The Whole Genome Sequence of a Novel Chrysovirus From *Beauveria Bassiana* Vuillemin, an Entomopathogenic Fungus

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Research Article

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Abstract

Beauveria bassiana, an entomopathogenic fungus, has a wide host range and is used for arthropod pest control worldwide. Here, we report the discovery and characterization of a novel double-stranded RNA (dsRNA) mycovirus Beauveria bassiana chrysovirus 2 (BbCV2), isolated from the *B. bassiana* from China. The genome of the virus was determined by metagenomic sequencing, RT-PCR, and RACE cloning comprises four dsRNA segments that are 3441bp, 2779bp, 2925bp, and 2688bp long, respectively, each of them contains a single ORF, the first one (ORF1) encoding a 1115-amino-acid-long protein (122.65 kDa) with a conserved RNA-dependent RNA polymerase (RdRp) motif, its sequences showed the highest identity of only 16.13% to that of the Beauveria bassiana chrysovirus 1. The second ORF (ORF-2) encoding a 807-amino-acid-long coat protein (CP) (88.77 kDa). The virus constitutes a new member of the *chrysoviridea* family from *B. bassiana*.

Background

Beauveria bassiana Vuillemin is an important member of the entomopathogenic fungus *Beauveria*. Since the establishment of genus *Beauveria* in 1912, the number of *B. bassiana* species has continued to increase, except for a small number of saprophytic in soil, plant rhizosphere, and endophytic plants, the most are pathogenic fungi parasitic on insects[1].*B. bassiana* has the characteristics of broadspectrum insecticidal, strong pathogenicity, and easy culture, so it is one of the most widely used insecticidal fungi in the biological control of insect pests [2]. However, poor stability, a certain temperature, humidity, and nutrition are needed in the process of conidial formation, spore germination, and mycelium growth, all of these affected the virulence against insect pests of *B. bassiana* [3], and also, its virulence was influenced by many other factors, adaptability to host diversity and virus parasitism will cause the uncertain change of the virulence of *B. bassiana* [4, 5]. Mycoviruses can replicate and proliferate in fungal cells and exists widely in fungi. In the 1840s, three different types of virions have been detected in the abnormally growing *Agaricus bisporus*, the presence of fungal viruses was first reported[6]. Subsequently, more and more DNA and RNA viruses were reported in fungi, such as Magnaporthe oryzae virus 1, Isaria javanica chrysovirus-1, Rosellinia necatrix hypovirus 2, *etc* [7-11] . Most of the mycoviruses do not affect the host, while with further research, it has been found that the fungal virus gave either favorable or adverse effects on the host, thus it is important to discover new mycoviruses and establish their sequence properties and structure for mechanism research of the interaction between viruses and fungal hosts.

Provenance and sequencing of the dsRNA virus from *Beauveria bassiana*

B. bassiana strains were isolated from dead body of *Ostrinia furnacalis* larva collected in the corn field in Gongzhuling city, Jilin Province, China (N 43° 30′ 46″, E 124° 48′ 6″), the conidia from which were cultured and subcultured at 26℃ on potato dextrose agar (PDA) medium in dark and identified as *B. bassiana* based on morphological characteristics and molecular data[12]. The fungi strain stored on PDA slants at 4℃ and asexual spores produced on PDA medium were collected in 25% glycerol at -80℃. Genomic DNAs were extracted with a DNA extraction kit (Sangon, Shanghai, China) and then amplified

