Molecular characterization of a novel ourmia-like virus from the phytopathogenic fungus Botryosphaeria dothidea

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Abstract

Here, we describe a novel ourmia-like virus, Botryosphaeria dothidea ourmia-like virus 2 (BdOLV2), derived from phytopathogenic fungus *Botryosphaeria dothidea* strain ZM180192-1, infecting maize in Henan province of China. The complete genome sequence of BdOLV2 consists of a positive-sense single-stranded RNA (+ ssRNA) segment with a length of 2,532 nucleotides (nt). The sequence contains a large open reading frame (ORF), encoding a putative RNA-dependent RNA polymerase (RdRp) including 605 amino acids (aa) with a molecular mass of 68.59 kDa. This RdRp protein contains eight typical conserved domain motifs associated with ourmia-like virus. BLASTp analysis revealed that the RdRp protein of BdOLV2 had the highest similarity (62.10%, 58.15%, and 55.75% identity, respectively) with Botourmiaviridae sp., Macrophomina phaseolina ourmia-like virus 2 and Macrophomina phaseolina ourmia-like virus 2-A. Phylogenetic analysis based on the RdRp aa sequence indicated that BdOLV2 is a new member of the genus *Magoulivirus* with in the family *Botourmiaviridae*.

Introduction

Botryosphaeria dothidea is one of the most important pathogens and infects a broad plant host range including apple, peach, pear, olives, kiwifruit, *etc* [1–3]. The Fruit rot and stem canker caused by *B. dothidea* especially reduce the yield and quality of fruit which can hinder the healthy development of fruit industries and cause great economic losses [4–6]. Now, *B. dothidea* is mainly controlled by spraying fungicides, but masses of chemical fungicides will harm human health in the long term which also increases pathogen resistance and cause environmental pollution at the same time [7–9]. In recent years, people pay more and more attention to harmful chemical agents, and it is urgent to take measures to avoid the risk of chemical agents. Biological control is friendly to the environment and has the promising prospect of preventing the great blast of *B. dothidea*, and mycoviruses (or fungal viruses) are one of the important biological control resources [10, 11].

Mycoviruses can infect and spread in all major groups of fungi which also can replicate in them [11–13]. At present, most of the reported mycoviruses include double-stranded RNA (dsRNA), positive-sense single-stranded RNA (+ ssRNA), negative-sense single-stranded RNA (-ssRNA), and single-stranded DNA (ssDNA) according to their genome types [11, 12, 14]. Most mycoviruses do not have any effect on their hosts, but some mycoviruses can decrease the pathogenicity of their hosts. For example, Cryphonectria hypovirus 1(CHV1) was used to control chestnut blight, and Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1(SsHADV-1) was used to transform *Sclerotinia sclerotiorum* into beneficial endophytes [15–18]. With the application of high-throughput sequencing technology, more and more mycoviruses have been sequenced and identified in many species [19, 20]. Up to now, a total of 18 mycoviruses have been reported in the pathogen *B. dothidea*, and four of them are related to host virulence including Botryosphaeria dothidea chrysovirus 1(BdCV1), Bipolaris maydis botybirnavirus 1 strain BdEW220 (BmRV1-BdEW220), Botryosphaeria dothidea botrexvirus 1 (BdBV1), and Botryosphaeria dothidea RNA virus 1 (BdRV1) [13, 21–25].

According to the International Committee on Taxonomy of Viruses (ICTV), the family *Botourmiaviridae* comprises 12 genera including *Betabotoulivirus*, *Betarhizoulivirus*, *Betascleroulivirus*, *Botoulivirus*, *Deltascleroulivirus*, *Epsilonscleroulivirus*, *Gammascleroulivirus*, *Magoulivirus*, *Ourmiavirus*, *Penoulivirus*, *Rhizoulivirus*, and *Scleroulivirus* [26]. The members of the genus *Ourmiavirus* only contain plant viruses and have three + ssRNA segments encoding RNA-dependent RNA polymerase (RdRp), coat protein (CP), and movement protein (MP), while the remaining 11 genera infect mycovirus and contain an open reading frame (ORF) encoding RdRp [26].

