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Degenerate Primed Polymerase Chain Reaction for Detection of Both Nucleopolyhedrovirus and Granulovirus

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ABSTRACT

A technique using the polymerase chain reaction (PCR) was developed for simultaneous detection of the nucleopolyhedrovirus (NPV) and granulovirus (GV). Ninety one and 73 amino acid sequences of polyhedrin and granulin genes were compared in pairwise and multiple alignment sequences. Seven highly conserved DNA sequences within the coding region of the polyhedrin/granulin genes were identified. Four candidate regions were targeted for amplification and consequently one pair of degenerate PCR primers was designed to produce fragments of about 384 bp. The baculoviruses tested by this technique were *Autographa californica* (*Ac*MNPV), *Bombyx mori* NPV (*Bm*NPV), *Lymantria dispar* NPV (*Ld*NPV), *Spodoptera littoralis* NPV (*Spli*NPV), *S. littoralis* GV (*Spli*GV), *Pieris rapae* GV (*Pr*GV), and two local GV isolates (GV_{G213} and GV_{F115}). Furthermore, four randomly chosen PCR products were cloned and sequenced. The sequencing data showed that the four PCR products were fragments of polyhedrin and granulin genes. Conclusively, this technique would be useful in monitoring the environmental fate, distribution of *Baculovirus* species, release of the wild type and recombinant *Baculovirus* and quality control studies of *Baculovirus* insecticides, as well.

Keywords: Nucleopolyhedrovirus, Granulovirus, Baculovirus, PCR.

INTRODUCTION

Baculoviruses have a large circular double-stranded DNA genome ranging from approximately 80 to 180 kb in size (Blissard and Rohrmann, 1990). The family *Baculoviridae* is taxonomically divided into two genera, *Nucleopolyhedrovirus* and *Granulovirus* (Theilmann *et al.*, 2005). Although Murphy *et al.* (1995) have reported baculovirus infections in over 600 insect species in the orders: Lepidoptera, Hymenoptera, Diptera, Coleoptera, Neuroptera, Trichopera and Thysanura, as well as in the Crustaceae order Decapoda (shrimps), it is recently confirmed that only those derived from orders Lepidoptera, Hymenoptera, and Diptera are members of the family *Baculoviridae* (ICTV, 2009). An updated classification of the family *Baculoviridae*, which includes four genera: *Alphabaculovirus* (lepidopteran-specific NPV), *Betabaculovirus* (lepidopteran-specific GV), *Gammabaculovirus* (hymenopteran-specific NPV) and *Deltabaculovirus* (dipteran-specific NPV), was thus proposed (Jehle *et al.*, 2006).

Baculoviruses are being extensively studied for their potential use as bioinsecticides around the world and as expression vectors for heterologous gene expression in insect-derived cells, as well as in host caterpillars (Summers and Smith, 1987, Choi *et al*., 1999, Wood and Robert, 1991). Recently, some nucleopolyhedrovirus has been successfully developed for surface display of recombinant proteins (Rahman and Gopinathan, 2003), and for potential use as gene therapy vectors (Condreay and Kost, 2007; Huser and Hofmann, 2003; Tani *et al.*, 2003). In addition, the commercialization and release of recombinant viruses in the environment

created the concern that they might cause ecological disturbances, such as displacement of native microorganisms, adverse effects on non-target organisms and the horizontal transfer of DNA into non-target organisms (Leung *et al*., 1994). For the above mentioned reasons, many authors were interested in developing an accurate and easy diagnostic method for early, reliable and rapid detection of NPV and GV infections (Wang *et al*., 2000, Christian *et al*., 2001, Moraes and Maruniak, 2001, Woo, 2001, Ikuno *et al*., 2004, Lange *et al*., 2004, Jehle *et al*., 2006, Murillo *et al*., 2006, Kundu *et al*., 2008, Manzán *et al*., 2008, Galal, 2009, Hewson *et al*., 2011, Ravikumar *et al*., 2011, Arneodo *et al*., 2012). Several methods have been employed to detect wild type or recombinant baculoviruses, such as microscopic diagnosis (Traverner and Connor, 1992), serological techniques (Brown *et al*., 1982, Naser and Miltenburger, 1983, Webb and Shelton, 1990), radioimmunoassay techniques (Smith and Summers, 1981, Knell *et al*., 1983), and DNA dot blot hybridization assays (Ward *et al*., 1987, Keating *et al*., 1989). The use of these techniques has been limited because they are either tedious and unreliable, or because they utilize radioactive materials. Polyhedrin and granulin are the major matrix proteins of NPVs and GVs. Lepidopteran polyhedrin gene has been repoted to share 50% amino acid identity with granulin gene (Rohrmann, 1992, Seufi, 2008). Up to date, there was published data on the complete genome sequence of more than 60 baculoviruses (NPVs and GVs). Polyhedrin and granulin are proteins of about 245 to 250 amino acids, and appeared to be the most highly conserved and similar baculovirus proteins. These characteristics lead to the use of polyhedrin and granulin sequences as the base of baculovirus phylogenetic studies (Zanotto *et al*., 1993), as well as in polymerase chain reaction (PCR) studies. PCR is a highly sensitive technique, which amplifies target DNA sequences and does not employ radioactive material. PCR has been extensively used to detect many organisms such as animal, human, plant and various pathogens. Many authors reported the use of PCR technology to screen baculoviruses in different approaches over the last three decades (Webb *et al*., 1991, Burand *et al*., 1992, Kundu *et al*., 2003, Ikuno *et al*., 2004, Kundu *et al*., 2008, Galal, 2009, Manzán *et al*., 2008, Hewson *et al*., 2011, Ravikumar *et al*., 2011, Arneodo *et al*., 2012).

