Evidence for a Novel Partitivirus Isolated From Entomopathogenic Nematode *Steinernema Ceratophorum*

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Research Article

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Abstract

Nematodes are abundant, yet little is known about their viruses. In this study, we report a novel partitivirus isolated from Entomopathogenic nematode specie "*Steinernema ceratophorum*, named as Steinernema ceratophorum Partiti-like Virus 1 (ScPV1). The complete genome of ScPV1 is comprised of two dsRNA segments, dsRNA1 (2352 bp) and dsRNA2 (2196 bp) in length. Both dsRNAs contained a single open reading frame (ORF), encoding a putative RNA-dependent RNA polymerase (RdRp) and a coat protein (CP), respectively. The sequences of the RdRp and CP showed the highest similarity (47% and 33% identity, respectively) to Plasmopara viticola associated Partitivirus 7. Multiple sequence alignments and phylogenetic analysis of RdRp of ScPV1 with other selected viruses indicated that ScPV1 is the new member of genus *Betapartitivirus* in the family *Partitiviridae*.

Introduction

Nematodes were one group of organisms that were previously thought to be immune to viral infections and has not been adequately explored for infectious viruses. Recent advances in genomic approaches has led to the rapid identification of unknown viruses from recalcitrant environments and organisms [2, 3]. The first nematode viruses were detected from *Caenorhabditis elegans* and *C.briggsae* [4, 5]. Orsay, the first known infectious virus of nematodes was isolated from *C. elegans* [4]. In addition, Santeuil and Le Blanc viruses were identified from *C.briggsae* [5]. Sequence analysis of all three nematode viruses revealed that they belong to family *Nodaviridae* [4, 5]. Soon afterwards, first infectious viruses of plant parasitic nematodes were reported from *Heterodera glycines* (soybean cyst nematode; SCN) [6, 7]. The identification of viruses from natural nematode populations supports the notion that nematode viruses might be common but overlooked [4]. EPNs are known to control wide range of insect pests and are safe alternatives to chemical insecticides which are known to be hazardous to environment and human health. [1].

Above evidence indicates that EPNs may also be infected with viruses, in this study, we report a dsRNA virus isolated from *Steinernema ceratophorum* strain D43. Steinernema ceratophorum Partiti-like Virus 1 belongs to the family *Partitiviridae*, which is consists of five genera, *Alphapartitivirus*, *Betapartitivirus*, *Cryspovirus*, *Deltapartitivirus*, and *Gammapartitivirus* [8]. Recently, two new genera, "Epsilonpartitivirus" and "Zetapartitivirus", have been proposed in this family [9, 10]. Phylogenetic analysis of the RdRp of ScPV1 and other related viruses confirmed that ScPV1 is a new member of the genus *Betapartitivirus*

Provenance Of Viral Material

The strain D43 was stored at 4°C refrigerator, was cultivated on the larvae of *Galleria mellonella* for the purpose of multiplication of D43. Wax moths were incubated at 23°C for 28 hours. Nematodes were collected in nematode collection dish from dead wax moth placed in dark for 5–7 days and stored at 4°C. The dsRNA was extracted using TransZol RNA extraction kit following the described kit procedures.

Extracted RNA was stored at -80°C for the construction of cDNA libraries. The strain D43 was identified by method proposed by Bekal *et al* [6].

The dsRNA was extracted using CF-11 cellulose (Sigma) chromatography [11]. The contamination of DNA and rRNA was removed by digestion with DNase I and S1 nuclease, respectively, (Takara, Dalian, China), purified dsRNAs were electrophoresed in a 1% (w/v) agarose gel. The strain D43 was found to harbor several dsRNA segments. A cDNA library was constructed using the tagged random primer dN6 (5'-GACGTCCAGATCGCGAAT TCNNNNNN-3') and reverse transcriptase [12]. Resulting cDNAs were amplified by using a specific primer set (Forward primer 5'-CCAGGTCATTAGGTTGCTGA-3'/Reverse primer 5'-GCAGGTTCTCTATTGGGTGG-3') and PrimeSTAR HS DNA Polymerase (TaKaRa, Dalian, China). The 5'- and 3'-terminal sequences of ScPV1 were cloned by using RNA-ligase-mediated rapid amplification of cDNA ends (RLM-RACE). All of the PCR products were cloned into the PMD™19-T vector (Takara, Dalian, China) and sequenced by the Sanger method. Positive clones were selected for sequencing and analyzed using the DNAMAN program and the BLASTx program on the NCBI website.

Putative ORFs were predicted by using ORF finder program in NCBI (<https://www.ncbi.nlm.nih.gov/orffinder/>). Conserved domain findings and homology searches were performed by using the NCBI's conserved domain search and the BLASTp program. Multiple sequence alignment of ScPV1 with homologues partitiviruses was performed by using the MAFFT program (<https://www.ebi.ac.uk/Tools/msa/mafft/>). The complete nucleotide (nt) sequences of the both dsRNAs were deposited in the GenBank database under the accession numbers MZ964623 for dsRNA1 and MZ964624 for dsRNA2. A phylogenetic tree was constructed by the maximum likelihood (ML) method with 1000 bootstrap samples, with MEGA 7.0 software [13]

Sequence Properties

The complete genome of ScPV1 is comprised of two segments here named as dsRNA1 and dsRNA2, are 2352 bp and 2196 bp in length, respectively, with poly (A) tails at their 3'-termini. The 5'- untranslated region (UTR) and 3'- UTR of dsRNA1 were 84 bp and 210 bp long, respectively (Fig. 1A). Whereas 5'- and 3'- UTRs of dsRNA2 were 58 bp and 303 bp long, respectively (Fig. 1A). Alignment of the 5'- and 3'-UTR sequences of both ScPV1 dsRNAs showed that they were conservative each other (Fig. 1B). Sequence analysis revealed that both dsRNAs contain single ORF, ORF1 and ORF2 respectively.

