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Taxonomic implications for Fijiviruses based on the terminal sequences of Fiji disease fijivirus

Brief Report

J. A. McMahon, J. L. Dale, and **R. M. Harding**

Centre for Molecular Biotechnology, School of Life Sciences, Queensland University of Technology, Brisbane, Queensland, Australia

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Summary. The 5′ and 3′ terminal sequences of the plus strand of Fiji disease fijivirus (FDV) segments 2, 3, 9 and 10 possess the conserved terminal sequences, 5′AAGUUUUU.....CAGCAGAUGUC 3′. The 5′ sequence is identical to that of maize rough dwarf fijivirus (MRDV) and rice black-streaked dwarf fijivirus (RBSDV), whereas the FDV 3′ sequence shares the consensus, CAGCNNNNGUC, with MRDV and RBSDV. The FDV terminal sequences, and the amino acid sequences from FDV segment 9, are more closely related to those from MRDV and RBSDV than to those from oat sterile dwarf fijivirus (OSDV) and *Nilaparvata lugens* reovirus (NLRV; a putative Fijivirus).

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Plant-infecting reoviruses are classified into three genera, *Phytoreovirus, Fijivirus* and *Oryzavirus*, based on the number and size of genome segments and their electrophoretic profile, virion morphology, serology and insect vectors [2]. Fiji disease fijivirus (FDV) is the type member of the genus *Fijivirus* and the sole member of its serogroup 1. Serogroup 2 fijiviruses include rice black-streaked dwarf virus (RBSDV), maize rough dwarf virus (MRDV) and pangola stunt virus, whereas the sole member of serogroup 3 is oat sterile dwarf virus (OSDV) [7]. Sequence analyses of several segments of MRDV and RBSDV have shown that the 5′ and 3′ terminal sequences, 5′AAGUUUUU.....GUC 3′, are found in the genomes of the two serogroup 2 viruses [6, 1]. Recently, analysis of the sequences of OSDV (serogroup 3) revealed the terminal sequence, 5′AACGAAAAA....UUUUUUUU AGUC 3′ [3], which only shares the sequence, 5′AA.....GUC 3′, with serogroup 2 genome segments. In addition, *Nilaparvata lugens* reovirus (NLRV), a putative member of the genus *Fijivirus* [8], possesses the terminal conserved sequences, 5′AGU.....GUUGUC 3′ [9].

	5' sequence	3' sequence
FDV		
S2	**AAGUUUUU**	**CAGC**AGAU**GUC**
S3	**AAGUUUUU**	**CAGC**AGAU**GUC**
S9	**AAGUUUUU**	**CAGC**AGAU**GUC**
S10	**AAGUUUUU**	**CAGC**AGAU**GUC**
RBSDV		
S7	**AAGUUUUU**	**CAGC**UGAU**GUC**
S8	**AAGUUUUU**	**CAGC**UAUU**GUC**
S9	**AAGUUUUU**	**CAGC**UAUC**GUC**
S10	**AAGUUUUU**	**CAGC**UAUU**GUC**
MRDV		
S6	**AAGUUUUU**	**CAGC**UGAU**GUC**
S7	**AAGUUUUU**	**CAGC**UAUU**GUC**
S8	**AAGUUUUU**	**CAGC**UGAU**GUC**
S10	**AAGUUUUU**	**CAGC**UAUU**GUC**
Consensus:	**AAGUUUUU**	**CAGC**NNNN**GUC**

(a)

MRDV/RBSDV/FDV consensus:	**A**AGUUUUU.....AGCNNNNN**GUC**
OSDV consensus:	**A**ACGAAAAA.....UUUUUUUU**AGUC**
NLRV consensus:	**A**GU.....GUU**GUC**
Fijivirus consensus:	**A**.....**GUC**

(b)

Fig. 1. Comparison of the terminal sequences of **a** FDV, MRDV and RBSDV, **b** FDV, MRDV, RBSDV, OSDV and NLRV. Conserved bases are in bold type

We have partially characterised the FDV genome and obtained the terminal sequences of segments 2 and 3 (S2 and S3) (J.A. McMahon, J.L. Dale and R.M. Harding, unpublished results), S9 [12] and S10 (P. Burns, J.A. McMahon, G.R. Smith, J.L. Dale and R.M. Harding, unpubl. res.). From these segments, we have derived a consensus sequence to compare with the terminal sequences derived from fijiviruses belonging to serogroups 2 and 3.

FDV segment-specific clones were isolated from a FDV cDNA library and terminal sequences were obtained by anchor-ligated PCR as described by Soo et al. [12]. The terminal sequences from S2, 3, 9 and 10 were aligned and compared (Fig. 1a). The 5′ 8 nucleotide (nt) sequence, AAGUUUUU, and the 3′ 11 nt sequence, CAGCAGAUGUC, were found in all the four FDV segments. The terminal sequences of RBSDV S7, 8, 9 and 10 (GenBank S63917 (S7), S63914 (S8), ABO11403 (S9), D00606 (S10)) and MRDV S6, 7, 8 and 10 (GenBank X55701 (S6), L76562 (S7), L76561 (S8), L76560 (S10) were also aligned, and it was found that the three viruses had the consensus sequence of 5′AAGUUUUU.....CAGCNNNNGUC 3′ (Fig. 1a). This 3′ consensus sequence differed in two positions from the MRDV and RBSDV 3′ consensus sequence, AGCUXXXGUC, reported by Isogai et al. [4]. The first difference is that there is a conserved C at the beginning of the consensus sequence. The second is that

although there is a conserved U at nt 4 of the sequence reported by Isogai et al. [4], and also found in the 3′ terminal sequence of MRDV and RBSDV, is not found in FDV. Another difference in the 3′ terminal sequences is that, for MRDV and RBSDV, nt 6–8 of the newly proposed consensus sequence is not the same in different viruses, whereas this region is found in all FDV segments sequenced to date.

