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Molecular Characterization of a Novel Putative Partitivirus Infecting *Cytospora sacchari*, a Plant Pathogenic Fungus

Mahtab Peyambari¹, Mina Koohi Habibi¹, Khalil-Berdi Fotouhifar¹, Akbar Dizadji¹ and Marilyn J. Roossinck^{2*}

¹Department of Plant Protection, Faculty of Agricultural Science and Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

²The Huck Institutes of the Life Sciences, Center for Infectious Disease Dynamics, Millennium Science Complex, The Pennsylvania State University, University Park, PA 16802, USA

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Three double-stranded RNAs (dsRNAs), approximately 1.85, 1.65 and 1.27 kb in size, were detected in an isolate of *Cytospora sacchari* from Iran. Partial nucleotide sequence revealed a 1,284 bp segment containing one ORF that potentially encodes a 405 aa protein. This protein contains conserved motifs related to RNA dependent RNA polymerases (RdRp) that showed similarity to RdRps of partitiviruses. The results indicate that these dsRNAs represent a novel Partitivirus that we tentatively designate *Cytospora sacchari partitivirus* (CsPV). Treatment of the fungal strain by cyclohexamide and also hyphal tip culture had no effect on removing the putative virus. Phylogenetic analysis of putative RdRp of CsPV and other partitiviruses places CsPV as a member of the genus *Partitivirus* in the family *Partitiviridae*, and clustering with *Aspergillus ochraceous virus 1*.

Keywords : *Cytospora*, mycovirus, partitivirus, virus evolution

Fungal viruses or mycoviruses are widespread among all major taxa of fungi (Ghabrial and Suzuki, 2009). Although many Mycoviruses are apparently symptomless in their hosts, some affect mycelial growth, sporulation, pigmentation and, for pathogenic fungi, virulence of the fungal hosts (Pearson et al., 2009). Virus effects on fungal virulence can be up-regulating or down-regulating (called hypervirulence and hypovirulence, respectively). Attenuation of virulence by mycoviruses has led to their use as biological control for phytopathogenic fungi (Nuss, 1992). The fungus-mycovirus system *Cryphonectria parasitica*, the casual agent of chestnut blight disease, and *Cryphonectria hypovirus 1*

(CHV1) is a well-characterized example of hypovirulence (Milgroom and Cortesi 2004; Nuss, 2005). *Rosellinia necatrix megabirnavirus 1* is also a potential viral agent for biological control of the white root rot disease caused by *Rosellinia necatrix* (Chiba et al., 2009). An example of hypervirulence is the double-stranded RNA (dsRNA) element of *Nectria radicicola* that is associated with enhanced virulence of the host fungus (Ahn and Lee, 2001).

Mycoviruses appear to lack any extracellular phase in their life cycle so their transmission is thought to be intracellular, horizontally by hyphal anastomosis, and vertically by spores (Cortesi et al., 2001; Ghabrial and Suzuki, 2009). As with all viruses, they are dependent on their host for their replication (Park et al., 2006). Most mycoviruses have either single-stranded RNA (ssRNA) or dsRNA genomes (Ghabrial, 1998), although recently, a novel ssDNA mycovirus was discovered in *Sclerotinia sclerotiorum* (Yu et al., 2010). The ssRNA mycoviruses are classified into the families *Hypoviridae*, *Narnaviridae*, *Endornaviridae*, *Alphaflexiviridae* and *Gammapflexiviridae* (Ghabrial and Suzuki, 2009; King et al., 2012). Some ssRNA mycoviruses have not yet been classified into families. The dsRNA mycoviruses are classified into four families: *Partitiviridae*, *Totiviridae*, *Reoviridae* and *Chrysoviridae*, based on the number of genome segments, capsid structure and nucleotide sequences (King et al., 2012). *Partitiviridae* and *Totiviridae* members have a wide range of fungal host species, compared with the members of *Reoviridae* and *Chrysoviridae* families. Recently some novel dsRNA mycoviruses were reported that do not classify in any of these families, e.g., *Rosellinia necatrix megabirnavirus 1* (Chiba et al., 2009) and *Fusarium graminearum virus 3* (Yu et al., 2009). Increasing numbers of reported novel mycoviruses reflects the high diversity of mycoviruses and might help to increase our knowledge about the origin, ecology and evolutionary pathways of mycoviruses.

