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Biological and molecular characterization of a multicapsid nucleopolyhedrovirus from *Thysanoplusia orichalcea* (L.) (Lepidoptera: Noctuidae)☆

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Abstract

A multicapsid nucleopolyhedrovirus (ThorMNPV) that was co-isolated with a single nucleocapid ThorSNPV from mixed infected larvae of *Thysanoplusia orichalcea* L. (Lepidoptea: Noctuidae) is characterized. Scanning electron microscopy of ThorMNPV showed a dodecahedral-shaped occlusion body (OB). The occluded virions contained one to as many as eight nucleocapsids/virion. Virion band profiles in gradient centrifugation were consistent in at least 10 rounds of centrifugation from different virion sample preparations. The ThorMNPV had high virulence to third instar *Trichoplusia ni* and *Pseudoplusia includens* with LD_{50} values of 17 and 242 OBs per larva, respectively. However, ThorMNPV did not cause mortality in *Spodoptera exigua*, *Spodoptera frugiperda*, *Spodoptera eridania*, *Anticarsia gemmatalis*, and *Helicoverpa zea*. ThorMNPV replicates in cells of various tissues such as the fat body and tracheal epithelium cells. *T. ni* High 5 cells were permissive to ThorMNPV in terms of infection and viral DNA transfection, but SF-21 was less permissive and the infection process was slower. Production of OBs by ThorMNPV in the nuclei of SF-21 was not well pronounced. The genome size of ThorMNPV was estimated to be 136 kb. The polyhedrin gene open reading frame (ORF) was cloned and completely sequenced. The promoter sequence is identical to that of *Autographa californica* MNPV. Phylogenetic analyses using partial sequences of the *polh*, *lef-8*, and *lef-9* revealed that ThorMNPV is a member of the Group I NPVs and is related but distinct from the AcMNPV/*Rachiplusia ou* NPV/*Bombyx mori* NPV cluster.

Keywords: *Thysanoplusia orichalcea* MNPV; Host range; Virus infectivity; *Pseudoplusia includens*; *Trichoplusia ni*; DNA restriction profiles; Biological control; Phylogenetic analysis

1. Introduction

By virtue of their specificity, virulence and safety for non-target species, insect viruses, particularly baculoviruses, have become promising alternative candidates to chemicals for controlling agricultural and forest insect pests (Podgwaite, 1985). Several baculoviruses have been in general use around the world for the control of insect pest populations (Bedford, 1981; Cunningham and Entwistle, 1981; Moscardi, 1999; Shepard and Shepard, 1997).

Larvae of a looper species, *Thysanoplusia orichalcea*, infected with a nucleopolyhedrovirus (NPV) were collected from carrots in 1992 in West Java, Indonesia and

☆ The sequences reported in this paper has been deposited in GenBank/NCBI with the Accession Nos. AF169480, AY706587, AY706652, and AY706719.

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were brought to Clemson University, South Carolina, USA to test for infectivity against the soybean looper, *Pseudoplusia includens*. Two NPVs were isolated, one with a tetrahedral occlusion body (OB) and the other with a polyhedral OB. Analyses of the tetrahedral-shaped NPV revealed a single nucleocapsid NPV belonging to Group II NPVs (Cheng and Carner, 2000; Cheng et al., 1998; Jehle, 2004). In this paper, we report the characterization of the second virus with polyhedral OBs.

2. Materials and methods

2.1. Insects

Pseudoplusia includens eggs were obtained from the USDA Southern Insect Management Laboratory in Stoneville, Mississippi, and other insects used in this study were colonies maintained on pinto bean artificial diet in 31-ml (one ounce) plastic cups with paper lids in our insect rearing facility (Burton, 1969). The insect cultures were maintained according to the methods of Cheng and Carner (2000).

