

Isolation of Nucleopolyhedrovirus (NPV) from the Beet armyworm *Spodoptera exigua* (Hübner) (*SpexNPV*)

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ARTICLE INFO

Article History

Received: Jun 23, 2013

Accepted: Sept. 18, 2013

Available online: Dec. 2013

Keywords

Isolation,
Nucleopolyhedrovirus (NPV),
Beet armyworm,
Spodoptera exigua (Hübner),
Light traps.

ABSTRACT

Primary isolation of Nucleopolyhedrovirus (NPV) from the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) was carried out in Dep. Cutworms and Mole Cricket, PPRI, ARC from a field-collected eggs- mass, at Menofea Governorate, Egypt as described by Khattab (2003). Light microscope and Transmission Electron Microscope examinations as well as histopathological studies were directed. Light microscope examination (1000X) cleared that the polyhedral occlusion bodies (POBs) were isolated from infected dead larvae (hatched from the collected eggs mass) with an average of 1.5 μm (n= 20). In addition, Electron microscopy photos of tissues from infected larvae revealed multiple POBs in the nuclei of infected cells. POBs were seen to contain many virions, and multiple nucleocapsids were capsulated within the membrane of each virion, indicating that this virus is a multicapsid nuclear-polyhedrovirus (MNPV). The amplified and sequenced the partial polyhedrin sequence of the detected virus indicated that it is identical 100% to *Spodoptera litura* NPV. According to the potentiality and the high virulence of *SeMNPV* it could be considered as an alternative agent for control of *S. exigua*.

1. INTRODUCTION

The beet armyworm is one of the most important species of Noctuid moths whose larvae are called *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). It has a wide host range throughout tropical and subtropical regions of the world (Metcalf and Metcalf, 1992) and occurring as a serious pest of vegetable, field, and flower crops. Among susceptible vegetable crops are asparagus, bean, beet, broccoli, cabbage, cauliflower, celery, chickpea, eggplant, lettuce, onion, pea, pepper, potato, radish, spinach, sweet potato, tomato, and turnip. Field crops damaged include alfalfa, corn, cotton, sugar beet, etc. Weeds also are suitable for larval development (Smith, 1989). The larvae feed on the foliage of plants and attack flowers, buds and fruits of some crops. Difficulty of *S. exigua* Control with chemical insecticides may be due to differences in susceptibility of different populations and / or the development of resistance (Wang *et al.*, 2006).

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ISSN 2156-7530

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The beet armyworm *Spodoptera exigua* has also been shown to be susceptible to a number of nucleopolyhedroviruses including those of *Autographa californica* (Speyer) (Vail and Jay 1973) *Helicoverpa* (= *Heliothis*) *armigera* (Hübner) (Hamm, 1982), *Mamestra brassicae* (MbMNPV) (Smits and Vlak 1987), *Anagrapha falcifera* (Kirby) (Hostetter and Puttler 1991) and *Spodoptera littoralis* (SlMNPV), *Spodoptera frugiperda* (SfMNPV) (Munä OZ and Caballero, 1999), among others. However, the NPV isolated from *S. exigua* (SeMNPV) has been demonstrated to be more pathogenic and to have shorter speed of kill than any other NPV that is infectious to *S. exigua*.

S. exigua multiple nucleopolyhedrovirus (SeMNPV) is commonly found in the environment and frequently causes a high prevalence of infection to the beet armyworm populations feeding on crop and natural plant hosts (Gelernter and Federici, 1986 and Caballero *et al.*, 1992). Several *S. exigua* MNPV (SeMNPV) strains was isolated from collected larvae in different parts of the world as Egypt and the Netherlands (Murillo *et al.*, 2001), California (Gelernter and Federici, 1986); Spain (Caballero *et al.*, 1992), Florida (Kolodny-Hirsch *et al.*, 1993), Japan and Thailand (Hara *et al.*, 1994; Kondo *et al.*, 1994 and Takatsuka & Kuimi, 2002) and Uzbekistan (Murillo *et al.*, 2001) MNPV of *S. exigua* differs than any other baculoviruses in that it is monospecific and highly virulent against first instars and older larvae (Hunter & Hall, 1968, Chautani and Rehnborg, 1971). However, the molecular mechanism associated with these properties is unknown (Smits, 1987). Certain isolation of SeMNPV has been marketed as a biological insecticide in several countries. The virus is commercially available in the USA, Thailand, Spain and the Netherlands (Moscardi, 1999).