*Corresponding author.

Phone) +1-814-865-2292, FAX) +1-814-863-7217

E-mail) mjr25@psu.edu

The family *Partitiviridae* consists of four genera that have bipartite genomes (Ghabrial et al., 2008; Nibert et al., 2009); the members of the genus *Partivirus* are reported to specifically infect fungi (King et al., 2012). Partitiviruses have isometric virions consisting of two unrelated, linear dsRNA segments, in the size range of 1.4–2.3 kbp. Each genome segment is packaged inside a separate viral capsid (Nibert et al., 2013). RNA1 codes for the RNA-dependent RNA polymerase (RdRp) and RNA2 codes for the capsid protein (CP) (Ghabrial et al., 2008; King et al., 2012). However, an additional segment has been identified in some members of this family, which is presumed to be a satellite RNA (Chiba et al., 2013; Ghabrial et al., 2008).

Species of the fungus *Cytospora* Ehrenb. cause canker and dieback diseases in trees, shrubs and, rarely, herbaceous plants. The diseases affect cultivation, productivity and longevity of fruit trees, and ornamental and forest shrubs and trees (Adams et al., 2006; Fotouhifar et al., 2010). *Cytospora* species are classified into Valsaceae, Diaporthales, Ascomycota (Adams et al., 2005). Two species, *C. tritici* and *C. sacchari*, were reported from the monocotyledonous plants sugarcane (*Saccharum officinarum* L.) and wheat (*Triticum aestivum* L.), respectively (Adams et al., 2005). In Iran *C. sacchari* was reported as the causal agent of sheath rot disease of sugarcane from Khuzestan province (Taher-Khani et al., 2004). This species can also infect *S. spontaneum* and *Holcus sorghum* (*Sorghum vulgare*) (Sivanesan, 1983). A sexual form has not been reported for *C. sacchari*. Infected plants have leaves that become dry from the tips downwards and their sheaths are brown to black (Sivanesan, 1983). In severe infection, the whole sheath turns reddish-brown and eventually becomes necrotic (Taher-Khani et al., 2004). This infection can result in significant yield losses. The attempts to control *Cytospora* cankers with fungicides or resistant cultivars have had limited success (Snyder et al., 1989). Attenuated virulence in some plant pathogenic fungi is associated with the presence of dsRNA that leads to biological control of diseases. Hammar et al. (1989) reported that a hypovirulent isolate of *Leucostoma persoonii*, casual agent of peach canker, contained dsRNAs and exhibited abnormal culture morphology and reduced virulence. Recently a new *Hypovirus*, *Valsa ceratosperma hypovirus 1* (VcHV1), has been identified in an isolate of *Valsa ceratosperma* (Yaegashi et al., 2012). However, VcHV1 did not affect colony morphology or fungal virulence in *V. ceratosperma*. To our knowledge, no dsRNA has yet been reported for *C. sacchari*.

In the present study, we identify and partially characterize a dsRNA mycovirus, tentatively named *Cytospora sac-*

chari partitivirus (CsPV), isolated from *C. sacchari* isolate 125, which was originally obtained from sugarcane in Khuzestan, Iran. Partial nucleotide sequence of this virus, sequence comparisons and phylogenetic analysis of putative RdRp indicate that CsPV is closely related to members of the genus *Partivirus*, e.g., *Penicillium stoloniferom virus S* (PsVS), *Aspergillus ochraceous virus 1* (AoV1) and *Ophiostoma Partitivirus 1* (OPV1) that belong to the family *Partitiviridae*. Attempts to remove the virus by treatment of the fungus with cyclohexamide and hyphal tip culture were unsuccessful.

