Universität Hamburg – Fakultät für Mathematik, Informatik und Naturwissenschaften – Department Biologie
Biozentrum Klein Flottbek und Botanischer Garten

A novel double-stranded RNA mycovirus from *Fusarium graminearum*; Nucleic acid sequence and genomic structure

O. Darissa, P. Willingmann, W. Schäfer and G. Adam

Introduction

Mycoviruses (fungal viruses) have been described in many fungal species including phytopathogenic fungi (Pearson et al., 2009). Since the first report of a fungal virus which was in 1962 in diseased mushroom, Agaricus bisporus (Hollings), more than 200 mycoviruses classified into 10 families have been reported (Ghabrial and Suzuki, 2009). Although the majority of the mycoviruses are associated with dsRNA genomes and to a lesser extent with ss (+) RNA genomes, few mycoviruses with ssDNA, or dsDNA genomes have been reported (Yu et al., 2010). With the exception of few cases, most of the reported mycoviruses have been associated with cryptic or latent infections of their hosts (Buck, 1998). In Figure 1, some properties of the major taxonomic families with mycovirus members are shown. Mycoviruses have limited routes of transmission. These include the intercellular routes such as hyphal anastomosis and heterokaryosis or via sexual and asexual spores (Xie et al., 2006; Chu et al., 2004; Buck, 1998). These transmission limitations are also reflected on the natural host range of mycoviruses, which is restricted to fungal individuals who are vegetatively compatible.

	Totiviridae	*Partitiviridae*	*Chrysoviridae*	*Reoviridae*	*Hypoviridae*
Genome	monopartite	bipartite	tetrapartite	multisegmented	monopartite
Genome size	4.6 - 7 kbp	1.4 - 2.3 kbp	2.4 - 3.6 kbp	0.7 - 5 kbp	9 - 13 kb
Number of components					
Envelope	-	-	-	-	Unencapsidated membrane vesicle
Particle size	40-43 nm	30-40 nm	35-40 nm	~80 nm	
Associated hypovirulence	Yes	No	Yes	Yes	Yes
Host	fungi protozoa	fungi plants	fungi	fungi plants protozoa vertebrates	fungi

Fig. 1: Properties of the major viral families encompassing mycovirus members.

Results

Here, we present the molecular characterization of a novel dsRNA mycovirus purified from a *F. graminearum* isolate from china. The virus has been called *F. graminearum* mycovirus china 9 (FgV-ch9).

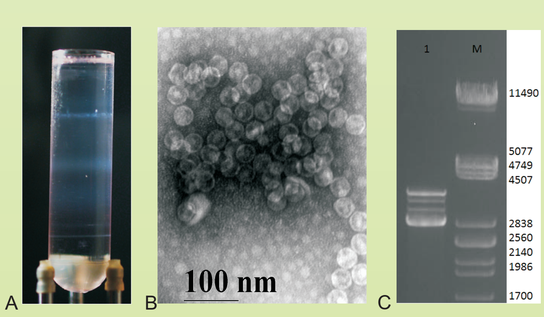
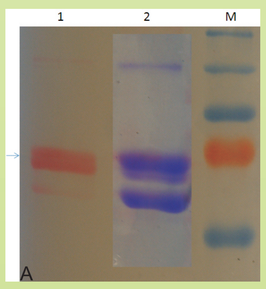
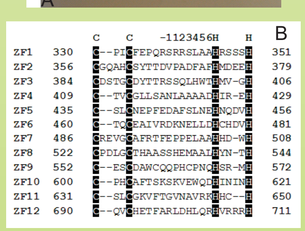


Fig. 2:Purification and Particle Properties:

A: Banding pattern of FgV-ch9 after cesiumchloride gradient centrifugation. Multiple bands are common for mycoviruses and is thought to be attributed to VLPs of different replicative stages
B: Virus Like Particles (VLPs) of FgV-ch9 purified by CsCl-gradient ultracentrifugation.
C:Agarose gel electrophoresis of dsRNA segments isolated from purified FgV-ch9. M: λ-PstI marker