ORF1 extends from nt position 85 to 2226, was predicted to encode a 713-aa protein with predicted molecular mass of 83 KDa (Fig. 1A). BLASTp search indicated that dsRNA1 product has the highest similarities with RdRps of *Partitiviridae* family members particularly with *Plasmopara viticola* associated Partitivirus 7 (GenBank accession number QHD64796.1; E value = 2e-142, 98% Query coverage and 47% Percent identity). A Conserved Domain Database (CDD) search showed that RdRp contains a conserved viral RdRp domain (RT_like super family, cl02808). Multiple sequence alignment of RdRps based amino acid sequences of ScPV1 and other related viruses indicated six conserved motifs (III-VIII) (Fig. 2A). ORF2 begins from 59 bp and ends at 1951 bp, encoding a 630-aa protein with calculated molecular mass of 71

KDa (Fig. 1A). A BLASTp search indicated that this protein was most closely related to the CP of *Plasmopara viticola* associated Partitivirus 7, with 33% aa sequence identity of (E value 1e-109; 96% Query coverage). In addition, multiple sequence alignment based on coat proteins of ScPV1 and other closely related members of *Partitiviridae* also showed conservation of few regions (Fig. S1A).

The phylogenetic analysis based on RdRp (Fig. 2B) and CP (Fig. S1B) sequences showed that ScPV1 grouped with members of genus *Betapartitivirus*. Selected members of family *Partitiviridae* were divided into four recognized genera (*Alphapartitivirus*, *Betapartitivirus*, *Gammapartitivirus*, and *Deltapartitivirus*) and two proposed genera (*Epsilonpartitivirus* and *Zetapartitivirus*) (Fig. 2B) (Fig. S1B). Therefore, ScPV1 can be considered a new member of the genus *Betapartitivirus*.

Declarations

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical approval This article does not contain any studies involving human participants or animals.

References

1. Askary TH, Abd-Elgawad M (2017) Beneficial nematodes in agroecosystems: a global perspective. *Biocontrol Agents: entomopathogenic and slug parasitic nematodes* CAB International:3-25
2. Culley AI, Lang AS, Suttle CA (2006) Metagenomic analysis of coastal RNA virus communities. *Science* 312:1795-1798
3. Edwards R, Rohwer F (2005) Viral metagenomics. *Nat. Rev. Microbiol* 3:504-510
4. Félix M-A, Ashe A, Piffaretti J, Wu G, Nuez I, Bélicard T, Jiang Y, Zhao G, Franz CJ, Goldstein LD (2011) Natural and experimental infection of *Caenorhabditis* nematodes by novel viruses related to *nodaviruses*. *PLoS Biol* 9:e1000586
5. Franz CJ, Zhao G, Félix M-A, Wang D (2012) Complete genome sequence of Le Blanc virus, a third *Caenorhabditis* nematode-infecting virus. *Am Soc Microbiol*,
6. Bekal S, Domier LL, Niblack TL, Lambert KN (2011) Discovery and initial analysis of novel viral genomes in the soybean cyst nematode. *J Gen Virol* 92:1870-1879
7. Bekal S, Domier LL, Gonfa B, McCoppin NK, Lambert KN, Bhalerao K (2014) A novel *flavivirus* in the soybean cyst nematode. *J Gen Virol* 95:1272-1280
8. Vainio EJ, Chiba S, Ghabrial SA, Maiss E, Roossinck M, Sabanadzovic S, Suzuki N, Xie J, Nibert M (2018) ICTV virus taxonomy profile: *Partitiviridae*. *J Gen Virol* 99:17-18

- ## Figures

Figure 1

(A) Schematic representation of the ScPV1 genomic structure. ORF1 encodes an RdRp, and ORF2 encodes a hypothetical protein. The black lines indicate the 5' and 3' UTRs. (B) Comparison of the 5'- and 3'-terminal sequences of dsRNA1 and dsRNA2. The conserved parts of the 5'-UTR and 3'-UTR sequences of ScPV1 dsRNA1 and dsRNA2 are shaded by the black and grey color.

Figure 2

(A) Multiple alignment of the amino acid sequences of RdRp of ScPV1 and other selected members of Partitiviridae. The horizontal lines above the alignment indicate the six conserved motifs. Similar amino acids are shaded black and grey. (B) Phylogenetic analysis based on RdRP sequences of ScPV1 and other members of the family Partitiviridae. Bootstrap values higher than 60% are shown. The Maximum Likelihood (ML) tree was constructed in MEGA 7.0 with 1000 bootstrap replicates. ScPV1 is indicated by a red circle

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