Materials and Methods

Fungal isolate and growth conditions. *C. sacchari* 125 was originally isolated from diseased sugarcane that was collected from Khuzestan province, Iran (Fotouhifar, 2007). Isolate 125 was identified as a strain of *C. sacchari* by amplifying and sequencing of the internal transcribed spacer (ITS) region, ITS1-5.8S-ITS2, and comparing the sequence (GenBank accession no. EF447388) with those of other strains in Genbank (Fotouhifar et al., 2010). *C. sacchari* 125 was cultured on potato dextrose agar (PDA) at 25–27°C for 4–5 days. Then five mycelial agar discs from the PDA plate were inoculated to 150 ml potato dextrose broth (PDB) and cultured for 3–4 days at 25–27°C with shaking. The biomass was extracted with Buchner funnel, lyophilized and then kept in –20°C for extracting dsRNA.

dsRNA extraction. Viral dsRNA was extracted from isolate 125 by CF11 (Whatman, UK) cellulose chromatography as described by Dodds et al. (1984). In brief, mycelial tissue was ground by mortar and pestle in liquid nitrogen to a fine powder. Total nucleic acids were extracted from 40 mg of mycelial powder using 800 µl 2×STE buffer (20 ml of 1 M Tris (pH 8.0), 40 ml of 5 M NaCl, 4 ml of 0.5 M EDTA (pH 8.0) and 936 ml of sterile distilled water) and proteins were precipitated with 80 µl 10% sodium dodecyl sulfate (SDS) and 800 µl phenol-chloroform-isoamylalcohol (12:12:1). The resulting mixture was centrifuged at 5,000×g for 10 min, and exactly 700 µl of the aqueous phase was mixed with 700 µl chloroform-isoamylalcohol (24:1), followed by centrifugation to separate phases for 5 min, 13,000×g. Exactly 600 µl of the aqueous phase was transferred to a 1.5 ml microfuge tube, and the extracted nucleic acids adjusted with absolute ethanol to a final concentration of 15%. The dsRNA was purified by chromatography on CF11 cellulose; the cellulose CF-11 was washed with 1×STE/15% ethanol and the dsRNA was eluted with 1×STE. The dsRNA was precipitated in 0.3 M sodium ac-

etate (pH 4) and 70% ethanol and was collected by centrifugation. The pellet was rinsed with 70% ethanol. The final dsRNA pellet was dissolved in 20 µl RNase-free water and digested with DNase I and RNase A at high and low salt concentrations as described by Howitt et al. (1995). The final dsRNA was electrophoresed on a 0.8% agarose gel in TBE buffer stained with ethidium bromide, and observed under UV illumination. The dsRNA sample was stored at -20°C for further steps.

Curing experiments. Cycloheximide treatments were used to eliminate dsRNA in isolate 125. These treatments were carried out on PDA plates containing 25 and 50 µg cycloheximide (Sigma, USA) per milliliter (Hammar et al., 1989). Mycelial plugs of isolate 125 were transferred onto treated PDA plates. Two replicates of each concentration were incubated at $25\text{--}27^{\circ}\text{C}$. After 3 days, hyphal tips from the margins of the colonies were transferred to fresh PDA. This was repeated two times. Then mycelial plugs were cultured in PDB for 3–4 days. The dsRNA was extracted to investigate the effect of cycloheximide and hyphal tip culture on mycelial infection as described above.

