Molecular characterization of a novel victorivirus isolated from the phytopathogenic fungus Phaeobotryon rhois

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Abstract

Phaeobotryon rhois is an important pathogenic fungus that causes dieback and canker disease of woody hosts. Here, a novel mycovirus, tentatively named Phaeobotryon rhois victorivirus 1 (PrVV1), was identified from *P.rhois* strain sx8-4. The PrVV1 has a double-stranded RNA (dsRNA) genome of 5,224 nucleotides long and it contains two open reading frames (ORF1 and ORF2), which overlap at a AUGA sequence. ORF1 encodes a polypeptide of 786 amino acids (aa) with a conserved a coat protein (CP) domain, while ORF2, encodes a large polypeptide of 826 aa with a conserved RNA-dependent RNA polymerase (RdRp) domain. Our analysis of the genomic structure, homology searches, and phylogeny indicated that PrVV1 is a novel member of the genus *Victorivirus* in the family *Totiviridae*. This is the first study to report the complete genome sequence of a victorivirus that infects *P.rhois*.

Introduction

Mycoviruses can infect almost all types of fungi, including fungi, yeasts, and various oomycetes, such as phytopathogenic fungi, biocontrol fungi, and endophytic fungi [1–3]. Mycoviruses can have different genomic structures, including double-stranded (dsRNA), positive single-stranded RNA (+ ssRNA), negative single-stranded RNA (-ssRNA), and single-stranded DNA (ssDNA) [1, 4]. The International Committee on Taxonomy of Viruses (ICTV) classifies dsRNA mycoviruses into nine families including *Amalgaviridae*, *Botybirnaviridae*, *Chrysoviridae*, *Hypoviridae*, *Megabirnaviridae*, *Partitiviridae*, *Quadriviridae*, *Reoviridae*, *Totivirida* by the International Committee on Taxonomy of Viruses (ICTV). Mycoviruses in the family Totiviridae belong to five established genera, namely *Totivirus*, *Victorivirus*, *Trichomonasvirus*, *Giardiavirus*, and *Leishmaniavirus* [5–6]. To date, members of the genera *Victorivirus* only infect fungi [6], including various phytopathogenic fungi, such as *Aspergillus foetidus*, *Fusarium asiaticum*, *Nigrospora oryzae*, *Ustilaginoidea virens* [7–10]. The genome of there viruses is nearly 5 kbp in size, and generally contains two ORFs, with the 5'-proximal ORF encoding the CP and the 3'-proximal ORF encoding the RdRp [5, 11]. The termination codon of the CP overlaps with the initiation codon of the RdRp with junction sequence, typically AUGA or UAAUG. Furthermore, the CP has an Ala/ Gly/Pro-rich region near their C-terminus, which are the typical characteristics of members of the genus *Victorivirus* [6, 12–14].

Phaeobotryon rhois, belonging to the family *Botryosphaeriaceae*, is an important pathogenic fungus that cause dieback and canker disease in woody hosts [15–16]. Interestingly, mycoviruses have been reported in many types fungi that cause these types of diseases [17–19], but none reported in *P. rhois*.

Here, reports a novel dsRNA mycovirus isolated from *P. rhois* strain SX8-4, tentatively named "Phaeobotryon rhois victorivirus 1" (PrVV1). Genomic structure analysis nd phylogeny showed that PrVV1 is closely related to members of the genera *Victorivirus*. To our knowledge, it's the first mycovirus of the family *Totiviridae* that infects *P.rhois*.

Provenance And Sequencing Of Strains

P. rhois strain SX8-4 was isolated from jujube tips collected from Shanxi province, China. The isolates were cultured on a PDA plate for five days at 28ºC (Fig. 1a). The RNA samples were extracted by culturing the fungi overlaid with cellophane membranes for five days, then harvesting and grinding the mycelia with liquid nitrogen to a fine powder for dsRNA extraction using CF-11 cellulose powder method as previously described [20]. The extracted nucleic acids were digested with DNase I and S1 nuclease to eliminate DNA and ssRNA. The purified dsRNA was separated on a 1.2% gel at 130 V and imaging with a gel documentation system.

The cDNA library of *P. rhois* strain SX8-4 dsRNA was constructed using Reverse transcription box (TransGen Biotech) following the manufacturer's protocols. The sequences cloning were executed using a random primer (5'- CGATCGATCATGATGCAATGCNNNNNN-3') amplification method, and the resulting cDNAs were amplified using a single specific primer (5'-CGATCGATCATGATGCAATGC-3'). The sequencing and analysis of the cDNA sequences were done following methods that were described previously [20]. The gaps were filled by RT-PCR using l primers of obtained sequences and the terminal sequences were completed with ligase-mediated rapid amplification of cDNA ends [21]. All the amplified PCR products were cloned into pMD18-T and introduced into *E. coli* DH5α cells using the heat shock transformation method. The clones containing the inserts were sequenced at TsingKe Biological Technology Co. Ltd. (Beijing, China) for sequencing.