The present study aimed to screen the presence of occluded viruses of *S. exigua* obtained from collected one eggs- mass in light trap at Monofea governorate.

2. MATERIALS AND METHODS

2.1 Experimental insect:

Fresh eggs- mass of the beet armyworm, *Spodoptera exigua* (Hübner) was collected from paper strips lay on glass cage of light trap at Monofia Governorate, in March, 2008. The eggs – mass was kept in glass container covered with muslin cloth and incubated at constant conditions ($25 \pm 2^\circ\text{C}$ & $65 \pm 5\%$ R.H.) until hatching. Newly hatching larvae was transferred individually on semi-synthetic diet - synthetic diet described by Shorey and Hale (1965) poured into glass tubes (3 x 10 cm) using a fine hair brush and then capped by a piece of cotton wool. All tubes were incubated at constant conditions ($25 \pm 2^\circ\text{C}$ & $65 \pm 5\%$ R.H.). Daily inspection was conducted until adult emergence to determine mortality percentage of larvae and inspect the subsequent stages. Some emerged adult moths were dissected and identified by a Professional Taxonomist in PPRI, ARC, Dokki, Giza, as beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae). In addition, one pair of emerged moths was placed in glass cage fed on 20% sugar solution. The cage was examined daily to determine fecundity, larval mortality & % pupation and adult emergence (F1). A special bioassay plates (LICEFA, Bad-Salzuflen, Germany) were used to keep the diseased larvae due to virus infection (Khattab, 2007). All plates were incubated under the previous conditions.

2.2 Virus isolation:

Natural virus isolation, obtained from virus-dead larvae of the beet armyworm was triturated and occlusion bodies (OB's) were purified as described previously (Khattab, 2003).

Larvae were macerated in distilled water using a suitable mortar. The resulting suspension was filtered through two layers of muslin to remove the undesirable fragments and skin, followed by centrifugation at 4000 rpm for 20 minutes. Immediately, after centrifugation, the supernatant was stored while pellet was examined in a smear test using light microscope for the presence of

virus inclusion bodies (IB,s) of nucleopolyhedrovirus (NPV) and consequently determine the size of present isolated polyhedral inclusion bodies (PIB). The occlusion bodies (OB's) were suspended in distilled water, counted using a Neubauer improved chamber (Hawksly, Lancing, United Kingdom) and stored at 4°C prior to use.

2.3 Light microscope examination:

Moribund larvae showing distinct disease symptoms were individually examined for the presence of polyhedral inclusion bodies, using light microscope. A wet smear of the homogenized liquid using a drop of haemolymph or a small part of larval tissue was spread on a clean slide.

The slide was then dipped in 10% Giemsa's stain for 10 minutes. The excess stain was then washed with running water for 5-10 second (Wigley, 1976). The prepared smear was examined using the oil immersion of phase contrast microscope. The smear test would allow recognition of the occlusion bodies of nuclear or cytoplasmic polyhedrosis viruses (CPV or NPV). The detection of new viruses was largely depending on the number of naturally diseased larvae collected from natural populations.

2.4 Histopathology:

Tissues from infected larvae were dissected, fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3)-0.23M sucrose for 3hrs post fixation in 1% osmium tetroxide for 1hr, dehydrated through an ethanolpropylene oxide series, and embedded in Epon-Araldite resin at the Research Institute of Ophthalmology. Thin sections were stained and examined with the light microscope. Also, Transmission Electron Microscope (TEM) examination was directed to the thin tissues sections.

2.5 Transmission Electron Microscope examination:

Separated virus was fixed in buffered 4% glutaraldehyde for 1 hour then postfixed in 2% osmic acid for 1 hour, dehydrated in

ascending alcohol, then embedded in epoxy resin. Ultrathin sections were performed using Leica Ultramicrotomie (Leica Microsystems GmbH, Ernst-Leitz-Strasse, Austria). The sections were stained with uranyl acetate and lead citrate and examined using Philips EM 208S (Eindhoven, The Netherlands) (Grimaud *et al.*, 1980).