RT-PCR, cloning and sequencing. DsRNA from isolate 125 was used for cDNA synthesis with tagged random hexamer primers using a protocol based on that of Roossinck et al. (2010). A 9 µl aliquot of dsRNA was mixed with 1 µl 10 mM TE buffer (10 mM Tris, 10 mM EDTA, pH 7.5) and 2 µl (20 µM) random primer 5'-CCT GAA TTC GGA TCC TCC N6-3'. The dsRNA was placed 2 min in boiling water for denaturing, and then chilled on ice. Eight µl of reverse transcription mixture containing 4 µl $5\times$ superscript buffer, 1 µl dNTPs (10 mM each), 2 µl dithiothreitol (supplied by manufacturer) and 1 µl Superscript III (Invitrogen, USA) was added to the sample, and the mixture was incubated on ice for 15 min, and then placed at 50°C for 2 h. The samples were removed from 50°C and treated with 1 µl (10 mg/ml) of boiled ribonuclease A (Sigma, USA), and incubated at room temperature for 15 min. The sample then was heated to 85°C for 2 min, and the primers removed using a cycle pure kit (Omega, Bio-Tek, USA) according to the manufacturer's instructions. The sample was eluted in 30 µl water. A 1.5 µl aliquot of the cDNA was amplified in a total volume of 50 µl containing a final concentration of $10\times$ PCR buffer (30 mM MgCl_2 , Idaho Technologies), 0.2 mM dNTPs, 2 µM Tag primer 5'-CCT GAA TTC GGA TCC TCC-3', and 1 unit of Taq DNA polymerase (Promega, USA). The amplification was done in an Idaho Technologies Rapid Cycler II, using capillary tubes and the following program: 94°C for 1 min; 65°C 0 s; 72°C 5 s,

with a slope of 9, followed by 40 cycles of 94°C 0 s; 45°C 0 s; 72°C 30 s, with a slope of 5, and a final 5 min at 72°C and 5 min at 37°C . The amplified cDNAs were removed from capillary tubes, and were viewed after separation on 1.2% agarose gel and staining with ethidium bromide.

For cloning, the double stranded cDNAs were purified using a cycle pure kit (Omega, Bio-tek), and were ligated with pGEM-T vector using T4 DNA ligase according to manufacturer's instructions (Promega, USA). The recombinant vectors were transformed into competent cells of *Escherichia coli* strain JM101. The inserted clones were screened and amplified with M13 forward and reverse universal primers, using the program: 94°C for 15 s, followed by 30 cycles of 94°C 15 s; 54°C 6 s; 72°C 3 min and a final 5 min at 72°C and 5 min at 37°C . The amplified clones were sequenced using Applied Biosystems 3730XL. All clones were sequenced in both directions.

PCR was conducted to determine missing sequence gaps using specific primers, designed to amplifying a 300 bp fragment (Table 1), and Taq DNA polymerase. The PCR conditions included an initial denaturation step of 15 s at 94°C , followed by 35 cycles of 15 s at 94°C , 6 s at 53°C and 45 s at 72°C , with a final of 5 min at 72°C and 5 min at 37°C . PCR products, with a predicted size of 300 bp, were fractionated by gel electrophoresis on 1.2% agarose gels and stained with ethidium bromide. PCR products were cloned and sequenced as described above.

Sequence analysis. Sequence data were assembled using Geneious software version 6.1.2 (Drummond et al., 2011). The results were compared to databases with the both BlastN and BlastX against the GenBank nucleotide (nt) and non-redundant protein (nr) databases, respectively. The BLAST program is available in The National Center for Biotechnology Information (NCBI). The putative amino acid sequence of the isolate 125 RdRp gene was compared to databases with BlastP. This putative RdRp and those of closely related viruses were obtained from the NCBI database, and aligned using the ClustalW program. After hand editing of the alignments these were used for phylogenetic analyses and tree construction using MrBayes (Huelsenbeck

Table 1. Specific primers used in this study

Primer name	Sequence (5'-3')
125(1)-F	GAACCACGCTCAAGC
125(1)-R	GCACTGACATACCTCCTC
125(2)-F	CAATTTGATCGTGATGTG
125(2)-R	CTTGTCTTCGGATCAGAG

and Ronquist, 2001) via the Geneious plugin. The rate matrix was set to a Poisson distribution with a gamma rate variation. The total chain length was 10,000 and branch lengths were unconstrained.