2.2. Isolation of ThorMNPV

The ThorMNPV was separated from the ThorSNPV by inoculating *Trichoplusia ni* larvae with the original mixture of viruses since ThorSNPV was not able to replicate in *T. ni* larvae (Cheng and Carner, 2000). Seventy microliters of semi-purified original NPV inoculum ($\sim 10^7$ OBs/ml) were spread on the surface of the diet of third instar *T. ni* larvae and larvae were incubated at 27 °C. Infected larvae showing swollen body and pale color were collected in a container and stored at −20 °C. Propagation of ThorMNPV was carried out in either *T. ni* or *P. includens*. In the following studies, OBs from *P. includens* were used unless otherwise indicated.

OBs from ThorMNPV infected larvae of *P. includens* and *T. ni* were purified separately by differential and sucrose gradient centrifugation (Cheng et al., 1990). The purified OB pellet was suspended in 0.5 ml of distilled water and quantified using a hemocytometer. The virus preparation was used as a stock for DNA analyses and bioassays.

Virions were purified according to the methods of Harrap and Longworth (1974) and Cheng and Carner (2000). The purified ThorMNPV virions were used for DNA extraction or stored at −20 °C.

2.3. DNA analysis

DNA was extracted from purified virions of ThorMNPV either produced in *P. includens* or *T. ni* larvae by the phenol method. To check if the ThorMNPV was contaminated with ThorSNPV, the DNA samples were used as templates in PCRs where the primers used in ThorSNPV polyhedrin analysis were used for the amplification (Cheng and Carner, 2000; Cheng et al., 1998). ThorSNPV DNA was used as a positive control.

Restriction endonuclease (REN) analysis was carried out by digesting 500 or 200 ng of purified ThorMNPV DNA with *Hin*dIII, *Eco*RI, *Pst*I, and *Xba*I (Promega or Gibco-BRL) in 20 μl reaction volumes. Digested fragments were fractioned on 0.7 and 0.4% agarose gels, respectively. The gels were stained with ethidium bromide and photographed with an IS-1000 Digital Imaging System (Alpha Innotech, San Leandro, CA). The accompanying software (V. 2.02) was used to estimate the molecular weight of DNA fragments.

To locate DNA fragments containing the polyhedrin gene of ThorMNPV, 500 ng of viral DNA were cleaved with *Hin*dIII, *Eco*RI, *Pst*I, and *Xba*I and separated on a 0.7% agarose gel. The DNA fragments were transferred to a nylon membrane (Hybond-N^+, Amersham) by using the standard alkaline method (Chomczynski, 1992; Southern, 1975). A 2.5 kb *Sal*I fragment containing the polyhedrin gene from *Orgyia pseudotsugata* MNPV (OpMNPV) cloned in pBluescript vector was kindly provided by G.F. Rohrmann (Oregon State University). A 1.85 kb fragment containing most of the polyhedrin gene of OpMNPV was obtained by digestion of the plasmid with *Xba*I followed by fractionation on a 0.7% agarose gel (Rohrmann et al., 1982). The 1.85 kb fragment was gel-purified and labeled using the digoxygenin random primer extension method according to the manufacturer's protocol for the Genius I kit (Boehringer Mannheim).

DNA–DNA hybridization was performed at 50 °C with 20 ng/ml of the digoxigenin-labeled probe for 16 h. The blot was washed twice for 5 min in ample 2× SSC containing 0.1% SDS at 22 °C, and twice for 15 min in 0.1× SSC containing 0.1% SDS at 62 °C. Chromogenic detection (Genius I) was used to visualize the hybridized probe. Restriction fragments containing the polyhedrin gene of ThorMNPV were identified from the hybridization patterns.

To clone and sequence the polyhedrin gene of ThorMNPV, viral DNA was digested with REN and separated on a 0.7% agarose gel. The fragments containing the putative polyhedrin gene were cloned into a plasmid (pBluescript II SK+, Stratagene) and amplified in *Escherichia coli* (DH5α). Putative plasmids containing the polyhedrin gene were digested with the appropriate enzymes and fractionated on a 0.7% agarose gel followed by Southern analysis to screen for fragments hybridizing to the OpMNPV-polyhedrin gene probe. Hybridizing fragments were purified and cloned followed by DNA sequencing.