the sequence of the ITS region (ITS1□5□-TCCGTAGGTAGGTGAACCTG CGG-3□; ITS4: 5□-TCCTCCGCTTATTGATATGC3□) by PCR for molecular identification. Mycelia of identified *B. bassiana* strains were cultured on PDA medium for 5 days at 26°C in dark to obtain mycelial samples. The double-stranded RNA segments were extracted by cellulose (Sigma) chromatography [13]. The contaminating DNA and ssRNA materials were eliminated by digestion with DNase I and S1 nuclease (TaKaRa, Dalian, China) according to the manufacturer's instructions. The purified dsRNA was electrophoresed in 1% (w/v) agarose gels, stained with GelStain (TransGen, Beijing, China), and visualized under 350nm UV illumination [5]. The strain BbOFZK152 was selected for further study. After electrophoretic separation on agarose gels, dsRNAs were used as templates for cDNA synthesis and PCR amplification of products using random priming RACE3RT (5□-CGATCGATCATGATGCAATGCNNNNN-3□), sequence-specific priming and rapid amplification of cDNA ends (RACE). M-MLV Reverse Transcriptase and PrimeStar HS DNA polymerase (TaKaRa, Dalian, China) were used for cDNA synthesis and PCR. The products were ligated to pMD18-T vector and transformed into *Escherichia coli* strain DH5α (TaKaRa, Dalian, China) for sequencing. According to the obtained sequence, specific primers were designed to amplify the gap sequence [14].

Sequence properties

The four dsRNA segments were recovered from strain BbOFZK152, and the purified dsRNA was digested with DNase I and S1 nuclease to eliminate DNA and ssRNA contamination for further research (Fig. 1). The dsRNA was reversely transcribed to obtain cDNA by RT-PCR. The gene sequences of four ds RNA segments were amplified with random primers, the partial sequences were obtained and were spliced with DNAMAN 9.0. The gaps were amplified by PCR with known sequence fragments as specific primers, and the conserved regions at the ends of the four chains were obtained by RACE. The virus genome consists of four dsRNA segments that are 3441bp (MW314841.1), 2779bp (MW314842.1), 2925bp (MW314843.1), and 2688bp (MW314844.1), respectively. Each of them encoded a single ORF. The 5' and 3' untranslated regions (UTRs) of the each segment were 44bp and 49bp in length, respectively. The genomic organization of the virus is shown in Fig. 2a. Stem-loop structures in the 5□- and 3□-UTRs were predicted with an initial ΔG value of -9.77 kcal/mol and -4.60 kcal/mol (Fig. 2b), respectively, using the RNA-fold web server (http://rna.tbi.univie.ac.at/cgi-bin/RNAWe bSuit e/RNAfo ld.cgi).

Comparison of the untranslation Region (UTR) sequences at the 5'- and 3' -terminal the four dsRNA segments revealed the high conservation, and the 5'-terminal have been found the adenine-rich regions. Typical chrysoviruses have (CAA)n repeats in each of the four dsRNA segments□in this virus, the (CAA)3 repeats were found at 3'UTRs. The complete nucleotide sequence of ds RNA1 of the virus is 3441bp, harbors a single ORF1. ORF1 is 3348nt (nucleotide positions 45nt to 3392nt) long, encodes an 1115-AA protein (122.65 kDa). A BLASTX [15] searching indicated that the obtained protein sequences show the highest sequence consistency with the RdRp (RNA-dependent RNA polymerase) of Neofusicoccum parvum chrysovirus 1 (NpCV1, GenBank logon no.QDB74975.1) which was 73.27%. The results of constructing a phylogenetic tree based on the RdRp domain sequence also showed that BbCV2 is highly homologous to MoCV1-A (YP_003858286.1), came from *Magnaporthe oryzae*. The conservative domain

database (CDD) revealed that the ORF1-encoded protein contained a conservative RT-like super family domain. The result of phylogenetic treeanalysis of RdRp shows that the virus is a member of *betachrysovirus*, names as BbCV 2 (Fig. 3). The complete nucleotide sequence of dsRNA2 is 2779bp, dsRNA2 contains a single ORF, named ORF 2, and its 5 'UTR and 3' UTR are 143bp and 214bp, respectively. The ORF2 extending from 144nt to 2564nt encodes an 807-AA protein (88.77 kDa), a BLASTX[20] searching indicated that the protein sequences obtained had the highest sequence consistency of 64.67% with the putative coat protein of NpCV1(GenBank logon no.QDB74976.1) from *Neofusicoccum parvum*.