Results and Discussion

Identification of dsRNAs in *C. sacchari* 125. Viruses of the family *Partitiviridae* have bi-segmented genomes containing two linear dsRNA molecules, which encode two proteins: the smaller segment encodes the CP, and the larger one encodes the RdRp (Nibert et al., 2013). Extracting dsRNA from mycelial tissue of *C. sacchari* 125, recovered from sugarcane in Khuzestan, Iran, indicated a potential mycovirus infection. The electrophoresis analysis showed the extracted dsRNAs consist of more than the two linear dsRNAs of approximately 1.85, and 1.65 kbp that are presumed to be the viral genomic elements; they also contain a smaller dsRNA segment (about 1.27 kbp) that could be a satellite dsRNA or defective-interfering dsRNA (King et al., 2012) (Fig. 1). In some partitiviruses such as those infecting *Rosellinia necatrix*, *Discula destructiva*, *Gremminiella abietina* and *Atkinsonella hypoxylon*, more than two linear dsRNAs, have been detected, thought to be satellites or defective RNAs (Chiba et al., 2013; Ghabrial et al., 2008). Analysis of the effects of subviral RNAs in partitiviruses are difficult because no reverse genetics system

has been established (Chiba et al., 2013).

For confirming the identity of these fragments as dsRNAs, they were treated with DNase I and RNase A in high and low ionic strength buffer. These fragments were resistant to treatment by DNase I and RNase A in high salt concentrations, but they were sensitive to RNase A treatment in low salt concentrations (not shown). These results confirmed the identity of these elements as dsRNAs.

Elimination of dsRNAs. Many methods for eliminating dsRNA viruses from fungi have been reported (Márquez et al., 2007; Hammar et al., 1989). These methods met with varying degrees of success. Hammar et al. (1989) used cyclohexamide to eliminate dsRNAs in a strain of *Leucostoma persoonii*, some segments of dsRNAs were eliminated and partially cured strain was obtained. Márquez et al. (2007) successfully eliminated dsRNAs from *Curvularia protuberata* by desiccation and freeze-thawing. Elias and Coty (1996) used a single conidial transfer method for removing dsRNAs from six isolates of *Aspergillus section flavi*; except one isolate, these were cured completely or partially.

We attempted to eliminate CsPV from isolate 125 using treatment with cycloheximide in two concentrations (25 and 50 µg) and hyphal tip culture. The results showed that neither 25 µg nor 50 µg of cycloheximide affected the potential mycovirus infection in isolate 125, and also hyphal tip culture had no effect on infected mycelia, and the dsRNAs were retained in *C. sacchari* isolate 125 (data not shown). Details of mycovirus biology are still scant, but it is clear that there is a great deal of variation, with some viruses being extremely unstable in culture, and others being recalcitrant to curing (M. Roossinck, unpublished). This makes detailed analysis of the effects of these viruses on their hosts very challenging.

Molecular characterization of dsRNA. The partial nucleotide sequence of isolate 125 was obtained by sequencing recombinant plasmid clones derived from a cDNA library. Twenty-four cDNA plasmid clones were randomly selected for sequencing in two directions. Sequence assembly of these cDNA clones and comparison with known mycoviruses using the BLAST program indicated that there was a gap, which was filled by PCR with specific primers, and we obtained a 1,284 base pair (bp) sequence. The analysis of the sequence with BlastN and BlastX programs revealed significant sequence similarities with the RNA-dependent RNA polymerase (RdRp) of viruses of the family *Partitiviridae*. Sequence similarity among partitiviruses and the obtained sequence of isolate 125 indicate that it is a novel

Fig. 1. Agarose gel electrophoresis of dsRNA elements associated with isolate 125 of *Cytospora sacchari*. Lane M, 1 kb DNA marker; numbers in left and right indicate bp.