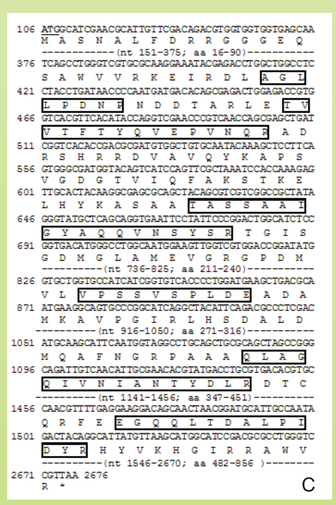
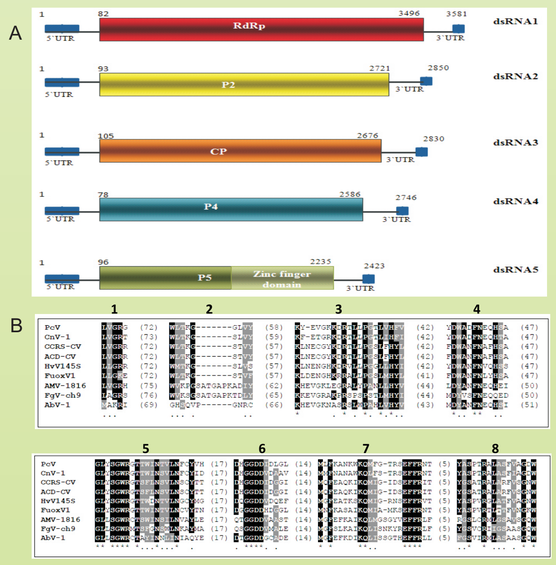


Fig. 3: Structural Proteins and possible Functions
A: SDS-PAGE and surface protein labeling of purified VLPs with NHS-Biotin. 1: Surface-labeled proteins. 2: Structural proteins of FgV-ch9. M: Prestained protein marker. Note the prefered labeling of the CP band (arrow) which appears as a double band, probably due to protein degradation or modification.
B: Multiple alignment of the 12 C2H2 zinc finger domain present at the C-terminus of the protein encoded by dsRNA5 of FgV-ch9.
C: CP of FgV-ch9. The deduced amino acid sequence is shown under the nucleotide sequence. The tryptic peptide-derived amino acid sequences, isolated by reverse-phase HPLC, are boxed.



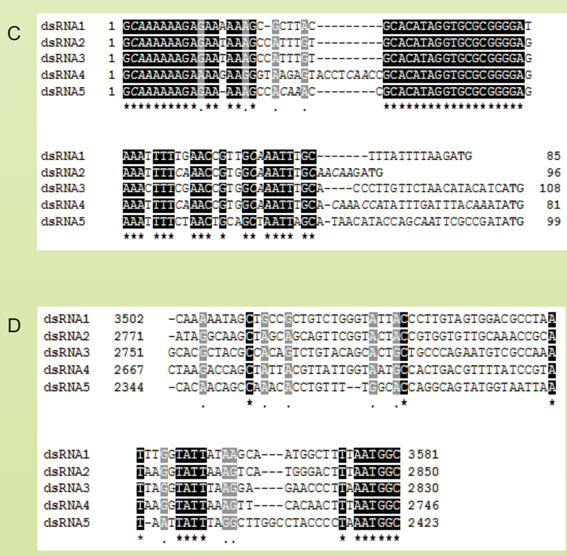
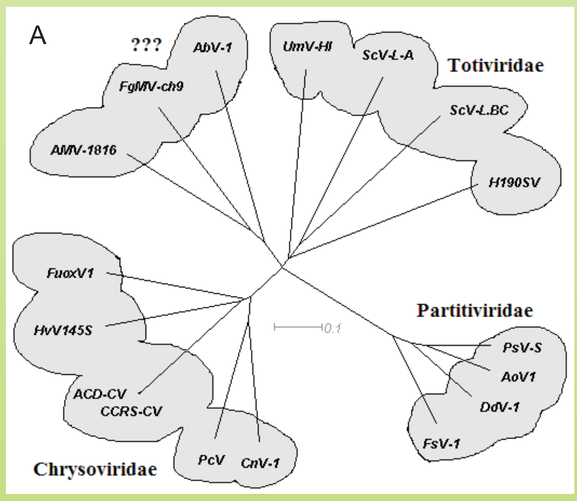


Fig. 4: Genome organisation and some characteristic features of FgV-ch9.

