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GENOME SEQUENCES

Genome Sequence of *Sclerotinia sclerotiorum* Hypovirulence-Associated DNA Virus 1 Found in the Fungus *Penicillium olsonii* Isolated from Washington State, USA

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ABSTRACT We report the discovery of a *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1) isolate, named SsHADV1_PO, from the fungus *Penicillium olsonii* isolated from Washington state, USA. The genome of SsHADV1_PO is 2,166 bp and contains two open reading frames, with more than 98% nucleotide identity with respect to reported SsHADV-1 isolates.

Mycoviruses are widely distributed in all major groups of fungi. The majority of mycoviruses possess either double-stranded or single-stranded RNA genomes but seldom possess single-stranded DNA (ssDNA) (1, 2). The first circular ssDNA mycovirus, named *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1), was discovered in strain DT-8 of *Sclerotinia sclerotiorum* in China (2). SsHADV-1 is the type species of the family *Genomoviridae* (3). The genome of SsHADV-1 is 2,166 nucleotides (nt) and contains two open reading frames, encoding a capsid protein (CP) and a replication-associated protein (Rep) (2). Subsequently, sequences similar to SsHADV-1 were detected in river sediments in New Zealand (4), in the insects dragonflies and damselflies in the United States (5), and in *Sclerotinia sclerotiorum* in Australia (6). Here, we report the discovery of a SsHADV-1 isolate from the fungus *Penicillium olsonii* from Washington, USA.

During the study of a collection of *Penicillium* isolates isolated from tomato fruits and insects collected in Kennewick and Pullman, Washington, USA, a SsHADV-1-like virus was detected in a strain (QM2-1) of *Penicillium olsonii* by PCR screening (Fig. 1A and B and Table 1). This strain was from the insect *Bradysia impatiens* and was initially isolated on potato dextrose agar (PDA), purified from a single conidium, and identified based on morphology and DNA sequences of three loci (*TUB2*, *CAL*, and the internal transcribed spacer [ITS]) (7). Total genomic DNA was isolated from 7-day-old mycelia cultured on PDA at 22°C using the cetyltrimethylammonium bromide method (8). The partial CP gene was amplified with the PCR primers CP-F and CP-RP (Table 1) in two separate amplifications and sequenced by Sanger sequencing in both orientations. The sequences were found to be identical and were used to design two back-to-back primers (725F and 704R) for inverse PCR to amplify the entire viral genome, using the Q5 high-fidelity DNA polymerase (New England Biolabs, USA). The ~2.2-kb PCR product was cloned into the pMiniT 2.0 vector (New England Biolabs) (Fig. 1C). Two independent clones were sequenced in both directions by Sanger sequencing with primer walking (Table 1). Additionally, the viral DNA was enriched by rolling circle amplification using TempliPhi (GE Healthcare) and sequenced completely in one direction by Sanger sequencing using primer walking (Table 1). Ambiguous sequences at both ends of the sequence reads were trimmed by DNAMAN v.9.0 (Lynnon Biosoft). Vector NTI software (Invitrogen, USA) was used for assembly of the sequences. The resulting viral genome sequence was identical to that generated by inverse PCR.

The complete nucleotide sequence of SsHADV-1 in *Penicillium olsonii* (named SsHADV1_PO) is 2,166 nt, with a GC content of 47.4%; it encodes a CP on the sense

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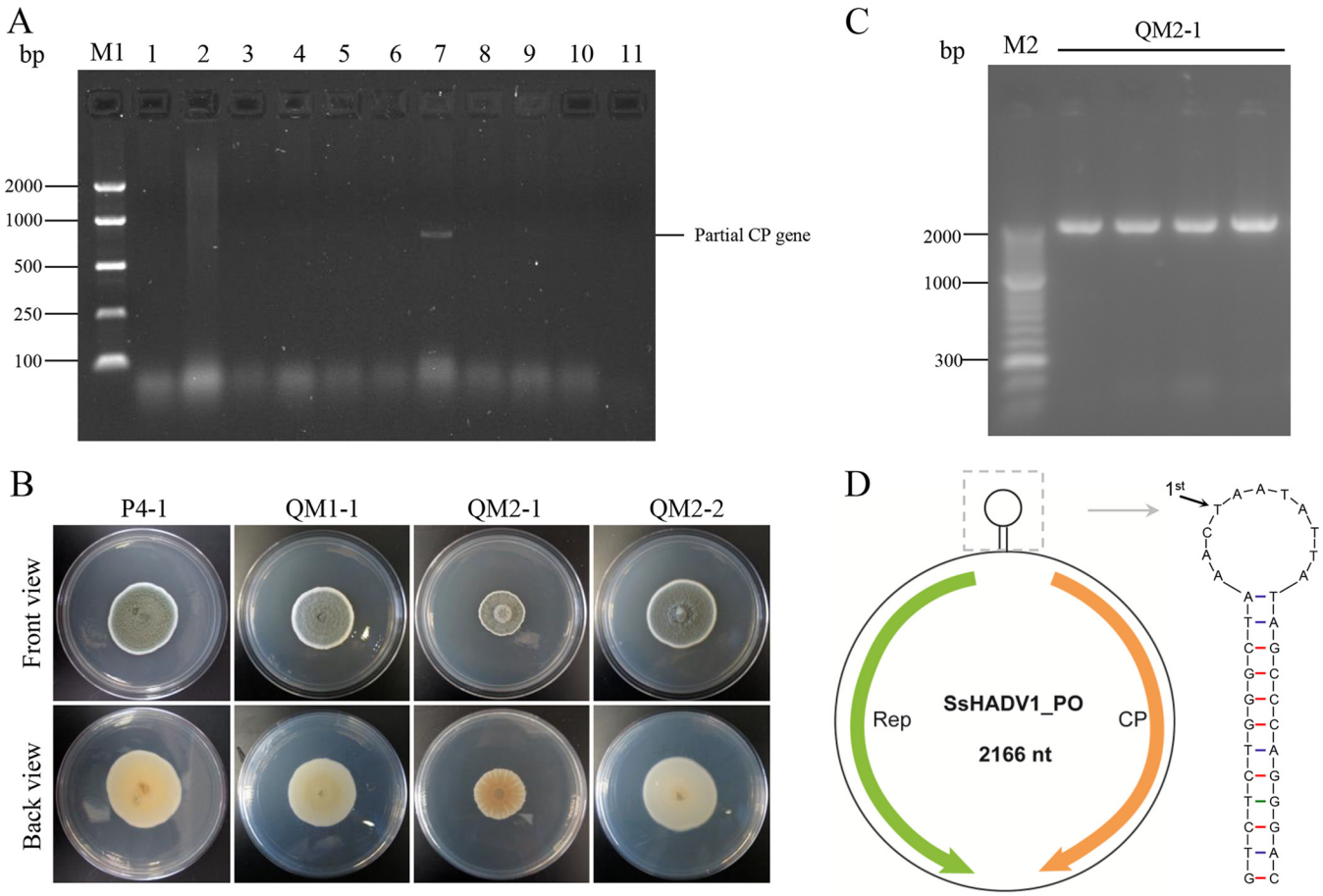