DsRNA viruses have been isolated from various fungi, showed different effects on the pathogenicity and other physiological properties of host fungi[5], especially in phytopathogenic fungi, such as *Magnaporthe oryzae* [16], *Alternaria alternate* [17], *Penicillium italicum* [18], and so on, while rarely reported in entomopathogenic fungi. To the best of our knowledge, there are a few dsRNA viruses were reported in *Metarhizium brunneum* and *B. bassiana* so far [19-24]. For chrysovirus, there was only BbCV1 was found in *B. bassiana* [25]. Its genome comprises four dsRNA genome segments that are 3478bp (MK279433.1),3143bp (MK279434.1),3069bp (MK279435.1) and 2070bp (MK279436.1) long. Here we have isolated and provided a whole sequence of a strain of chrysovirus (BbCV2 from *B. bassiana*, which indicated a nucleic acid sequence homology only 16.13% of RdRp gene compared to that of BbCV1. Taxonomically, BbCV1 belongs to alphachrysovirus, while BbCV2 belongs to betachrysovirus. Therefore, we found a new strain of chrysovirus from *B. bassiana*.

Declarations

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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

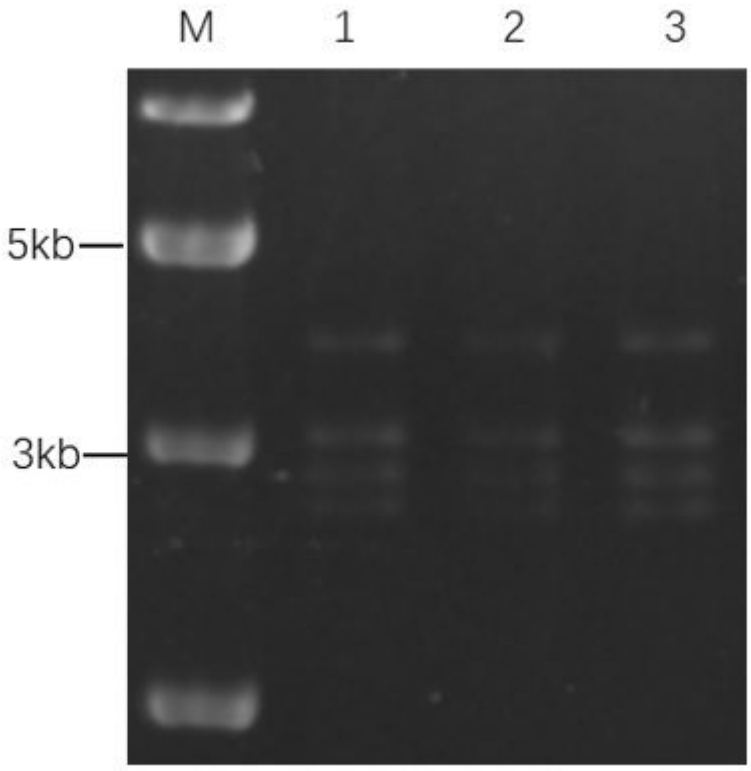

Figure 1

DsRNA fraction extracted from B. bassiana strain BbOFZK152 was electrophoresed in a 1% agarose gel and visualized under UV light after staining with ethidium bromide. Lane M, DNA marker; Lane 1-3, dsRNA sample after treatment with S1 nuclease and RNase-free DNase I.

