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GENOME ANNOUNCEMENT

Iteravirus-Like Genome Organization of a Densovirus from *Sibine fusca* Stoll

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The complete genome of *Sibine fusca* densovirus was cloned and sequenced. The genome contained 5,012 nucleotides (nt), including inverted terminal repeats (ITRs) of 230 nt with terminal hairpins of 161 nt. Its DNA sequence and monosense organization with 3 open reading frames (ORFs) is typical of the genus *Iteravirus* in the subfamily *Densovirinae* of the *Parvoviridae*.

The slug caterpillar *Sibine fusca* Stoll (syn. *Acharia fusca*; Limacodidae), a major pest of oil palm, is widely distributed in Southern America. The larvae live gregariously in colonies of up to 60 individuals and disperse just before pupation. Virus-infected larvae showed nuclear lesions typical of a densovirus infection (2, 8). Electron microscopy revealed isometric particles with about 20-nm diameters, also characteristic of densoviruses. Infected larvae of *S. fusca* drifted from the feeding colony, and considerable proliferation of cells, resembling tumors, was observed in the midgut (8). Thus far, this virus has not been further characterized, cloned, or sequenced but has been used effectively in biological control (4).

The virus was partially purified by the method described for *Galleria mellonella* densovirus (GmDNV) (7) from an infected larva. A sequence-independent, single-primer amplification (SISPA) method (9) was used in a preliminary genome characterization. DNA, extracted under conditions of high ionic strength to anneal the single-stranded DNA (ssDNA), had a size of around 5 kb. This DNA was digested with the Csp6I restriction enzyme, ligated with an adaptor, amplified by PCR as described elsewhere (1), and cloned into the PCR2.1 vector by the TA cloning method (5). Amplicon inserts were sequenced by Sanger's method as described previously (11). A unique ClaI restriction site was observed near the middle of a preliminary 4.7-kb sequence. DNA from the virus was then blunt-ended by a mixture of Klenow fragment and T4 DNA polymerase, digested with ClaI, and cloned into EcoRV and ClaI sites in the pBluescriptSK(−) vector, yielding clones with a 2.6-kb insert and clones with a 2.4-kb insert. Four inserts of each set were sequenced in both directions using Sanger's method and the primer-walking method as described before (11). Insert sequences were identical in each set except for the flip-flop sequences in the hairpins.

The *Sibine fusca* densovirus (SfDNV) genome contained inverted terminal repeats (ITRs) typical of the three members (*Bombyx mori* densovirus type 1 [BmDNV-1], *Casphalia extranea* densovirus [CeDNV], and *Dendrolimus punctatus* densovirus [DpDNV]) of the *Iteravirus* genus and with a length of 230 nucleotides (nt) (10). The terminal J-shaped hairpins of 161 nt were about 90% conserved between BmDNV-1 (6), CeDNV (3), and DpDNV (12). In the hairpins, nt 60 to 102 and nt 4911 to 4953 occurred in two orientations, "flip" and its reverse complement orientation "flop," that were identical to the flip-flop of CeDNV and 98% identical to that of BmDNV. The overall sequence was about 85% identical to CeDNV, about 78% identical to BmDNV, and about 72% identical to DpDNV.

The monosense genome contained three intronless genes that were virtually identical in position and size to those of other iteraviruses. The largest open reading frame (ORF), ORF1 (nt 354 to 2615), had a coding capacity of 753 amino acids (aa) and the typical NTPase motif for NS1 (3). ORF2 (nt 2669 to 4714), with the phospholipase A2 motif characteristic for VP (13), had a coding capacity of 681 aa. ORF3 corresponded to NS2 with a 452-aa coding capacity and typically overlapped the N terminus of NS1 (nt 481 to 1839). As a comparison, for the other iteraviruses, the NS1 is 753 to 775 aa, the NS2 is 451 to 453 aa, and the VP is 668 to 678 aa.

Nucleotide sequence accession number. The GenBank accession number of SfDNV is JX020762.

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