The Full-length Genome Sequence of a Novel Mitovirus From the Causal Agent *Botryosphaeria Dothidea* of Pear Ring Rot Disease

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Research Article

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Abstract

Here, we describe a novel mycovirus, *Botryosphaeria dothidea* mitovirus 2 (tentatively designated as BdMV2), isolated from *Botryosphaeria dothidea* FJ strain causing pear ring rot disease in Fujian Province, China. The complete genome nucleotide sequence of BdMV2 is 2538 nt in length and contains a single 2070 nt open reading frame (ORF) encoding a putative RNA-dependent RNA polymerase (RdRp) of 689 amino acid (aa) using fungal mitochondrial genetic code. BLASTp analysis revealed that the RdRp of BdMV2 shares 28.91%–69.36% (query sequence coverage more than 90%) sequence identity to those of members in the genus *Mitovirus*, and the closest similarity is 69.36% and 68.79% with the corresponding protein aa sequences of *Rhizoctonia solani* mitovirus 10 and *Macrophomina phaseolina* mitovirus 4, respectively. Phylogenetic analysis based on the RdRp aa sequences further revealed that BdMV2 is a newly member in the genus *Mitovirus* of the family *Narnaviridae*. To our knowledge, BdMV2 is thus a novel mitovirus from the causal agent *B. dothidea* of pear ring rot disease.

Background

Botryosphaeria dothidea is the important and widespread trunk pathogen, which causes pear ring rot disease in the pear production regions in China. *Botryosphaeria dothidea* with an extensive host range not only harms pear trees by causing severe symptoms such as stem wart and canker, branch dieback and fruit rot, but also other fruit trees including apple, Jujube and grapevine [1–6]. The ring rot disease is becoming increasingly serious and distributes throughout all pear producing areas at present, which seriously affects the yield and quality of pear to further hind the healthy development of pear industry [6–7]. It is urgent to find safe and effective measure to biological control of pear ring rot diseases. As mycoviruses can infect and proliferate in fungi and some mycoviruses present hypovirulence-associated traits, they are potential biological control agents for the control of these fungal disease. Indeed, many mycoviruses with potential biocontrol value have been found and identified from pathogenic fungi infecting fruit trees [8]. Therefore, it is particularly important to find and identify mycoviruses in *Botryosphaeria dothidea* strains casing pear ring rot disease. To identify and characterize mycovirus, high through-put sequencing techniques and subsequent bioinformatics have been shown as a very effective approach [9–11]. So far, eleven mycoviruses have been identified in *B. dothidea* strains and characterized. In our lab, *Botryosphaeria dothidea* chrysovirus 1 (BdCV1), *Bipolaris maydis* botybirnavirus 1 strain BdEW220 (BmRV1-BdEW220), *Botryosphaeria dothidea* RNA virus 1 (BdRV1) involved in hypovirulence [12–14], *Botryosphaeria dothidea* partitivirus 1 (BdPV1), *Botryosphaeria dothidea* victorivirus 1 (BdVV1), *Botryosphaeria dothidea* victorivirus 2 (BdVV2) and *Botryosphaeria dothidea* botourmiavirus 1 (BdBOV-1) with no obvious effect on host, have been identified in *B. dothidea* strains causing pear ring rot disease [12, 15–17]. *Botryosphaeria dothidea* bipartite mycovirus 1 (BdBMV1), *Botryosphaeria dothidea* mitovirus 1 (BdMV1), *Botryosphaeria dothidea* fusarivirus 1 (BdFV1) and BdCV1-G1 have been also identified in *B. dothidea* strains isolated from *Kerria japonica*, pepper and apple hosts, respectively [18–21]. The *Narnaviridae* genome has positive single strand RNA (+ssRNA) with approximately 2.2–3.6 kb in length

and encodes only one RNA-dependent RNA polymerase (RdRp). The *Narnaviridae* family is divided into two genera, *Narnavirus* and *Mitovirus* (Mitochondrial viruses), which replicate in the cytoplasm and mitochondria, respectively. The mycoviruses in *Narnaviridae* exist widely in fungi, but it is not clear if they have any effect on all kinds of fungi [22–23]. Here, we report the identification of a novel mitovirus isolated from *B. dothidea* strain, the causal pathogen of pear ring rot disease. We further determined its +ssRNA full-length genome sequence and molecular characteristics. This mycovirus was tentatively designated as *Botryosphaeria dothidea* mitovirus 2 (BdMV2).