Table 2. Amino acid identity between CsPV and other members of the genus *Partitivirus*

Partitivirus ^a	Sigla	% Identity	Overlap	Genbank accession no.
<i>Cytospora sacchari</i> partitivirus	CsPV	—	—	KF697164
<i>Penicillium stoloniferum</i> virus S	PsV-S	76%	100%	CAJ01909.1
<i>Aspergillus ochraceus</i> virus I	AoV1	71%	99%	ABC86749.1
<i>Botryotinia fuckeliana</i> partitivirus I	BfPV1	70%	99%	YP_001686789.1
<i>Gremmeniella abietina</i> RNA virus MS1	GaRV-MS1	71%	99%	NP_659027.1
<i>Aspergillus fumigatus</i> partitivirus I	AfPV1	71%	100%	CAY25801.2
<i>Verticillium dahlia</i> partitivirus 1	VdPV1	70%	100%	AGI52210.1
<i>Discula destructiva</i> virus 2	DdV2	67%	99%	NP_620301.1
<i>Discula destructiva</i> virus 1	DdV1	67%	100%	NP_116716.1
<i>Ophiostoma</i> partitivirus I	OPV1	67%	97%	CAJ31886.1

^aThe top nine distinct viruses returned by BLASTp are shown. For each virus, a representative isolate was selected for use in this table.

virus in the genus *Partitivirus* in the family *Partitiviridae*; we have tentatively designated it as *Cytospora sacchari* partitivirus (CsPV). The partial nucleotide sequence of CsPV has been deposited in GeneBank (accession no. KF697164).

The obtained sequence of CsPV can potentially encode a 405 amino acid (aa) protein. The search in BLASTP showed that this protein has similarity with RdRps of a number of fungal partitiviruses. Pairwise aa identities between CsPV and previously identified partitiviruses are presented in Table 2. The highest aa identity (76%) is between CsPV and *Penicillium stoloniferum* virus S (PsV-S). Blast search with deduced amino acid sequence reveals that this sequence has a single ORF. Search of the conserved domain

database (CDD) revealed a significant match with conserved domains related to the *Partitivirus* RdRp (RNA-dep-RNAP, cd01699). To define the relationship among the putative RdRp of this virus and the previously characterized partitiviruses, multiple sequence alignment was conducted. The alignment shows four amino acid conserved domains (I-IV) characteristic of partitivirus RdRps including the highly conserved GDD motif typical of RdRps in motif IV (Fig. 2).

A Bayesian phylogenetic analysis was conducted based on alignments of the putative amino acid sequence of RdRps of CsPV and selected members of the genus *Partitivirus* (Fig. 3). As expected, CsPV was included in a clade within the partitiviruses. Based on this analysis CsPV was clus-

	I			II			III			IV		
CsPV	PKTRLVW	55		DFSAFD TTVP	61		GVP SGSWWTQMVD SVVN	19		VLGDDS		
Psv-s	PKTRLVW	55		DFSGFD TKVP	61		GVP SGSWFTQMID SVVN	19		VLGDDS		
AoV1	PKTRLVW	55		DFSSF DSKVP	61		GVP SGSWWTQMVD SVVN	19		VLGDDS		
GaRV-MS1	PKTRLVW	55		DFSSF DTKVP	61		GVP SGSWWTQMVD SVVN	19		VLGDDS		
BfPV1	PKTRLVW	55		DFSSF DTKVP	61		GVP SGSWWTQMID SVVN	19		VLGDDS		
VdPV1	PKTRLVW	55		DFSAFD TKVP	61		GVP SGSWWTQMID SVVN	19		VLGDDS		
DdV1	PKTRLVW	55		DFSAFD SKVP	61		GVP SGSWWTQIIDS VVN	19		VLGDDS		
DdV2	PKTRLIW	55		DFSAFD SKVP	61		GVP SGSWWTQIIDS VVN	19		VLGDDS		
AfPV1	PKTRLVW	55		DFSAFD ARVP	61		GVP SGSWWTQMIYS VVN	19		VLGDDS		
OPV1	PKTRLVW	55		DFSSF DTKVP	61		GVP SGSWWTQMID SVVN	19		VLGDDS		
	*****:			***.***:	**		*****:***: *			*****		

