses, the BbBV1 virus from *B. bassiana* was proposed as a new member of the unassigned virus group.

Declarations

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

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

Informed consent Not applicable.

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