In this study, we report the complete genome of a novel ourmia-like virus from *B. dothidea* strain ZM180192-1 and tentatively named “Botryosphaeria dothidea ourmia-like virus 2” (BdOLV2). Phylogenetic analysis showed that BdOLV2 is closely related to the members of the genus *Magoulivirus* with in the family *Botourmiaviridae*.

Provenance Of The Virus Material

The strain ZM180192-1 was first obtained from maize leaf in Henan Province of China in 2018 and identified as *B. dothidea* based on its internal transcribed spacer (ITS), elongation factor-1α (EF-1α), and β-tubulin sequences. The strain was cultured on potato glucose agar at 25° C for 3 days in the dark to observe the colony morphology.

Total RNA was extracted from 230 *Botryosphaeriaceae* samples, including strain ZM180192-1, and sent to Novogene Bioinformatics Technology Co., Ltd for sequencing. After sequencing by the Illumina method, the adapter sequences and low-quality bases in the viral clones were further removed [25]. Finally, we obtained high-quality viral contigs. Among these contigs, we focused on the viral c36267_g1_i1 sequence of BdOLV2, which shared the highest sequence identity to Botourmiaviridae sp. (Partial genome, identity: 62.15%, Query Cover: 80%, Gen Bank accession number: No. URG17163.1) (Supplementary File S1). Total RNA was extracted with TRIzol™ Reagent (Adlai, Beijing, China). On this basis, cDNA was synthesized by SuperScript III First-Strand Synthesis System Kit (Vazyme, Nanjing, China). Specific primers were designed based on the c36267_g1_i1 sequence, and positive results were identified in the target strain (ZM180192-1) by RT-PCR (Supplementary Table S2). Another sequence, c37748_g1_i1, BLASTx alignment shares the highest identity to Plasmopara viticola lesion associated narnavirus 4 (Complete coding genome, identity: 71.48%, Query Cover: 99%, GenBank accession number: No. QIR30283.1) and they are also positive in strain ZM180192-1. The dsRNA was extracted from the mycelium by column separation method, with minor modifications [27]. Then, the dsRNA was digested with DNase I and S1 nuclease (Takara, Dalian, China) to eliminate DNA and ssRNA. Subsequently, the treated dsRNA was electrophoresed in a 1.0% (w/v) agarose gel stained with Goldview and viewed on a UV transilluminator (Fig. 1A). The terminal sequence of BdOLV2 was obtained by using nested primers designed according to the central sequences of BdOLV2 and ligase-mediated rapid amplification of cDNA ends (RLM-RACE) [28]. The PCR products were purified and ligated into the pMD18-T vector (Takara, Dalian, China), which were then transformed into chemical competent cells of *Escherichia coli* DH5α

(Tsingke, Beijing, China). At least three independent positive recombinant plasmids of each product were sequenced in both orientations to ensure the accuracy of the sequence (Sangon, Shanghai, China).

The obtained virus sequences were spliced using DNAMAN software (version 5.2.2). ORF Finder (http://www.unafold.org/mfold/applications/rna-folding-form.php) and Conserved Domain Database (CDD) Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) were used to predict the potential ORFs and conserved domains of BdOLV2 genome, respectively. The NCBI database was used for sequence homology comparison and reference sequences were downloaded. All sequences were multiple-aligned using CLUSTALX 2.0 and then annotated and colored with the GeneDoc software. The phylogenetic tree was constructed in MEGA 6.0 software using the maximum-likelihood method (ML), with a bootstrap test consisting of 1000 replicates.