2.6 Polyhedrin sequence) attached in Appendix):

The partial polyhedrin sequence of the virus sample was identified by Prof. Doctor Johannes A. Jehle (DLR Rheinpfalz Breitenweg 71, Germany).

3. RESULTS AND DISCUSSION

The initial source of virus – sample of *Spodoptera exigua* was obtained from only one eggs mass (about 18 eggs) collected from cage glass of light trap at Monofea governorate during March, 2008.

The collected eggs are greenish to white in color and covered with a layer of whitish scales that gives the egg mass cottony appearance. Eggs hatch in three days, after hatching the newly hatched larvae hanging by a thin thread on the surface of the cage glass like the cotton leaf worm *Spodoptera littroralis*. The life cycle and development of the different stages are identical to those described by Fye *et al.* (1972).

Data presented in Table (1) show larval mortality (%), (%) pupation & adult emergency (%) of *S. exigua* that collected as eggs mass from light trap. The respective % was 83.33%, 16.66% and 16.66%. Three days after pairing and feeding emerged moths egg- masses was laid, collected & incubated until hatching. Sixty newly hatching larvae were reared as described previously. Data in Table (1) demonstrate that larval mortality and (%) pupation was 93.33%. and 6.66, respectively.

The present observations demonstrate that diseased larvae due to virus dead in the fifth instar, (about 91.02%), and the rest died during the pupal stage (6.66 %).

Table 1: Response percentages of beet armyworm *Spodoptera exigua* (Hübner) collected and reared eggs by light trap at Monofea Governorate during 2008.

Source of eggs	No. of eggs	% hatchability	% larval Mortality	% Pupation	% Adult emergence	No. of laid eggs
Collected (field)	18.0	100.0	83.33	16.66	100.0	60.0
Resulted (Reared) F1	60.0	100.0	93.33	6.66	00.0	-

3.1 Biological activity:

The larval infected by *S.exigua* virus showed the typical symptom of nucleopolyhedrovirus (NPV) infection at a late stage of disease. The dead larvae have a

characteristic shiny-oily appearance. They are extremely fragile to the touch, rupturing to release fluid filled with infective virus particles. At this time larvae are whiten creamy (Fig. 1 D).

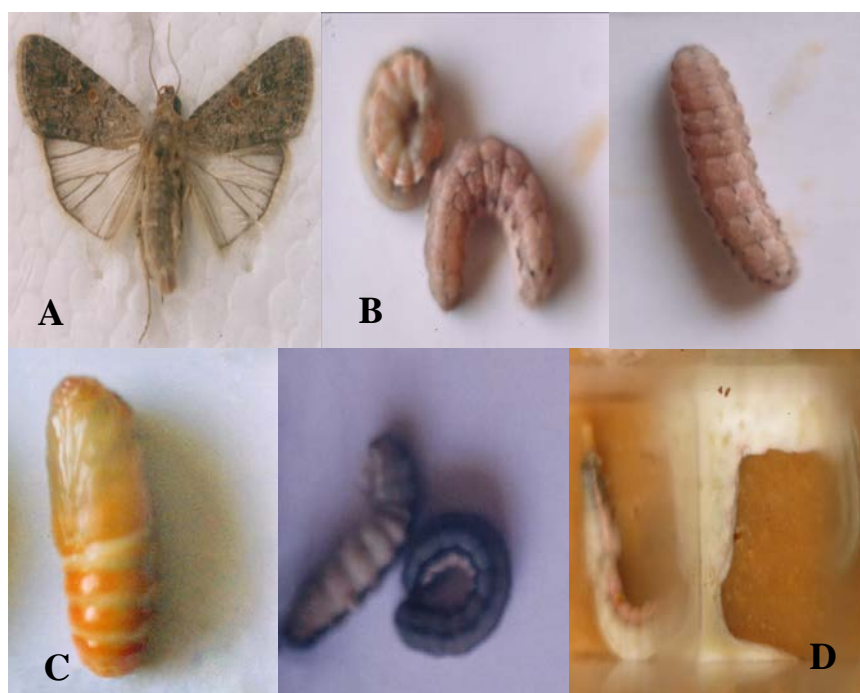


Fig. 1: A- Adult, beet armyworm *Spodoptera exigua* (Hübner)
 B- Typical mature larvae of *S. exigua*
 C- Normal color and size of *S. exigua* pupa
 D- The natural dead whiten creamy larvae of *S.exigua* infected with virus

3.2 Ultrastructural studies:

Light microscope examination of smears from individual maturely diseased larvae by phase contrast microscope revealed the presence of virus particles. The fluid inside the dead larvae is composed largely of virus-particles- many billions are produced inside of one larva (Fig. 2).