Fig. 2. Conserved amino acid motifs (I-IV) in RdRps of *Cytospora sacchari* partitivirus and other members of *Partitivirus*. Asterisks indicate identical residues; conserved and semi-conserved amino acid residues are indicated by colons and dots, respectively, as defined in the ClustalW program. Numbers between motifs correspond to the number of amino acid residues separating the motifs. Abbreviations of virus names and sequence accession numbers are shown in Table 2.

Fig. 3. Phylogenetic analysis based on amino acid sequences of RdRps of CsPV and selected members of *Partitiviridae*. Bayesian analysis was done using the MrBayes plugin in Geneious software. Posterior probabilities are indicated by numbers at the nodes. The accession numbers of sequences used in the analyses are given in the Table 2. Mycovirus FusoV, *Fusarium solani* virus 1 (accession no. BAA09520), was used as an outgroup.

tered with *Aspergillus ochraceous* virus 1 (ABC86749.1) in a clade with 0.9631 posterior probability.

Based on a recent phylogenetic analysis it seems that the taxonomy of the family *Partitiviridae* is due for some revisions (Crawford et al., 2006; Nibert et al., 2013). In the current classification of *Partitiviridae*, host range has an important role for genus divisions; fungal partitiviruses have been classified in the genus *Partitivirus* while plant partitiviruses have been placed in *Alphacryptovirus* or *Betacryptovirus*. However the discovery of plant-infecting partitiviruses in *Primula malacoides* (Li et al., 2009), and *Cannabis sativa* (Ziegler et al., 2012), and the recent phylogenetic results suggest that this criterion may not be appropriate for genus divisions in the family *Partitiviridae* (Nibert et al., 2013; Roossinck, 2010). The existence of

plant-infecting viruses in the genus *Partitivirus* supports the possibility of horizontal transfer of these viruses between plants and fungi (Crawford et al., 2006; Ghabrial et al., 2008; Roossinck, 1997; Roossinck, 2010). In addition, evidence has been recently found for integration of partitivirus sequences into plant genomes (Chiba et al., 2011; Liu et al., 2010). Phylogenetic analysis based on aa sequences of RdRps of members of family *Partitiviridae* led to identification of two clusters in the genus *Partitivirus*, comprised of a mixture of fungal viruses and plant viruses (Ghabrial et al., 2008; Nibert et al., 2013), these two clusters may be marked as two subgroups (subgroup 1 and 2) in this genus (Crawford et al., 2006; Ghabrial et al., 2008). One of the taxonomic revisions based on these results is to divide the genus *Partitivirus* into two new genera (Nibert et al., 2013), although other revisions of the *Partitiviridae* family are probably also necessary (Roossinck, 2010).

Phylogenetic analysis of aa sequences of RdRps of the established members of genus *Partitivirus* and putative RdRp of CsPV showed that CsPV would be placed in one of these subgroups that contains fungal viruses, whereas the other one consists of a mixture of fungal viruses and plant viruses. Based on suggested revision, each of these subgroups could be considered as a new genus in the family *Partitiviridae*. CsPV with AoV1 form a cluster in one of these new genera.

In summary, we have identified a putative partitivirus associated with *C. sacchari* that we tentatively named CsPV. The attempts for removing the dsRNAs with treatment by cyclohexamide and hyphal tip culture were unsuccessful. This study is the first report of partitivirus infection in the plant pathogenic fungus *C. sacchari*.

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