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Occurrence and characterization of a tetrahedral nucleopolyhedrovirus from *Spilarctia obliqua* (Walker)

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ABSTRACT

Spilarctia obliqua Walker (Lepidoptera: Arctiidae) is a polyphagous insect pest damaging pulses, oil seeds, cereals, vegetables and medicinal and aromatic plants in India. The pest also infests turmeric and ginger sporadically in Kerala. We observed an epizootic caused by a nucleopolyhedrovirus (NPV) in field populations of the insects in December 2013. The NPV was purified and characterized. The isolate was tetrahedral in shape and belonged to multicapsid NPV. The REN profile of the SpobNPV genome with *Pst* I, *Xho* I and *Hin*dIII enzymes showed a genome size of 99.1 ± 3.9 kbp. Partial*polh*, *lef-8* and *lef-9* gene sequences of the isolate showed a close relationship with HycuNPV and SpphNPV. Phylogram and K-2-P distances between similar isolates suggested inclusion of the present SpobNPV isolate to group I NPV. The biological activity of the isolate was tested under laboratory conditions against third instar larvae of *S. obliqua* and the LC_{50} was 4.37×10^3 OBs/ml occlusion bodies (OBs) per ml. The median survival time (ST_{50}) was 181 h at a dose of 1×10^6 OBs/ml and 167 h at a dose of 1×10^8 OBs/ml. SpobNPV merits further field evaluation as a potential biological control agent of *S. obliqua*, a serious pest of many agriculturally important crops in the Oriental region.

1. Introduction

Spilarctia obliqua (Walker) (Arctiidae: Lepidoptera), is a sporadic and polyphagous insect pest widely distributed throughout the Oriental region (CPC, 2004). It was reported to attack 126 plant species belonging to 25 families, including pulses, oilseeds, cereals, vegetables, mulberry, turmeric, medicinal and aromatic plants, causing heavy economic loss (Gupta and Bhattacharya, 2008; Senthil Kumar et al., 2011). The caterpillars feed gregariously during the early (first to third instar) larval stages and solitarily in the late (fourth to fifth instar) larval stages. Chemical control of this pest is difficult and uneconomical because the pest feeds on several weed plants (Gupta and Bhattacharya, 2008). In a survey for natural enemies of the pest, we observed a nucleopolyhedrovirus (NPV) (Family: Baculoviridae) causing epizootic of the insect in Kozhikode, Kerala, India.

Baculoviruses comprise of the most diverse family of double stranded DNA viruses and more than 90% of the 600 reported baculoviruses are from the insect Order Lepidoptera (Martignoni and Iwai, 1981). The persistence of bacuoloviruses in the environment for horizontal transmission (Miller, 1997) along with their high pathogenicity to host insects and narrow host range make them highly potential biocontrol agents against lepidoptern pests (Jakubowska et al., 2005). The NPVs that infect lepidopteran insects are further classified into taxonomic group I or group II (Herniou et al., 2001). The NPVs that infect the same host insect in different geographical regions may differ. Thus, the isolates of AgseNPV infecting *Agrotis segetum* (Denis & Schiffermüller) in France and Poland were distinct (Allaway and Payne, 1983). It has also been shown that the large collections of NPV isolates of *Spodoptera littoralis* (Boisduval), SlNPV from Israel belonged to two distinct genotypes, SlNPV-A and SlNPV-B (Cherry and Summers, 1985).

In this paper, we report a new tetrahedral shaped multicapsid NPV infecting *S. obliqua* from India. The virus was characterized based on nucleocapsid packaging, electron microscope (EM) studies, restriction enzyme analysis of the genome, and by sequence analyses of the partial *polh*, *lef-8* and *lef-9* genes. The biological activity of this NPV against *S. obliqua* and its utility in biological control is also discussed.

2. Materials and methods

2.1. Insects

Field collected *S. obliqua* first instar larvae were reared in the laboratory (at 26 °C, RH 70%) to adult moths on turmeric (*Curcuma*

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longa L.) leaves in plastic containers (4 l) secured with nylon mesh. The eggs laid by the moths were used to propagate healthy populations of the insect in the laboratory for experiments.

2.2. Virus source and propagation

The virus was isolated from dead *S. obliqua* larvae found hanging on plants with characteristic viral infection symptoms (Fig. 1) at the farm of ICAR-Indian Institute of Spices Research, Kozhikode (11°17′59.81″N, 75°50′30.76″E), Kerala during an epizootic in December 2013. The cadavers were individually transferred to sterile microfuge tubes and preserved at −20 °C until further use. A crude homogenate of the virus was prepared by grinding a single larval cadaver in distilled water. The homogenate was filtered through four layers of cheese cloth to remove larval debris. The number of occlusion bodies (OBs) in the filtrate was adjusted to 1×10^6 OBs/ml using a Neubauer haemocytometer in aqueous solution of 0.05% Tween 20 (v/v). Multiplication of the virus was done in fourth instar *S. obliqua* reared in the laboratory. For this, 1 ml of viral suspension was spread uniformly on a fresh turmeric leaf of 20 × 10 cm on both sides and air dried in a laminar flow hood. Ten fourth instar larvae starved for 2 h were allowed to feed on the contaminated leaf for 24 h in a plastic jar (4 l). The insects were transferred to a fresh diet afterwards in a clean container and maintained at 25 °C till death. The setup was replicated three times.