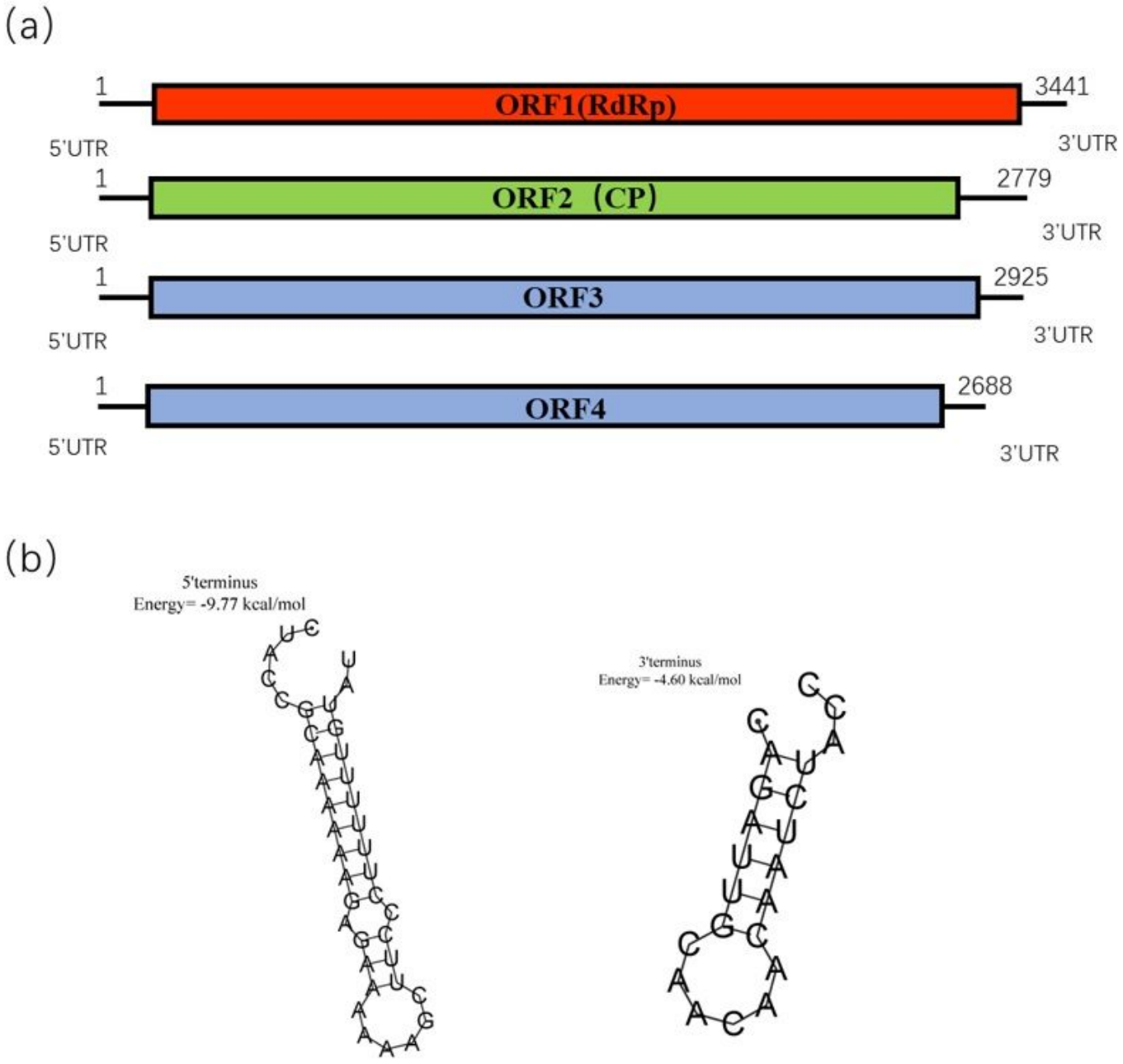

Figure 2

a. Schematic representation of the genomic organization of BbCV2. The BbCV2 genome consists of four dsRNAs, each containing one ORF (grey boxes) flanked by 5’- and 3’-UTRs. b. Predicted secondary structure of the 5 and 3 UTRs of BbCV2.

