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GENOME ANNOUNCEMENT

Pseudoplusia includens Densovirus Genome Organization and Expression Strategy

Oanh T. H. Huynh, Hanh T. Pham, Qian Yu, and Peter Tijssen

INRS-Institut Armand-Frappier, Laval, Quebec, Canada

The genome of a densovirus of a major phytophagous pest, *Pseudoplusia includens*, was analyzed. It contained 5,990 nucleotides (nt) and included inverted terminal repeats of 540 nt with terminal Y-shaped hairpins of 120 nt. Its DNA sequence and ambisense organization with 4 typical open reading frames demonstrated that it belonged to the genus *Densovirus* in the subfamily *Densovirinae* of the family *Parvoviridae*.

The distribution of the polyphagous soybean looper pest, *Pseudoplusia includens* (syn., *Chrysodeixis includens* [Hübner] [Noctuidae, Plusiinae, Lepidoptera]), is restricted to the Western Hemisphere, occurring from southern Canada to southern South America (1). In addition to the soybean, it may feed on a large number of crops of economic importance (8, 9). Previously, two small icosahedral viruses have been isolated from the soybean looper, a picornavirus and a smaller virus with biophysical properties that seem to match those of the densoviruses (2).

Densoviruses are notoriously unstable upon cloning (7, 10–13), and densovirus entries in GenBank, such as those from *Junonia coenia* (JcDNV) (3) and *Diatraea saccharalis* (DsDNV) (NC_001899), often lack significant parts of their inverted terminal repeats (ITRs). DNA purified from *Pseudoplusia includens* DNV (PiDNV) in phosphate-buffered saline (PBS) had a size of around 6 kb. This DNA was blunt ended by a mixture of Klenow fragment and T4 DNA polymerase and cloned into a linear pJazz vector (from Lucigen Corp.), which lacks transcription into the insert and torsional stress (5) to prevent recombination and deletion of insert fragments. Six clones, or about 0.3%, had full-length inserts and could be stably subcloned into circular vectors.

Four complete clones were sequenced in both directions, using Sanger's method and the primer-walking method as described before (11), and the contigs were assembled by the CAP3 program (http://pbil.univ-lyon1.fr/cap3.php/) (6). The difficulties encountered with sequencing of the terminal hairpins were solved by sequencing after (i) digestion near the middle of the hairpin with BstUI restriction enzyme or (ii) amplifying the hairpins by PCR in the presence three additives: 1.3 M betaine, 5% dimethyl sulfoxide, and 50 mM 7-deaza-dGTP. Sequences of the clones, except for the flip-flop regions in the hairpins, were identical. In the hairpins, nucleotides (nt) 46 to 75 and nt 5916 to 5945 occurred in two orientations, "flip" and its reverse complement orientation "flop." The ambisense PiDNV genome contained typical ITRs of members of the *Densovirus* genus with a length of 540 nt and terminal Y-shaped hairpins of 120 nt. The overall sequence of 5,990 nt was 83 to 87% identical with those of other viruses in the *Densovirus* genus but about 50 nt shorter.

The open reading frames (ORFs) were conserved with members of the *Densovirus* genus, and the putative splicing sites were conserved with those that have been identified for *Galleria mellonella* DNV (GmDNV) (11) and *Mythimna loreyi* DNV (MlDNV) (4). The large ORF1 (nt 1355 to 3019) on the plus strand had a coding capacity for NS1 of 554 amino acids (aa), ORF2 corresponded to NS2 (nt 1362 to 3019) with 275 aa, and ORF3 (nt 647 to 1348) corresponded to NS3 with 233 aa. On the complementary minus strand, a large ORF (also on the 5′ half at nt 3006 to 5423) with a potential coding region of 805 aa corresponded well to those of the VP structural proteins of related densoviruses. The distribution of the putative coding sequences implied an ambisense organization and expression, and PiDNV contained the typical NS-1 helicase superfamily III and VP phospholipase A2 (14) motifs observed in other parvoviruses.

Nucleotide sequence accession number. The GenBank accession number of PiDNV is JX645046.

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REFERENCES

1. **Alford RA, Hammond AM, Jr.** 1982. Plusiinae (Lepidoptera: Noctuidae) populations in Louisiana soybean ecosystems as determined with looplure-baited traps. J. Econ. Entomol. **75**:647–650.
2. **Chao Y-C, Young SY III, Kim KS, Scott HA.** 1985. A newly isolated densonucleosis virus from Pseudoplusia includens (Lepidoptera: Noctuidae). J. Invertebr. Pathol. **46**:70–82.
3. **Dumas B, Jourdan M, Pascaud AM, Bergoin M.** 1992. Complete nucleotide sequence of the cloned infectious genome of Junonia coenia densovirus reveals an organization unique among parvoviruses. Virology **191**: 202–222.
4. **Fediere G, El-Far M, Li Y, Bergoin M, Tijssen P.** 2004. Expression strategy of densonucleosis virus from Mythimna loreyi. Virology **320**:181–189.
5. **Godiska R, et al.** 2010. Linear plasmid vector for cloning of repetitive or unstable sequences in Escherichia coli. Nucleic Acids Res. **38**:e88.
6. **Huang X, Madan A.** 1999. CAP3: a DNA sequence assembly program. Genome Res. **9**:868–877.
7. **Liu K, et al.** 2011. The Acheta domesticus densovirus, isolated from the European house cricket, has evolved an expression strategy unique among parvoviruses. J. Virol. **85**:10069–10078.

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Address correspondence to Peter Tijssen, peter.tijssen@iaf.inrs.ca.

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8. **MacRae TC, et al.** 2005. Laboratory and field evaluations of transgenic soybean exhibiting high-dose expression of a synthetic Bacillus thuringiensis cry1A gene for control of Lepidoptera. J. Econ. Entomol. **98**:577–587.
9. **McPherson RM, MacRae TC.** 2009. Evaluation of transgenic soybean exhibiting high expression of a synthetic Bacillus thuringiensis cry1A transgene for suppressing lepidopteran population densities and crop injury. J. Econ. Entomol. **102**:1640–1648.
10. **Tijssen P, et al.** 2006. Evolution of densoviruses, p 55–68. *In* Kerr JR, Cotmore SF, Bloom ME, Linden RM, Parrish CR (ed), Parvoviruses. Hodder Arnold, London, United Kingdom.
11. **Tijssen P, et al.** 2003. Organization and expression strategy of the ambisense genome of densonucleosis virus of Galleria mellonella. J. Virol. **77**:10357–10365.
12. **Yu Q, Fediere G, Abd-Alla A, Bergoin M, Tijssen P.** 2012. Iteravirus-like genome organization of a densovirus from Sibine fusca Stoll. J. Virol. **86**:8897–8898.
13. **Yu Q, Hajek AE, Bergoin M, Tijssen P.** 2012. Papilio polyxenes densovirus has an iteravirus-like genome organization. J. Virol. **86**:9534–9535.
14. **Zadori Z, et al.** 2001. A viral phospholipase A2 is required for parvovirus infectivity. Dev. Cell **1**:291–302.