The size of virus particles in the present isolation was 1.5 μ m (n=20) (Fig. 2).

Transverse section of *S.exigua* larvae natural infected with virus examination by phase contrast microscope showing swollen nuclei and infected fat body (f) and Epidermis (EP). Serious infection of larval tissues, most cells lysing and larval tissues full of virus particles (Fig. 3).

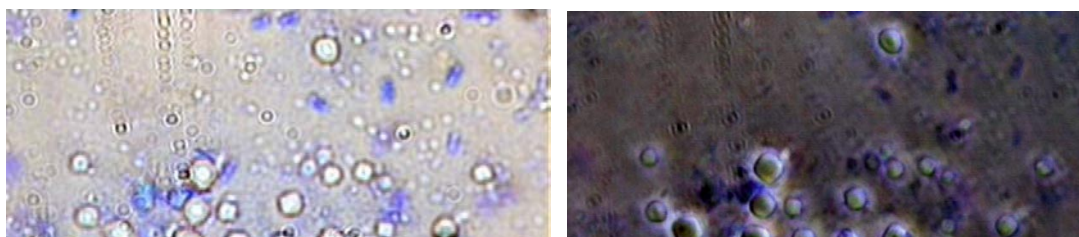


Fig. 2: Light microscopic examination of *S. exigua* purified suspension showing polyhedral inclusion bodies (1000X).

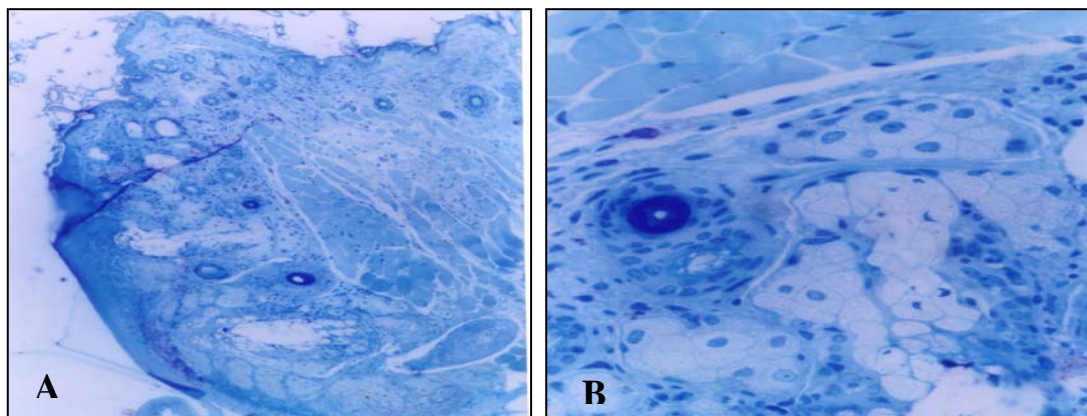


Fig. 3: Light micrograph of cross section of natural infected *S. exigua* larva (A=100X - B=400X).

3.3 Electron microscopy of tissues from larva infected with virus particles revealed multiple polyhedral occlusion bodies (POBs) with the nuclei of infected cells (Fig. 4). POBs were seen to contain many virions,

and multiple nucleocapsids were capsulated within the membrane of each virion, indicating that this virus is a multicapsid nuclear- polyhedrovirus (MNPV) (Volkman *et al.*, 1995) (Fig. 5).