FIG 1 SsHADV-1-like virus detected in a strain of *Penicillium olsonii*. (A) Detection of viral DNA from *Penicillium* cultures by PCR amplification with the SsHADV-1-specific primer pair CP-F/CP-RP. M1, EasyLadder I (Bioline Meridian Bioscience). Lanes 1 to 4, strains P1-1, P2-1, P3-1, and P4-1, respectively, from tomato fruits; lanes 5 to 10, strains QM1-1, QM1-2, QM2-1, QM2-2, QM3-1, and QM4-1, respectively, from *Bradysia impatiens*; lane 11, negative control (no DNA template). (B) Colony morphology of SsHADV1_PO-infected strain QM2-1 and three virus-free strains of *P. olsonii* grown on PDA plates at 22°C for 8 days. Mycelial discs (4-mm diameter) were taken from 2-day-old cultures on PDA plates of isolates all originating from a single conidium. (C) The full-length sequence of SsHADV1_PO amplified by inverse PCR using a back-to-back primer pair, 725F/704R. M2, HyperLadder 50 bp (Bioline Meridian Bioscience). (D) Genome organization of SsHADV1_PO. The stem-loop structure of the origin of virion-strand replication of SsHADV1_PO was predicted using the DNA folding form at the mfold web server (http://www.unafold.org/mfold/applications/dna-folding-form.php) with default settings.

strand and a Rep on the complementary-sense strand. A potential stem-loop structure was predicted in the origin of virion-strand replication of SsHADV1_PO (Fig. 1D). Based on BLASTn searches, SsHADV1_PO had >98% sequence identity to SsHADV-1 found in China (GenBank accession number GQ365709, 99.58%), Australia (GenBank accession

TABLE 1 Primers used in this study

Primer name	Sequence (5′ to 3′)	Purpose
CP-F	GGAGCATCCTCAACACGACATC	Amplification of partial CP gene of SsHADV1_PO; sequencing.
CP-RP	TACGAAGAAGGTCGGACGCC	
725F	CGATCCTATTATTGCCCCTCT	Amplification of complete genome of SsHADV1_PO; sequencing.
704R	GTCCAATCAACATTCTCTGCC	
1244F	AGGATCAGCGTTTGAACACC	Primer walking for sequencing.
1756F	CTGACGTAATGATAGCCCACT	
372F	TTTATTTGGTGCCCTACTGCAAT	Primer walking for sequencing of rolling circle amplification product.
557F	CGTTATGGCAACAGAGTCTCC	
794F	ATCTGGCAATACTAATGGGAT	
985F	ACCTTCTTCGTATGACCGCTA	
1244F	AGGATCAGCGTTTGAACACC	
1547F	CTCACTAGAGCCGATTCCAG	
2011F	TTCCAATGATGCACTCAGCTC	
2112R	ACATACCCTGCGATGAAACCAG	

number MF444288, 99.31%), New Zealand (GenBank accession number KF268026, 98.52%), and the United States (GenBank accession number KM598382, 98.43%). The mycovirus SsHADV-1 confers hypovirulence to strain DT-8, which can be used as a biocontrol agent for Sclerotinia white mold (9). The effects of the SsHADV-1_PO isolate on the biology and virulence of *Penicillium olsonii* remain to be investigated.

Data availability. The complete genome of SsHADV-1 described in this report has been deposited in NCBI GenBank under accession number OL542577.

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We declare that we have no conflicts of interests.

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