Provenance and sequencing of BdMV2

B. dothidea strain FJ was isolated from pear stem bark exhibiting wart symptoms in Fujian Province of China, in 2010, and the hypha blocks of FJ strain were stored in 25% glycerin solution at -80°C. The mycelium was grown on potato dextrose agar (PDA) plates overlaid with a layer of cellophane at 25 °C in the dark. The mycelium of *B. dothidea* strain FJ and other *Valsa* spp strains were together collected to be used for Illumina sequencing (Beckman company, Beijing, China). The obtained high-quality clean reads were assembled contigs, which were analyzed to search mycoviruses using Blastx. Among these contigs, Contig1603 with 2.5 kb in length shares the highest sequence similarity with 67.14% in comparison to that of *Macrophomina phaseolina* mitovirus 4. Total RNA was extracted by Trizol method (Aidlab Biotechnologies Co., Ltd, Beijing, China) as template for synthesize cDNA. Finally, the putative mycoviral genome intermediate sequence was determined by RT-PCR and obtained 2042 nt by sequencing in *B. dothidea* strain FJ using the designed primers based on Contig1603 sequence (Supplementary Table 1). The dsRNA was extracted from the cultured hypha by the column separation method [24]. The adaptor RACE-OLIGO was ligated to the 3' terminus of each strand of dsRNA, which were used for template to reversely transcribed cDNA using Oligo REV primer as described previously [25]. The terminal sequence amplification was performed by nested PCR using the primers designed based on obtained viral sequence and sequence-specific adaptor primers of O5RACE-2 and O5RACE-3, respectively (Supplementary Table 1). The obtained PCR product was purified, linked to pMD18-T vector (Takara, Dalian, China), and transformed into *Escherichia coli* Top10. For each PCR product, at least three independent identified positive clones were sequenced in both directions (Sangon Biotech Co., Ltd, Shanghai, China). Sequences obtained from clones were assembled using DNAMAN. The viral ORF was predicted using the ORF finder online website of <https://www.ncbi.nlm.nih.gov/orffinder>. Mycoviral sequences search was performed using NCBI database. Multiple mycoviral sequences were aligned using CLUSTAL X [26]. Phylogenetic analysis was conducted by using NJ method with 1000 replicates of MEGA 7.0, and RdRp domain motif sequences were analyzed using the GeneDoc software [27–28]. The secondary structures of BdMV2 genome terminus were predicated by online RNAfold software.

Sequence properties

The colony morphology of *B. dothidea* strain FJ was shown, which exhibited normal mycelia and the mycelia could grow to cover the whole plate in 5 days (Fig. 1A). RT-PCR and RNA-ligase-mediated rapid amplification of cDNA ends (RLM-RACE) in combination with cloning and sequence assembly were used

to obtain the BdMV2 full-length genome sequence from *B. dothidea* strain FJ. Its complete genome sequence was deposited in the NCBI GenBank database (Acc. No. MW553911), which was 2538 nt in length with a CG content of 47.24% for the genome. BdMV2 contains a single big ORF with AUG as the initiation codon at positions 176–178 nt and UAA as the termination codon at positions 2243–2245 nt of the genome by fungal mitochondrial codon usage. The ORF (2,070 nt in length) potentially encoded 689 aa RdRp protein with predicated molecular weight of 77.35 kDa. The 5′ and 3′ untranslated regions (UTRs) are 175 and 293 nt long, respectively (Fig. 1B). Using the RNAfold program, two potential stem-loop structures in the 5′ and 3′ UTRs and a potential panhandle structure of the BdMV2 positive-sense strand UTRs were predicted, respectively (Fig. 1C), which showed typical characteristic of reported members of the genus *Mitovirus* [22]. The BdMV2 contains a conserved RdRp domain at positions 164–495 aa of the genome (Mitovirus RNA-dependent RNA polymerase; pfam05919) by the Conserved Domain Database (CDD) search of NCBI. Multiple sequence alignment analysis of RdRp domain from BdMV2 with those members in *Mitovirus* showed that BdMV2 RdRp domain contains all six conserved motifs (I to VI), which are characteristics of members in the *Mitovirus* (Fig. 2A). A BLASTp comparison of the RdRp sequence of BdMV2 showed the highest similarity of 69.36% with that of RsMV10, and had identity 28.91–68.79% (query coverage more than 90%) to those of members in the genus *Mitovirus* (Supplementary Table 2). To determine the taxonomic status of BdMV2, phylogenetic analysis was performed using the RdRp aa sequences encoded by BdMV2 and other selected mycoviruses in *Mitovirus* and *Narnavirus* of the family *Narnaviridae*. The results showed that all mitoviruses were classified into two clades of clade I and clade II. It revealed that BdMV2 existed in a separate branch, which was closely clustered in the same subclade with those of RsMV10 and MpMV4 belonging to clade I in *Mitovirus* genus, distinct from members of the genus *Narnavirus* (Fig. 2B). In conclusion, based on its genome organization, sequence alignment and phylogenetic analysis, BdMV2 is a novel mitovirus in the *B. dothidea* pathogen causing pear ring rot disease.