The resulting sequences were analyzed using Bioedit software and the ORFs were predicted using ORFfinder at the National Center for Biotechnology Information (NCBI) website. The phylogenetic analysis was performed using MEGA 11 software [22]. All sequences used as reference were retrieved from the NCBI database.

Sequence Properties

Sequence analysis showed that *P. rhois* strain SX8-4 contained a dsRNA virus belonging to the genus *Victorivirus* in the family *Totiviridae*, which was tentatively named PrVV1, and it contained a single segment about 5,000 bp in size (Fig. 1b). The full genome sequence of PrVV1 was found to be 5,224 bp in length, containing 17.3% A, 35.5% C, 29.9% G, and 17.3% U. The PrVV1 genome contains two ORFs (Fig. 1c). ORF1 is 2,361 nt long (nt positions 298 to 2658) and encodes a 786- aa protein with a molecular weight of 81.6 kDa. Based on predictive analysis using the CDsearch program on the NCBI website, this protein is a conserved coat protein (nt positions 340 to 2421) domain. Meanwhile, ORF2 is 2481 nt long (nt positions 2,655 to 5,135) and encodes an 826-aa protein with a molecular weight of 91.1 kDa. This protein contains a conserved RdRp domain (nt positions 2,955 to 4,376) (Fig. 1c). The AUGA tetranucleotide (nt positions 2,655 to 2,658) is a motif for the translation of the down-stream ORF2 by a termination-re-initiation mechanism, which involves the ORF1 stop codon and ORF2 start codon (Fig. 1c). Furthermore, the tetranucleotide AUGA is widespread in victoriviruses [11, 13–14]. Besides, an H-type pseudoknot structure (GAAggagccgCggccGCUGCAggcc cggcuccCCAACAAUGA) at the upstream of the AUGA motif. The 5' untranslated region (UTR) and 3' UTR is 297 nt (nt positions 1 to 297) and 89 nt (nt

positions 5,136 to 5,224) long, respectively, and have stable secondary structures (Fig. 1d). The genome sequence of PrVV1 has been submitted to the GenBank database (accession number OP100309).

A homology search using BLASTp showed that the ORF1 of PrVV1 has significant similarities to the totivirus CP superfamily (pfam05518). The CP of PrVV1 has a high sequence similarity to the CP sequences encoded by Diplodia seriata victorivirus 1 (DsVV1; GenBank accession number UOK20172.1, 74.64% identity, 87% coverage, E-value = 0), Macrophomina phaseolina victorivirus 2 (MpVV2, GenBank accession number QKO02071.1, 72.01% identity, 87% coverage, E-value = 0), Sphaeropsis sapinea RNA virus 2 (SsRV2; GenBank accession number NP_047559.1, 71.70% identity, 86% coverage, E-value = 0), and Neofusicoccum parvum victorivirus 2 (NpVV2; GenBank accession number QTE76049.1, 67.01% identity, 87% coverage, E-value = 0). Moreover, these is an Ala/Gly/Pro-rich region (GGGAAPPPPP PPGGNPPPPP PPGPDGPSGG NPPPPPGGAY DVVDPLPAPH NPDEGAAAAA GPAPQQ) in the C-terminal sequence of PrVV1 CP, which was previously found to be a significant molecular feature of members of the genus *Victorivirus* [6, 14]. The ORF2 of PrVV1 has significant similarities to members of the RT-like superfamily (pfam02123). Furthermore, the PrVV1 RdRp is high similar to the RdRps encoded by DsVV1 (GenBank accession number UOK20171.1, 70.10% identity, 100% coverage, E-value = 0), MpVV2 (GenBank accession number QKO02072.1, 71.43% identity, 100% coverage, E-value = 0), SsRV2 (GenBank accession number NP_047560.1, 66.22% identity, 100% coverage, E-value = 0), and NpVV2 (GenBank accession number QTE76050.1, 66.22% identity, 100% coverage, E-value = 0).

Multiple aa sequence alignment of the RdRps of PrVV1 and some members of the family *Totiviridae* revealed several conserved aa motifs (Fig. 2a). The phylogenetic tree analysis based on the RdRp sequences of PrVV1 members of the family *Totiviridae*, and other selected mycoviruses was constructed using the ML method (Fig. 2b). PrVV1 clustered with three mycoviruses (MpVV2, DsVV1 and NpVV2) form a clade that, together with other two clades that, formed a larger clade representing the members of the genus Victorivirus. Moreover, phylogenetic tree based on the CP sequences of PrVV1 and other selected viruses was constructed too (Fig. 2c). PrVV1 also clustered with the three mycoviruses (MpVV2, DsVV1 and NpVV2) form a clade that, together with other four clades that, formed a larger clade representing the genus *Victorivirus*. The results showed that PrVV1 is a novel member of the genus *Victorivirus*, family *Totiviridae*.