Sequence Properties

The colony morphology of *B. dothidea* strain ZM180192-1 was normal, and the whole plate was covered within 3 days (Fig. 1B). The full-length genome sequence of BdOLV2 from strain ZM180192-1was obtained by RT-PCR and RNA-ligase-mediated rapid amplification of cDNA ends (RLM-RACE) method (Supplementary File S3), which was submitted to the GenBank database (accession number OP784426). The complete genome of BdOLV2 is 2532 nt in length, which consists of 18.44% A, 23.93% U, 31.48% G, and 26.15% C. The 5’ and 3’ terminals of the genome contain untranslated regions (UTRs) of 31 nt and 683 nt in length, respectively, and potential stem-loop structures were predicted by RNAfold (Fig. 1D). BdOLV2 genome contains an open reading frame (ORF) encoding 605 aa RdRp protein with a molecular mass of 68.59 kDa. The protein was also analyzed by Conserved Domain Database (CDD) Search and revealed the presence of a conserved RdRp catalytic core domain associated with + ssRNA viruses at the 149aa-355aa position (name: ps-ssRNAv RdRp-like Superfamily, accession number: CL40470, E value: 2.97e-72) (Fig. 1C). Multiple alignments of the RdRps sequence of BdOLV2 with that of other ourmia-like viruses in the *Botourmiaviridae* family revealed eight conserved motifs unique to the ourmia-like virus, including the GDD motif (Fig. 2A). The viruses reported name and accession number are as follows: Acremonium sclerotigenum ourmia-like virus 1 (AsOLV1), QDB75006.1; Botrytis cinerea ourmia-like virus 6 (BcOLV6), QJT73672.1; Macrophomina phaseolina ourmia-like virus 2-A (MpOLV2-A), QOE55588.1; Penicillium citrinum ourmia-like virus 1 (PcOLV1), AYP71797.1; Plasmopara viticola lesion associated ourmia-like virus 36 (PvlaOLV36), QGY72566.1; Plasmopara viticola lesion associated ourmia-like virus 54 (PvlaOLV54); Sclerotinia sclerotiorum ourmia-like virus 19 (SsOLV19); UCR95347.1.

BLASTp analysis revealed that the RdRp protein of BdOLV2 shares the highest identity with three viruses, including Botourmiaviridae sp. (Partial genome, identity: 62.10%, Query Cover: 77%, GenBank Accession No. URG17163.1), Macrophomina phaseolina ourmia-like virus 2 (Complete coding genome, identity: 58.15%, Query Cover: 74%, GenBank Accession No. QOE55587.1), and Macrophomina phaseolina ourmia-like virus 2-A (Complete coding genome, identity: 55.75%, Query Cover: 99%, GenBank Accession No. QOE55588.1) (Supplementary Table S4). The identity of BdOLV2 with these virus sequences is far less than the International Committee on Taxonomy of Viruses (ICTV)

(https://ictv.global/report/chapter/botourmiaviridae/botourmiaviridae/magoulivirus) criterion of the "RdRp proteins sequence identity < 90% among different species of the genus *Magoulivirus*". To further assess the taxonomic status of BdOLV2, a phylogenetic tree was constructed based on the RdRp aa sequence of BdOLV2 and other selected mycoviruses in the *Botourmiavridae* family. The results indicated that BdOLV2 was clustered closely (100% bootstrap support) with the published mycoviruses Macrophomina phaseolina ourmia-like virus 2-A and Macrophomina phaseolina ourmia-like virus 2 of the genus *Magoulivirus* (Fig. 2B).

In conclusion, based on the above results, we certainly identified a novel ourmia-like virus named BdOLV2 from *B. dothidea* strain ZM180192-1 which belongs to the genus *Magoulivirus* of the family *Botourmiaviridae*.

Declarations

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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.