Declarations

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Compliance with ethical standards

Conflict of interest

All authors declare that they have no any conflicts of interest in this manuscript.

Ethical approval

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

References

1. Marsberg A, Kemler M, Jami F, Nagel JH, Postma-Smidt A, Naidoo S, Wingfield MJ, Crous PW, Spatafora JW, Hesse CN, Robbertse B, Slippers B (2017) *Botryosphaeria dothidea*: a latent pathogen of global importance to woody plant health. *Mol Plant Pathol* 18(4):477–488
2. Ran L, Zhang M, Shen HJPD (2018) First report of Red Rot of Jujube fruit caused by *Botryosphaeria dothidea* in China. *Plant Dis* 102(7):1458
3. Slippers B, Wingfield MJ (2007) Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biol Rev* 21:90–106
4. Tang W, Ding Z, Zhou ZQ, Wang YZ, Guo LY (2012) Phylogenetic and Pathogenic analyses show that the causal agent of apple ring rot in China is *Botryosphaeria dothidea*. *Plant Dis* 96(4):486–496
5. Yan JY, Xie Y, Zhang W, Wang Y, Liu JK, Hyde KD, Seem RC, Zhang GZ, Wang ZY, Yao SW, Bai XJ, Dissanayake AJ (2013) Species of Botryosphaeriaceae involved in grapevine dieback in China. *Fungal Divers* 61(1):221–236
6. Zhai LF, Zhang MX, Lv G, Chen XR, Jia NN, Hong N, Wang GP (2014) Biological and molecular characterization of four *Botryosphaeria* species isolated from pear plants showing ring rot and stem canker in China. *Plant Dis* 98:716–726
7. Al-Haq MI, Seo Y, Oshita S, Kawagoe Y (2002) Disinfection effects of electrolyzed oxidizing water on suppressing fruit rot of pear caused by *Botryosphaeria berengeriana*. *Food Res Int* 35(7):657–664
8. Chiba S, Salaipeth L, Sasaki A, Kanemastu S, Suzuki N (2009) A novel bipartite dsRNA mycovirus from the white root rot fungus *Roselia necatrix*: molecular and biological characterization, taxonomic consideration, and potential for biological control. *J Virol* 83(24):128801–128812
9. Ghabrial SA, Castón JR, Jiang D, Nibert ML, Suzuki N (2015) 50-plus years of fungal viruses. *Virology* 479:356–368
10. Lefkowitz EJ, Dempsey DM, Hendrickson RC, Orton RJ, Siddell SG, Smith DB (2018) Virus taxonomy: the database of the International Committee on Taxonomy of Viruses (ICTV). *Nucleic Acids Res* 46(D1): D708–D717
11. Marzano SYL, Nelson BD, Ajayi-Oyetunde O, Bradley CA, Hughes TJ, Hartman GL, Eastburn DM, Domier LL (2016) Identification of diverse mycoviruses through metatranscriptomics characterization of the viromes of five major fungal plant pathogens. *J Virol* 90(15):6846–6863
12. Wang LP, Jiang JJ, Wang YF, Hong N, Zhang FP, Xu WX, Wang GP (2014) Hypovirulence of the phytopathogenic fungus *Botryosphaeria dothidea*: association with a coinfecting chrysovirus and a partitivirus. *J Virol* 88(13):7517–7527
13. Zhai L, Xiang J, Zhang M, Fu M, Yang Z, Hong N, Wang G (2016) Characterization of a novel double-stranded RNA mycovirus conferring hypovirulence from the phytopathogenic fungus *Botryosphaeria*