In conclusion, based on the sequence comparison, genome organization, ORFs characterization, and phylogenetic analysis, PrVV1 should be considered a new member of the genus *Victorivirus* in the family *Totiviridae*. To our knowledge, this is the first study to report a victorivirus infecting *P.rhois*, and the first mycovirus reported in *P.rhois.*

Declarations

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Authors' contributions YiZ, CaW and HM designed the research. HD, ZG, RZ, GW, ChW, YuZ, MW and XW performed the experimental work. YiZ, CaW, RZ, YL, and HM analyzed the data and wrote the manuscript.

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Informed Consent All the authors affirm that human research participants provided informed consent for publication of the manuscript and images in Figure(s) 1a, 1b, 1c, 1d,2a, 2b and2c.

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Figures

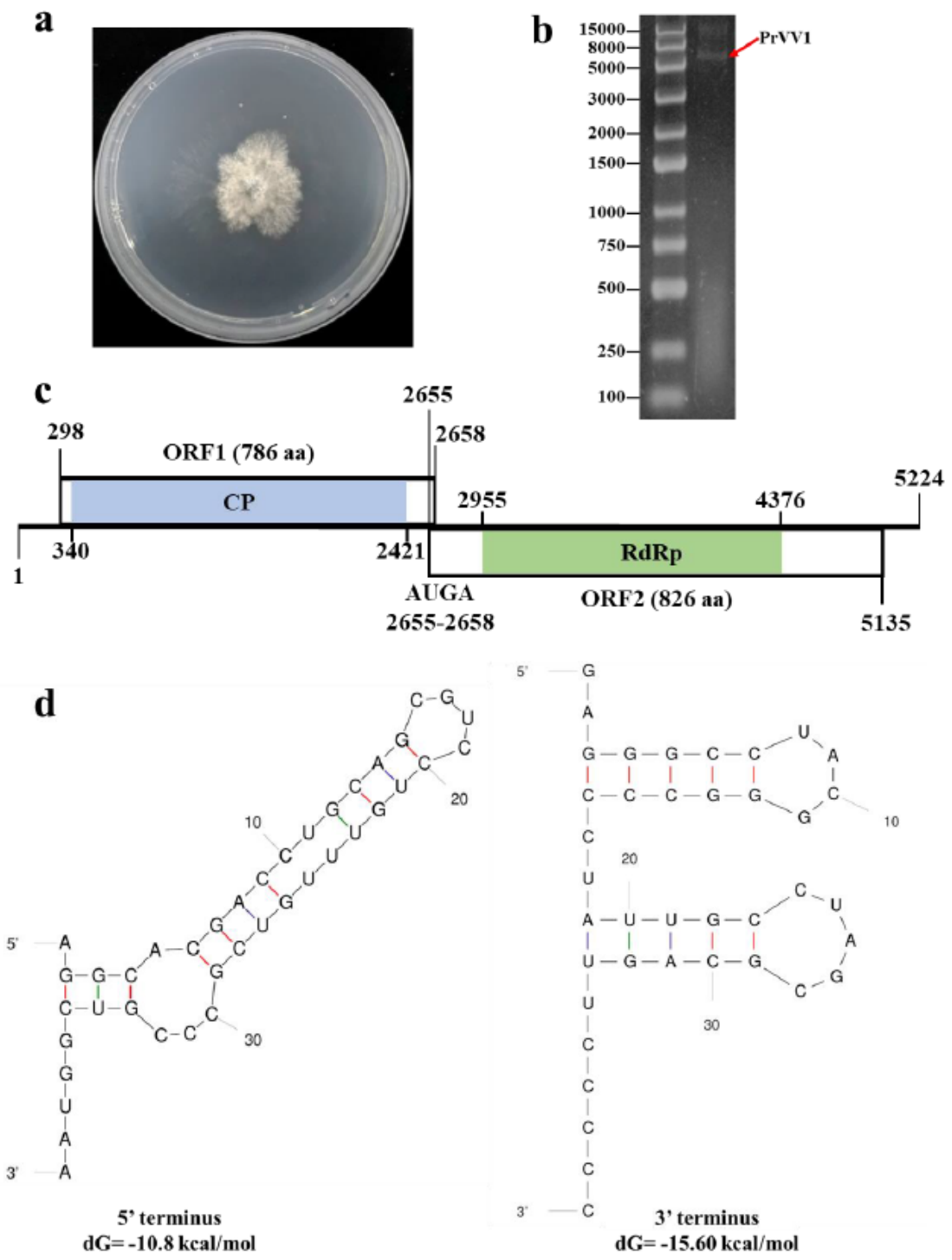

Figure 1

Properties of PrVV1. **(a)** Colony morphology of strain SX8-4. **(b)** Agarose gel electrophoresis of dsRNA extracted from strain SX8-4. **(c)** Genome organization of PrVV1. **(d)** The predicted secondary structures for the 5'-UTR and 3'-UTR of PrVV1.