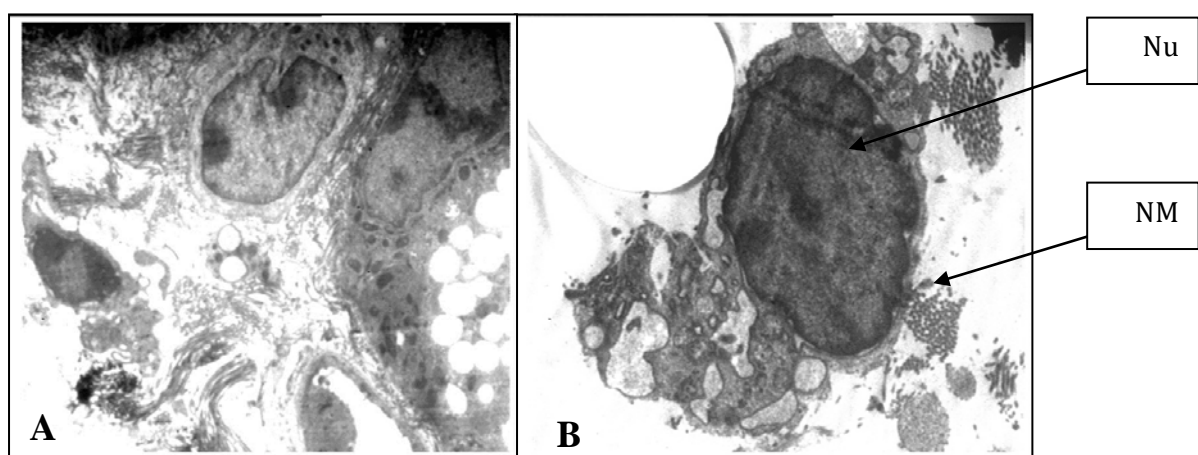
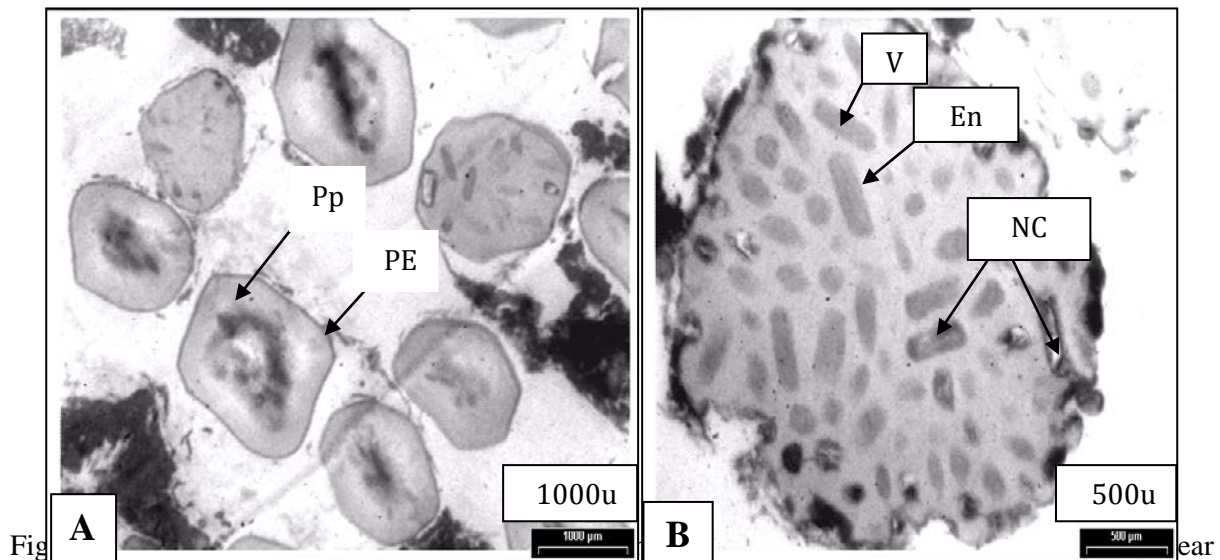


Fig. 4: Electron micrographs of cross section of natural infected *S. exigua* larva (A=1.800X - B=2.800X).

NM: nuclear membrane
Nu: nucleus



(A= 4.400 X-B= 8.900).