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Figures

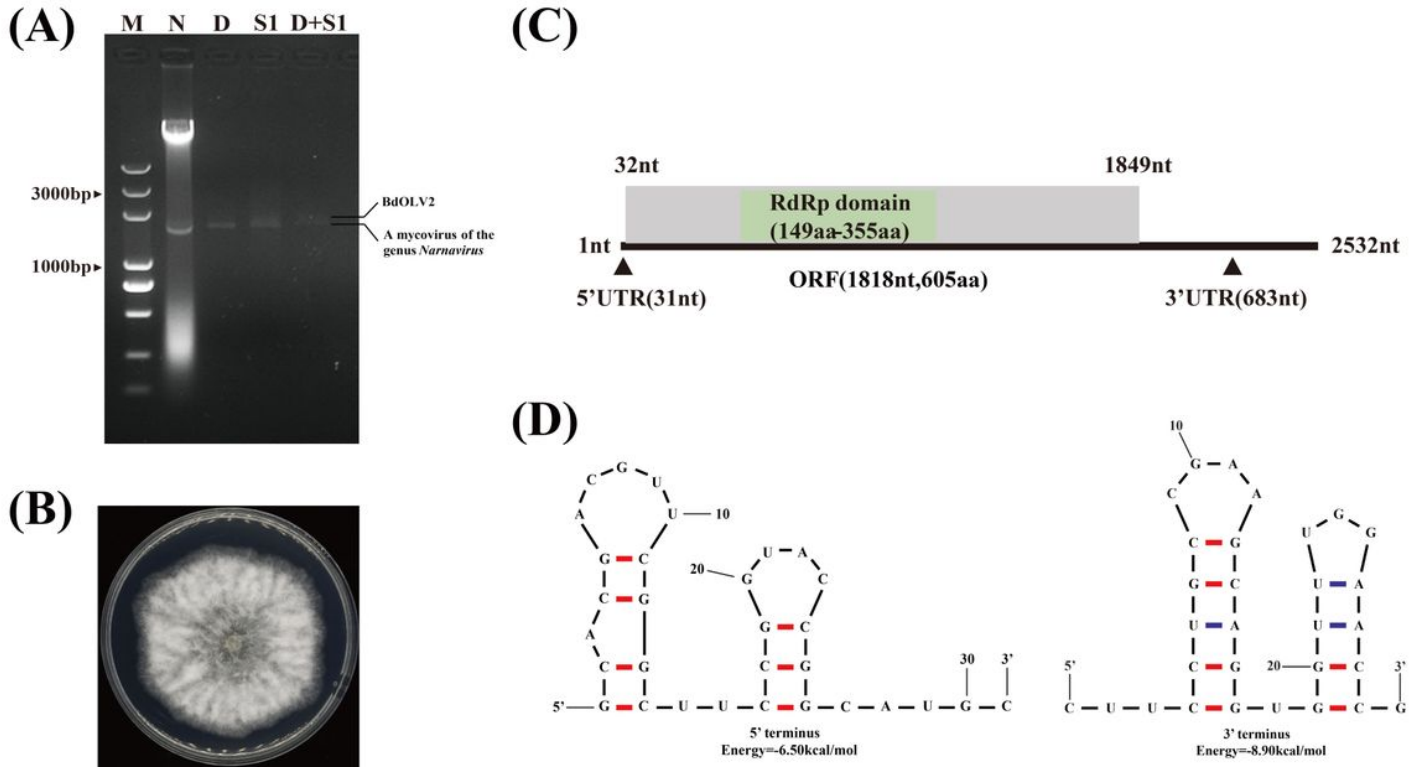

Figure 1

(A) Lane M, DL5000 DNA marker; Lane N, dsRNA extracted from strain ZM180192-1; Lanes D and S1, dsRNA treated with DNase I and S1 nuclease, respectively; lane D + S1, at the same time, dsRNA was treated with DNase I and S1 nuclease. (B) Colony morphology of *B. dothidea*strain ZM180192-1 grown in PDA medium at 25°C for 3 days. (C) Schematic representation of the genome organization of BdOLV2. (D) The putative secondary structures of the 5' and 3' termini of BdOLV2 UTRs.