dothidea. Virology 493:75–85

14. Zhai L, Yang M, Zhang M, Hong N, Wang G (2019) Characterization of a Botryovirus conferring hypovirulence in the phytopathogenic fungus *Botryosphaeria dothidea*. *Viruses* 11(3):266
15. Zhai L, Hong N, Zhang M, Wang G (2015) Complete dsRNA sequence of a novel victorivirus isolated from the pear stem wart fungus *Botryosphaeria dothidea*. *Arch Virol* 160(2):613–616
16. Yang M, Zhai L, Xiao F, Guo Y, Fu M, Hong N, Wang G (2019) Characterization of a novel victorivirus isolated from the phytopathogenic fungus *Botryosphaeria dothidea*. *Arch Virol* 164(6):1609–1617
17. Yang M, Zhou X, Zhai L, Xiao F, Hong N, Wang G (2020) Molecular characterization of a novel mycovirus infecting the phytopathogenic fungus *Botryosphaeria dothidea*. *Arch Virol* 165(7):1667–1670
18. Liu W, Hai D, Mu F, Yu X, Zhao Y, He B, Xie J, Jiang D, Liu H (2020) Molecular characterization of a novel fusarivirus infecting the plant pathogenic fungus *Botryosphaeria dothidea*. *Arch Virol* 165(4):1033–1037
19. Liu H, Wang H, Zhou Q (2021) A novel mycovirus isolated from the plant-pathogenic fungus *Botryosphaeria dothidea*. *Arch Virol* 166:1267–1272
20. Ding Z, Zhou T, Guo LY (2017) Characterization of a novel strain of *Botryosphaeria dothidea* chrysovirus 1 from the apple white rot pathogen *Botryosphaeria dothidea*. *Arch Virol* 162(7):2097–2102
21. Liu H, Liu M, Zhu H, Zhong J, Liao X, Zhou Q (2021) Molecular characterization of a novel mitovirus from the plant-pathogenic fungus *Botryosphaeria dothidea*. *Arch Virol* 166(2):633–637
22. Hillman BI, Cai G (2013) The family Narnaviridae: simplest of RNA viruses. *Adv Virus Res* 86:149–176
23. Hillman BI, Esteban R (2011) Narnaviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (eds) *Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses*. Elsevier, San Diego, pp 1055–1060
24. Yang F, Hong N, Wang GP. Mycovirus dsRNA extraction kit and its application: ZL201310072994.3. 2015–06–03
25. Liu H, Fu Y, Jiang D, Li G, Xie J, Peng Y, Yi X, Ghabrial SA (2009) A novel mycovirus that is related to the human pathogen hepatitis E virus and rubi-like viruses. *J. Virol* 83:1981–1991.
26. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23(21):2947–2948
27. Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30(12):2725–2729
28. Nicholas KB (1997) GeneDoc: analysis and visualization of genetic variation. *Embnew news* 4:14

Figures

Figure 1

(A) Colony morphology of *B. dothidea* strain FJ in PDA medium at 25 °C for 5d. (B) Schematic representation of the BdMV2 genome organization. The single ORF and UTRs are indicated by an open bar and black lines, respectively. The blue bar represented the RdRp conserved domain. (C) The putative secondary structures of the 5' and 3' termini and panhandle structure of BdMV2 UTRs.

Figure 2

(A) Sequence alignment of BdMV2 RdRp motifs with those of selected mycoviruses in the genus Mitovirus. Horizontal black lines above the alignment sequences indicate the conserved six motifs from I to VI. Shaded areas represented identical aa residues. (B) Phylogenetic tree analysis of BdMV2 in this study and selected mitoviruses and narnaviruses members specified as viral names followed by GenBank accession numbers were shown, based on the RdRp aa sequences using the neighbor-joining (NJ) method with 1000 bootstrap replicates. Bootstrap values with >50% are shown at the branches. The scale bar indicates a genetic distance of 0.2 aa substitutions per site. A red star indicates the newly mycovirus of BdMV2.

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