Pp: Polyhedral protein

PE: Polyhedral envelope

En: Enveloped virion

V: virion

NC: Nucleocapsid

Similar results were observed during an epizootic of *S. exigua* in California. A multinucleocapsid nuclear polyhedrovirus (MNPV) was isolated from a single larva of beet armyworm. Occlusion bodies (OBs) produced by this virus isolate, measured $1.52 \pm 0.11 \mu\text{m}$ in diameter and most contained virion, with two to four nucleocapsids per envelope. Additionally, *S. exigua* MNPV (*SeMNPV*) infects only a single insect species, *S. exigua* (Gelernter and Federici, 1986).

Amplified and sequenced the partial polyhedrin sequence of the virus sample showed that it is 100% identical *Spodoptera litura* NPV Appendix. These results are consistent with those reported previously for *S. exigua* NPV (*Se NPV*) and *S. litura* NPV (*Splt NPV*) (Feng *et al.*, 2007), where in *SeMNPV* and *SpltNPV* are genetically similar, but the larvae of *S. exigua* are not susceptible to *SpltNPV*.

Senthil Kumar *et al.* (2005) reported that the biological activity of *S. exigua* (*SeNPV*) harvest from dead larvae was about 50% more active than live. On the other hand, Evans (1986) showed that the infected one large corn earworm larvae *Helicoverpa*

(=*Heliothis*) *zea* (Boddie) can produce approximately 6×10^9 OBs. When these OBs contaminate feeding sites of susceptible larvae, the disease can spread rapidly through the host population.

Yu and Brown (1997) showed that, egg chorine of *S. exigua* consumed by a hatching larva averaged 5.7% of the egg surface area. They estimated that 18 OBs /egg were required to obtain LD_{80} for hatching larvae. Scanning electron microcopy revealed that most treated eggs had some observed polyhedra on the chorion. Also, *SeMNPV* produced significantly higher mortality among F_1 progeny from mating pairs with both partners contaminated than from mating pairs with single partners contaminated. Male- contaminated parents were almost as effective in transovum transmission as female contaminated parents. *S. exigua* MNPV induced much higher mortality via horizontal transmission among cohorts when hatched larvae from the same egg mass were reared together. Additionally, *S. exigua* offers a good opportunity for an autodissemination-based control because its larvae are gregarious during first to third instars, thereby encouraging NPV

transmission. Death of those larvae will in turn create a reservoir of virus in the soil or plants and that perpetuates control of insect through the growing season. The adults can be captured and contaminated easily using a light or pheromone trap with a contamination mechanism, possibly similar to that used by Jackson *et al.*, (1992).

However, the author named the present isolate of *S. exigua* in Egypt of (*SeMNPV*^{EG}). The present results supported the view that the *SeMNPV* can survive on the surface of the eggs.

Further studies are required for the nucleotide sequence of the DNA genome of the present natural isolate of *S. exigua*. Also, field studies are needed to evaluate the natural incidence of nucleopolyhedrovirus among *S. exigua* population in Egypt. In addition, the population dynamics of *S. exigua* is also important, in order to determine the proper timing of application that would ensure control during the early instars of the larvae, when they are more exposed and susceptible to the virus.

4. ACKNOWLEDGMENT

The author wishes to appreciate Prof. Dr. Mostafa Badr (PPRI, ARC, Dokki, Giza, Egypt) for his kind help and assistance in identifying larvae hatched from the collected eggs mass of *S. exigua*. Deep thanks are also extended to Prof. Dr. Johannes A. Jehle (State Education and Research Center for Agriculture, Viticulture and Horticulture (SLFA), Germany, for providing the *S. exigua* polyhedrin sequence. I wish to express my deepest thanks to Prof. Dr. Amira Rashad (PPRI, ARC, Dokki, Giza, Egypt) for her valuable help in giving guidance and advice and reviewing the manuscript.

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APPENDIX

Additionally: Polyhedrin sequence of the virus isolation from *S. exigua*.

We have amplified and sequenced the partial polyhedrin sequence of the virus sample you sent to us. A Blast search showed that it is 100% identical to *Spodoptera litura* NPV. This does not mean that the host insect was *S. litura* but the virus is definitely SpltNPV.