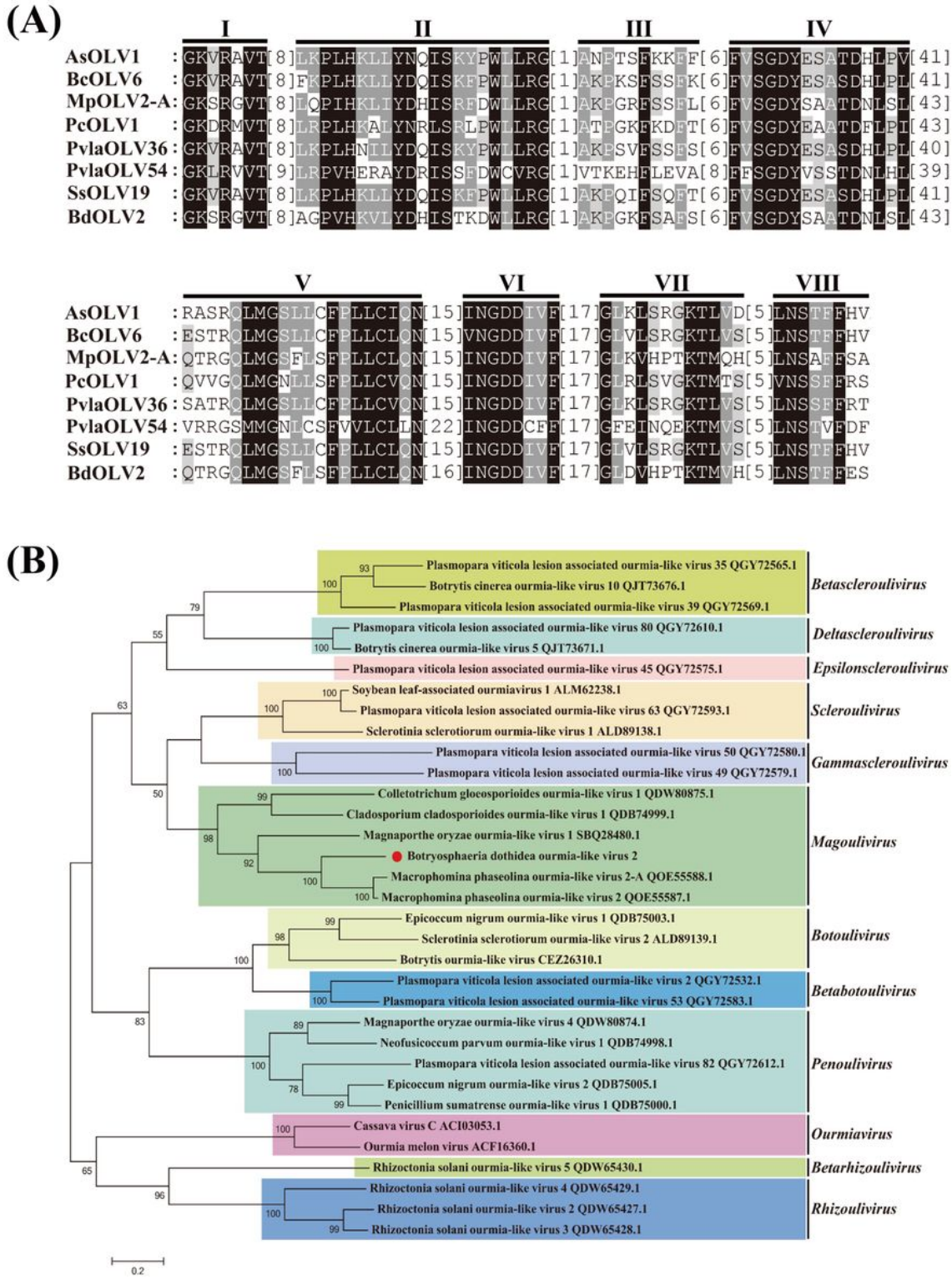

Figure 2

(A) Sequence alignment of the BdOLV2 RdRp motif with selected members of the *Botourmiaviridae*family. The horizontal black line above the sequence alignment represents 8 conserved motifs. The shaded areas represent the same amino acid residues. (B) A phylogenetic tree was constructed based on the RdRp sequences of BdOLV2 and some members of 12 genera of *Botourmiavridae*. Different genera are represented by different color blocks, and BdOLV2 is highlighted by

red circles. The tick mark represents the genetic distance of the 0.2 aa substitution at each locus. Less than 50% of the Bootstrap value will be hidden.

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