Psittacine circovirus DNA sequence analysis

Bassami M, Ypelaar I, Berryman D, Wilcox GE and Raidal SR[1]

Introduction

Psittacine circovirus (PsCV), is 17nm in diameter, non-enveloped, spherical and contains a circular ssDNA genome of <2kb. Determining the DNA sequence of the PsCV genome is important for defining the phylogenetic relationship among the plant and animal circoviruses and this was the main aim of this research.

Materials and Methods

PsCV DNA purified from the feathers of a PBFD-affected cockatoo was converted to dsDNA using random hexanucleotides, cloned into pUC18 and sequenced. PCR primers designed from this data were used to amplify overlapping segments of the PsCV genome to enable analysis of the complete genome and to study genetic variation in conserved regions from PsCV isolates from different psittacine birds from around Australia. The sequences of PCR products obtained from 10 PBFD affected birds (see Table) were analysed using Clustal program (ANGIS).

PBFD-affected birds sampled

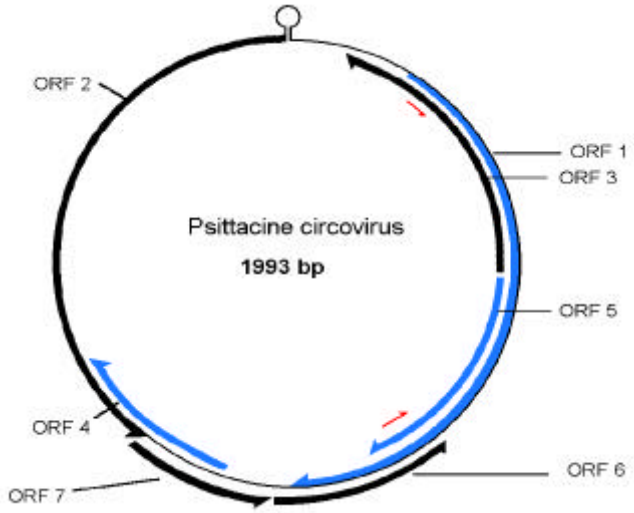
Budgerigar (n=3) (A-C)	Perth
sulphur crested cockatoo (D)	Northern Territory
sulphur crested cockatoo (E)	Perth
rainbow lorikeet (F)	Perth
rainbow lorikeet (G)	Victoria
blue bonnet (H)	Esperance
lovebird (I)	Perth
Major Mitchell's cockatoo (I)	Perth

Results

The PsCV genome comprised a circular molecule of 1993 bp (Figure 1) containing 7 ORF, which potentially encode 7 proteins >8.7 kDa. There was high aa sequence similarity between the potential protein product of ORF1 of PsCV and the replicase-associated protein (Rep) of porcine circovirus (PCV) and several plant circoviruses but not chicken aneamia virus (CAV). A potential stem-loop structure similar to that found in PCV and plant circoviruses was present in the putative encapsidated strand of the PsCV genome. At the top of this structure a nonanucleotide motif (TAGTATTAC) similar to that of PCV, plant circoviruses and geminiviruses. The DNA sequence of ORF1 of the 10 PsCV isolates was 94% identical (Figure 2).

[1] Division of Veterinary and Biomedical Sciences, Murdoch University, Perth, W.A. 6150

Figure 1. The putative replicative form of the PsCV genome demonstrating 7 open reading frames (ORF). ORFs in blue signify the putative encapsidated strand. Red bars indicate the position of PCR primers used for sequence analysis. A stem loop structure shown at the top of the figure containing a nonanucleotide motif is similar to that of PCV and plant circoviruses and is the initiation site for replication.



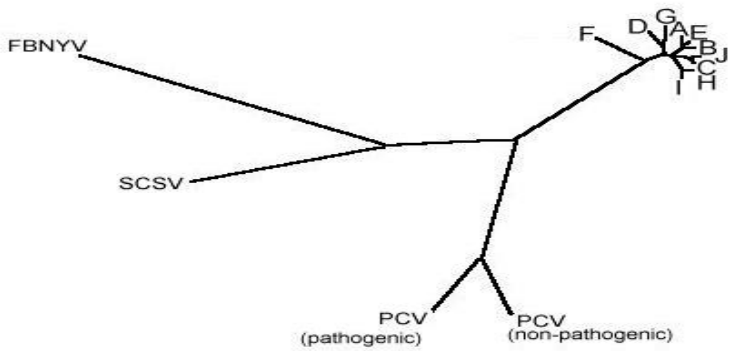


Figure 2. Phylogenetic relationships between ten samples of PsCV, PCV pathogenic and non-pathogenic strains, Subterranean clover stunt virus (SCSV) and Fava bean necrotic yellow virus (FBNYV). See table of birds sampled for origin and type of bird.

Discussion

Our findings provide evidence of a close relationship between PsCV, PCV and plant circoviruses but not with CAV. Comparison of the ORF1 of PsCV with the Rep protein genes of PCV and plant circoviruses suggests that PsCV replicates by a similar rolling circle replication mechanism to that employed by plant circoviruses, geminiviruses and many bacterial plasmids. Comparison of the DNA sequence of different PsCV isolates from different bird species and locations in Australia also suggests that PsCV is evolutionary well conserved Australia wide.

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