Regards,
Johannes

Sequence amplified:

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AAATCAaAAGTTGACTCTGTTCaAGGAGATCCGTAACGTGAAACCCGACACGaTgAAGCTGATCGTCAACTGG
AACGGCA
AAGAGTTTCTCCGTGAGACTTGGACCCGTTTCATGGAAGACAGCTTCCCCATCGTGAACGATCAAGAAGTGA
TGGACGTG
TTTCTAGTGGTGAACATGCGTCCCACTAGACCGAACCGTTGCTTTAGATTTTTGGCGCAACACGCGCTCCGA
TGCGACCC
CGAGTACGTTCCCCACGACGTGATCCGCATCGTCGAACCGTCGTACGTCCGGACCAACAATGAATACCGCA
TCAGTCTCG
CCAAGAAAGGTGGCGGCTGTCCCGTGATGAACCTGCACGCCGAATACACCACTTCGTTTGAGAGTTTCATCG
ACAAGGTG
ATATGGTACAACCTTTTACAAGCCCATCGTGTACGTGGGCACCGATTCCGGCCGAAGAGGAGGAGATCCTTCTC
GAAGTGTC
GCTCGTGTTCAAGATCAAAGArTtYgCkCCmGGTCATAGCTGTTTCCTGA
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> gb|DQ152923.1| Spodoptera litura nucleopolyhedrovirus polyhedrin gene,
complete
cds
Length=3173
Score = 926 bits (501), Expect = 0.0
Identities = 501/501 (100%), Gaps = 0/501 (0%)
Strand=Plus/Plus
Query 1 AAATCAAAAGTTGACTCTGTTCAAGGAGATCCGTAACGTGAAACCCGACACGATGAAGCT 60
|||||
Sbjct 1434 AAATCAAAAGTTGACTCTGTTCAAGGAGATCCGTAACGTGAAACCCGACACGATGAAGCT 1493
Query 61 GATCGTCAACTGGAACGGCAAAGAGTTTCTCCGTGAGACTTGGACCCGTTTCATGGAAGA 120
|||||
Sbjct 1494 GATCGTCAACTGGAACGGCAAAGAGTTTCTCCGTGAGACTTGGACCCGTTTCATGGAAGA 1553
Query 121 CAGCTTCCCCATCGTGAACGATCAAGAAGTGATGGACGTGTTTCTAGTGGTGAACATGCG 180
|||||
Sbjct 1554 CAGCTTCCCCATCGTGAACGATCAAGAAGTGATGGACGTGTTTCTAGTGGTGAACATGCG 1613
Query 181 TCCCACTAGACCGAACC GTTGTCTTAGATTTTTGGCGCAACACGCGTCCGATGCGACCC 240
|||||
Sbjct 1614 TCCCACTAGACCGAACC GTTGTCTTAGATTTTTGGCGCAACACGCGTCCGATGCGACCC 1673
Query 241 CGAGTACGTTCCCCACGACGTGATCCGCATCGTCGAACCGTCGTACGTCCGGACCAACAA 300
|||||
Sbjct 1674 CGAGTACGTTCCCCACGACGTGATCCGCATCGTCGAACCGTCGTACGTCCGGACCAACAA 1733
Query 301 TGAATACCGCATCAGTCTCGCCAAGAAAGGTGGCGGCTGTCCCGTGATGAACCTGCACGC 360
|||||
Sbjct 1734 TGAATACCGCATCAGTCTCGCCAAGAAAGGTGGCGGCTGTCCCGTGATGAACCTGCACGC 1793
Query 361 CGAATACACCACTTCGTTTGAGAGTTTCATCGACAAGGTGATATGGTACAACCTTTTACAA 420
|||||
Sbjct 1794 CGAATACACCACTTCGTTTGAGAGTTTCATCGACAAGGTGATATGGTACAACCTTTTACAA 1853
Query 421 GCCCATCGTGACGTGGGCACCGATTCCGGCCGAAGAGGAGGAGATCCTTCTCGAAGTGTC 480
|||||
Sbjct 1854 GCCCATCGTGACGTGGGCACCGATTCCGGCCGAAGAGGAGGAGATCCTTCTCGAAGTGTC 1913
Query 481 GCTCGTGTTCAAGATCAAAGA 501
|||||
Sbjct 1914 GCTCGTGTTCAAGATCAAAGA 1934
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