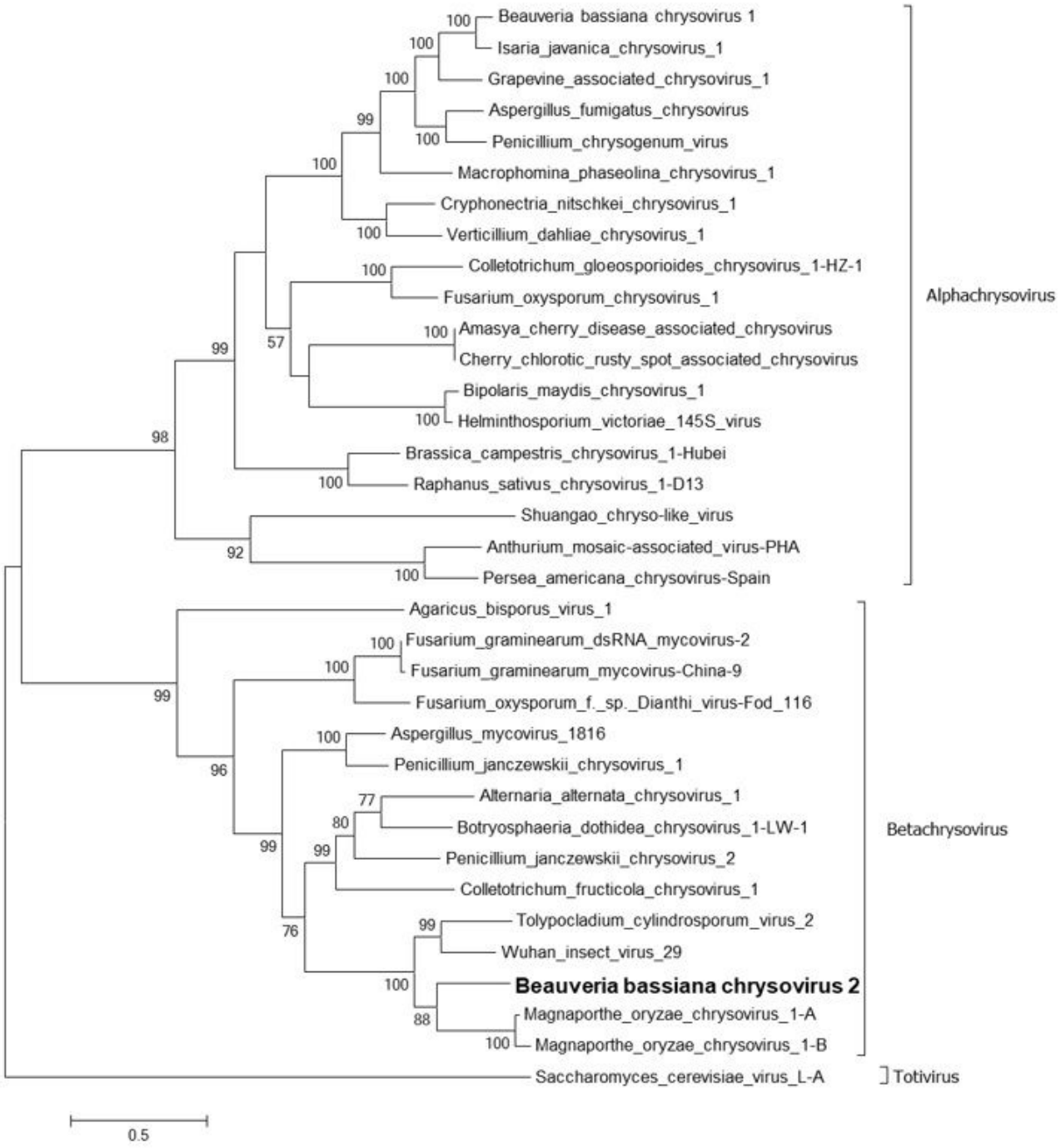

Figure 3

Phylogenetic analysis of members of the family Chrysoviridae and related viruses based on amino acid sequences of their RdRP. A multiple alignment of RdRP amino acid sequences was produced using MUSCLE, all positions with less than 30% site coverage were eliminated and the LG+G+I+F substitution model was used and a maximum likelihood phylogenetic tree was created by the program MEGA 6.0.