A. Genomic structure of FgV-ch9. The blue rectangels at 5` & 3` ends represent conserved regions. P2 & P4 are proteins with unidentified function. RdRP: RNA dependent RNA Polymerase (see B this Fig). CP: capsid protein (see Fig. 2 A).

B. Comparison of the conserved RdRPs motifs of selected dsRNA mycoviruses including FgV-ch9. Dark shading and asterisks signify identical amino acid residues.

C. and D.Comparison of the 5` (C) and 3`(D) UTRs of the 5 dsRNA's of FgV-ch9. Asterisks signify identical bases.



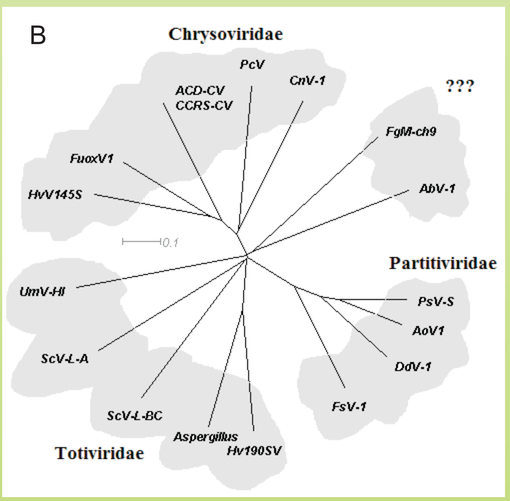


Fig. 5: Relationship of FgV-ch9 to other dsRNA Mycoviruses. Phylogenetic analysis of the RdRP (A) and the CP (B) of FgV-ch9. Multiple alignments of amino acid sequences were carried out using the CLUSTALX2 with the default parameters. Phylogenetic trees were constructed with the TreeView v0.5.0 program.

Summary

- **The novel mycovirus FgV-ch9 has 5 dsRNA segments associated with isometric virus particles of 35-40 nm in diameter.**
- **The complete nucleotide sequence of the 5 dsRNA segments was determined. Each of the segments was found to have a single unique ORF.**
- **BLAST searches of the deduced amino acid sequences showed that dsRNA1 encodes a putative RdRP closely related to those of dsRNA mycoviruses.**
- **The deduced amino acid sequences of dsRNA2 and 4 have no significant similarity to any published protein.**
- **Evidence that dsRNA3 encodes a putative outer capsid protein stem from two sources.**
  - **Surface protein labeling of a CsCl gradient-purified virions followed by SDS-PAGE and Western blot analysis showed preferential labeling of the protein band encoded by dsRNA3.**
  - **Internal peptide sequences of this protein band were identical to those deduced from the DNA clones of dsRNA3.**
  - **BLAST searches revealed relatively high sequence similarities between the protein encoded by dsRNA3 and the L3 protein (cp) of the Agaricus bisporus virus 1 associated with La France disease.**
- **The N-terminus of the protein encoded by dsRNA5 shares no significant similarity with any known protein, however, we identified 12 multiple-adjacent-C2H2 zinc fingers at its C-terminus.**
- **Relative quantitative PCR showed that the dsRNAs of FgV-ch9 are separately and unequally encapsidated. These results are supported by the presence of conserved terminal sequences at the 5`and 3`UTRs, an evidence of multipartite and multicomponent genomes.**
- **Phylogenetic analysis of the RdRP and the putative capsid protein of FgV-ch9 showed that the virus initiate a new cluster closely related to the family *Chrysoviridae*.**

In conclusion: The phylogenic analysis and genome organization of FgV-ch9 with 5 monocistronic dsRNAs and presence of zinc finger domain in the protein encoded by dsRNA5 distinguish this virus from the reported mycoviruses so far. Based on the results described above, we propose the establishment of a new genus or subfamily in *Chrysoviridae* to accommodate the 3 viruses forming the new phylogenic cluster (FgV-ch9, Aspergillus mycovirus 1816 and AbV-1).

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