2.2.1. Extraction of OBs

OBs were extracted from dead larvae by homogenizing the cadavers in water and purified by filtration and differential centrifugation (O'Reilly et al., 1992). Briefly, a larval cadaver was homogenized in 3 ml of 0.1% aqueous sodium dodecyl sulfate (SDS) (w/v) and the crude suspension was filtered through four layers of cheese cloth. The filtrate was centrifuged at 29×*g* for 30 s and the supernatant was further centrifuged at 2935×*g* for 5 min at 4 °C. The pellet was serially washed at 2935×*g* at 4 °C, once with 3 ml each of SDS (0.1%), 0.5 M aqueous sodium chloride and distilled water. The semi-purified virus was further purified by sucrose gradient (40–65% w/w) centrifugation at 96,000×*g*. The pellet containing OBs was finally suspended in double distilled water. The OB concentration was determined under phase contrast microscopy at 400× and stored at −20 °C for further use. The isolate was deposited in the Entomopathogenic virus repository of Indian Institute of Spices Research (IISR) with accession number IISR-NPV-02.

Fig. 1. Dead *S. obliqua* larva showing characteristic signs of NPV infection.

2.2.2. Nucleocapsid packaging

Nucleocapsid packaging of the isolate was studied following Bernal et al. (2013). Occlusion derived virions (ODVs) were released from purified OBs of 1×10^{10} OBs/ml by exposure to alkaline buffer (0.1 M Na_2CO_3; pH: 10.5) for 30 min at 28 °C. Polyhedrin and other debris were removed by low-speed centrifugation (2500×*g*, 2 min). The ODV containing supernatant was banded by density equilibrium centrifugation (30,000×*g*, 1 h at 4 °C) on 30–60% (w/w) continuous sucrose gradient. The banding pattern was visually inspected and photographed.

2.3. Extraction of viral DNA

Viral DNA was extracted following Bernal et al. (2013) with slight modifications. Briefly, virions were released from OBs by incubating a mixture of 100 µl of OB extract containing 10^9 OBs/ml, 100 µl 0.5 M Na_2CO_3, 50 µl 10% (w/v) SDS and 250 µl sterile distilled water for 10 min at 60 °C. Undissolved OBs and other debris were removed by low speed centrifugation (3800×*g*, 5 min). The supernatant containing the virions was treated with 25 µl proteinase K (20 mg/ml) for 1 h at 50 °C. Viral DNA was extracted by adding an equal volume of a mixture of phenol/chloroform/isoamyl alcohol (25:24:1) followed by high speed centrifugation at 16,627×*g* for 10 min at 4 °C. The DNA was precipitated from the supernatant by adding 0.1× of 3 M sodium acetate, pH 5.2 and 2.5 × of absolute ethanol and the contents were kept on ice for 15 min followed by centrifugation at 14,167×*g* for 15 min at 4 °C. The pellet containing the DNA was washed with 70% cold ethanol, suspended in Tris-EDTA (TE) buffer and stored at 4 °C.

2.3.1. REN analysis

Two µg of viral DNA was digested with *Pst* I, *Xho* I and *Hin*dIII (New England BioLabs) for 5–7 h at 37 °C under the conditions recommended by the supplier. Digested fragments were loaded together with λ DNA digested with *Hin*dIII and 1 kb ladder and electrophoresis was performed on 0.7% gel, stained with ethidium bromide and visualized on a UV trans-illuminator (BioRad) and photographed. The molecular size of the fragments was determined by the graphical method (Southern, 1979).

2.3.2. PCR amplification, sequencing of partial polh, lef-8 and lef-9 genes and phylogenetic analysis

PCRs were performed to amplify the partial *polh, lef-8* and *lef-9* genes with degenerate primer pairs (prPH-1, prPH-2, prL8-1, prL8-2 and prL9-1, prL9-2) as described by Lange et al. (2004) with slight modifications. Amplification reactions were performed in a 25 µl reaction under the following PCR conditions: for *polh* gene an initial denaturation at 95 °C for 3 min, followed by 36 cycles of 95 °C for 30 s, 53 °C for 1 min, 72 °C for 1 min, and a final extension step of 72 °C for 10 min; for partial *lef-8* gene, an initial denaturation step of 94 °C for 5 min and 35 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, and a final extension step of 72 °C for 10 min; for partial *lef-9* gene, an initial denaturation step of 94 °C for 5 min and 35 cycles of 94 °C for 1 min, 65 °C for 1 min, 72 °C for 30 s, and a final extension step of 72 °C for 10 min.