The FDV/MRDV/RBSDV consensus sequence was compared to the serogroup 3 (OSDV) consensus sequence [3] and to the consensus sequence for the putative fijivirus, NLRV [9] (Fig. 1b). The only sequence shared by all fijiviruses in their terminal sequences is 5′ A....GUC 3′. Similar analysis of the sequences of the phytoreoviruses rice dwarf, rice gall dwarf and wound tumour viruses, which differ in biological properties such as host range, showed that they only shared a terminal sequence of 5′ GG....GAU 3′ [5].

Alignment of the terminal sequences clearly indicates that FDV is more similar to the serogroup 2 fijiviruses than to OSDV (serogroup 3) or the putative fijivirus, NLRV. This is consistent with an earlier report by Reddy et al. [10, 11] where

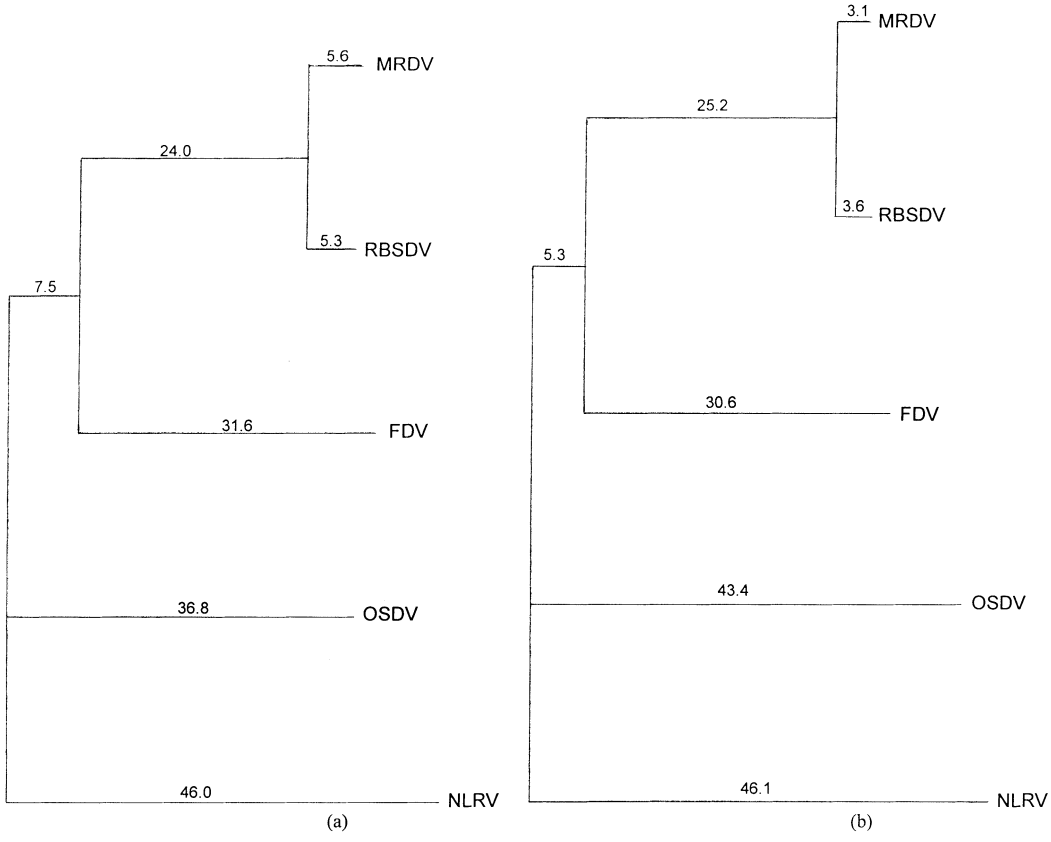

Fig. 2. CLUSTREE plots of similarities of the amino acid sequences of **a** ORF1, **b** ORF 2 from MRDV S8, RBSDV S9, FDV S9, NLRV S9 and OSDV S10. The numerical values on the branch lengths represent percentage of non-identical amino acids

the electrophoretic profiles of FDV, MRDV and RBSDV were found to be very similar.

The recent publication of genome sequences from RBSDV S9 and OSDV [3, 4] has allowed phylogenetic comparisons to be made with the sequence of FDV S9. In this study, comparisons were made between FDV S9 (GenBank AF050086), MRDV S8, RBSDV S9, OSDV S10 (GenBank AB011027) and NLRV S9 (GenBank D49700), all of which have two open reading frames. The predicted amino acid sequences of ORFs 1 and 2 from these viruses, respectively, were compared using the WebAngis programs PILEUP and CLUSTREE. Phylogenetic trees were constructed and bootstrapped (n = 1000) (Fig. 2a, b). The branching in each of these trees shows that FDV is more closely related to MRDV and RBSDV than to NLRV or OSDV. In all cases, NLRV is the most distantly related virus. The phylogenetic analyses were repeated using EPROTPARS and the same branching pattern was observed.

In conclusion, comparison of the terminal sequences of FDV indicates that FDV groups more closely to RBSDV and MRDV (serogroup 2) than to OSDV (serogroup 3) or NRLV (putative fijivirus). This grouping is further supported by phylogenetic analyses.

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Authors' address: Dr. R. M. Harding, Centre for Molecular Biotechnology, School of Life Sciences, Queensland University of Technology, G.P.O. Box 2434, Brisbane, Queensland 4001, Australia.

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