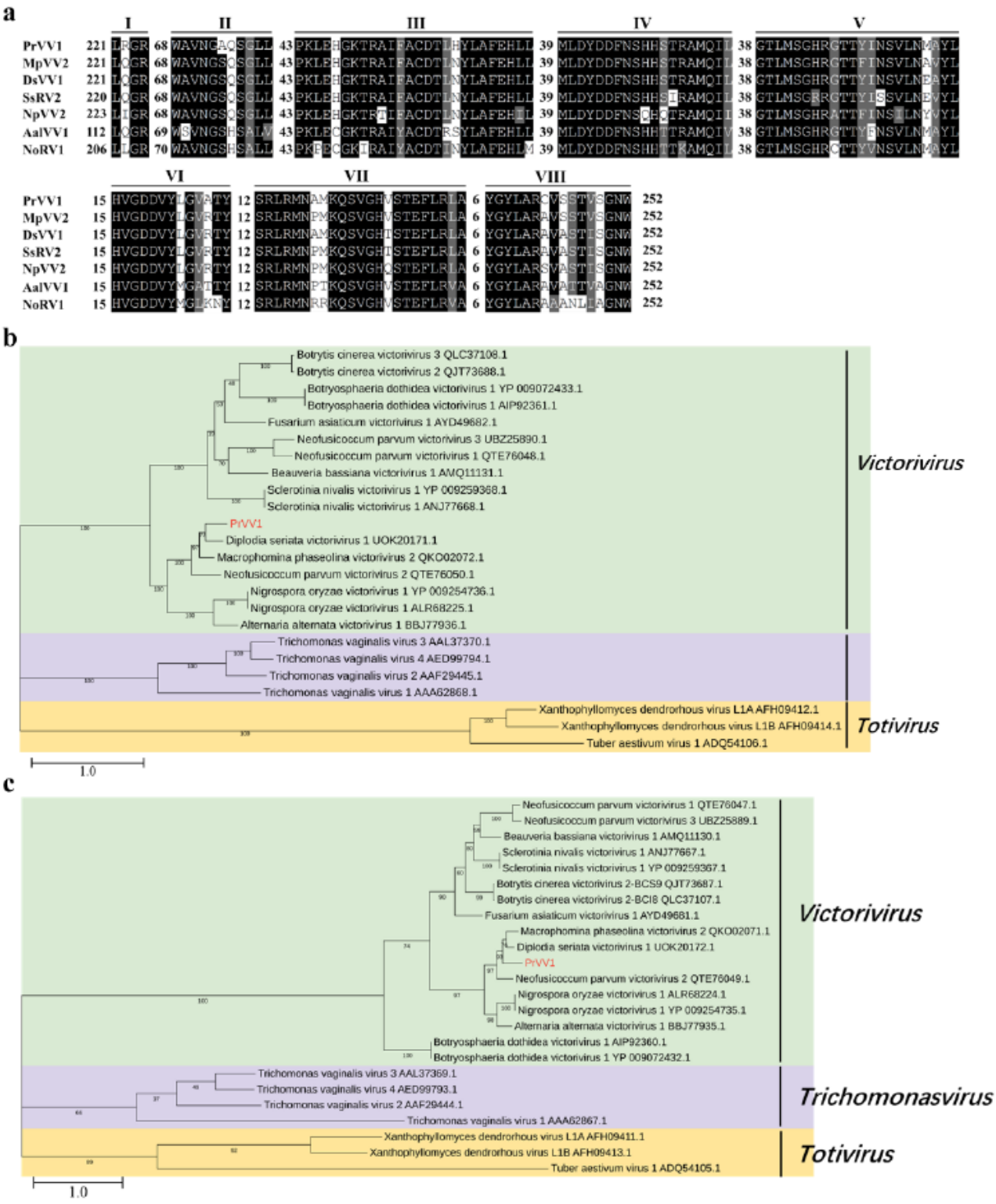

Figure 2

Phylogenetic analysis of PrVV1. **(a)** Multiple alignment of the amino acid sequences of RdRp of PrVV1 and other selected members of the family *Totiviridae*. **(b)** Phylogenetic analysis of AnV1 based on CP sequences. **(c)** Phylogenetic analysis of AnV1 based on RdRp sequences. The trees were constructed in MEGA 11 using the ML method with 1,000 bootstrap replicates.