The aim of the present study was to design degenerate primer set to detect multiple NPVs and GVs, simultaneously, using PCR technique. The present study will be useful tool in studies seeking to rapidly elucidate a polyhedrin/granulin gene structure, to monitor the release of the wild type as well as genetically engineered baculoviruses, and to isolate NPVs and GVs in the natural environment.

MATERIALS AND METHODS

Gene sequence data

All polyhedrin and granulin amino acid (a.a.) sequences available in January 2012 from GenBank, EMBL, and DDBJ were downloaded. 91 polyhedrin and 73 granulin a.a. sequences were aligned using Mega4 or ClustalX software. Neighbour joining trees for polyhedrin and granulin sequences were examined separately using Mega4 and all divergent sequences were excluded. In addition, the alignments were manually corrected by shifting sequences in places for some sequences possessed large spans of unique deletions or insertions which threw off the alignment algorithm. 100% identical sequences of the same species, with different accession numbers, were represented by only one sequence.

Selection of highly conserved genome regions for primer design

The term "conserved genomic regions" used here is defined as petidome regions that have most frequently presented a.a. sequences. To identify the highly conserved regions eligible for primer design, pairwise scan for the sequences was performed and point by point alignment output file was produced using Mega4 and/ or ClustalX software. The most frequently presented a.a. in the same coordinate for all sequences of the alignment was detected. The output (FASTA file) was then analyzed by ClustalX software to select candidate conserved regions for primer design. A "candidate region" was defined as a site within the polyhedrin/granulin open reading frame (*orf*) that had 6+ a.a. from the 3' end and with a a.a. frequency of 0.80+. Candidate conserved regions were subjected to calculation of redundancy scores and the average dominant base counts. Average dominant base counts were calculated by summing the number of occurrences of the most common base at each position in a window length of 20+ bases and averaging those counts across all positions in the window.

Primer design

The distance between conserved regions was taken into account when selecting conserved sites as was the potential for using mixed bases or deoxyinosines, to enhance bonding at variable positions. Standard nucleotides were preferred close to the 3' termini of the oligonucleotide. The different parameters of primer design (length and sequence, GC content, Tm,.....etc.) were taken into consideration. A set of degenerate primers common for the whole group was designed. Primers were designed to amplify 384 bp within the *orf* of polyhedrin/granulin sequence. The species and accession numbers of polyhedrin and granulin a.a. sequences used in this study are listed in Table (1).

Table 1: Baculovirus species (NPV or GV) and accession numbers of the amino acid sequences used in the present study.

GV Species	Acc#	GV Species	Acc#	NPV Species	Acc#	NPV Species	Acc#
Adoxophyes orana	AAL02082,	*Harrisina brillians*	AAF66610	*Adoxophyes honmai*	BAC67252	*Hyposidra talaca*	AEK86285
Agrotis exclamationis	AAW49149	*Helicoverpa armigera*	ABY47692	*Agrotis ipsilon*	AAY41433	*Leucoma salicis*	AAW66663
Agrotis segetum	AAS82737	*Hyphantria cunea*	AAW49159	*Agrotis segetum*	AAZ38167	*Lymantria xylina*	ADD73710
Andraca bipunctata	AAS86810	*Peridorma morpontora*	AAW49162	*Anagrapha falcifera*	AAB53357	*Malacosoma disstria*	AAD00095
Caloptilia theivora	BAJ24884	*Phthorimaea operculella*	AAM70199	*Antheraea pernyi*	ABF50243	*Mamestra configurata*	AAB51031
Choristoneura fumiferana	AAC69544	*Pieris rapae*	AAR06236	*Bombyx mori*	AAA46734	*Maruca vitrata*	ABL75953
Choristoneura murinana	AAW49153	*Pieris brassicae*	ACJ24910	*Buzura suppressaria*	CAA50194	*Neodiprion abietis*	AAM95580
Choristoneura occidentalis	ABC61135	*Plathypena scabra*	AAW49165	*Choristoneura fumiferana*	AAA93292	*Neodiprion lecontei*	YP_025198
Clostera anachoreta	AAW49154	*Pseudaletia unipuncta*	BAF45154	*Choristoneura rosaceana*	AAB51303	*Neodiprion sertifer*	AAQ96378
Cryptophlebia leucotreta	AAQ21599	*Scotogramma trifolii*	AAW49166	*Diaphania pulverulentalis*	ACS83600	*Orgyia leucostigma*	ABY65727
Cydia pomonella	AAK70668	*Spodoptera frugiperda*	AAW49167	*Ecotropis obliqua*	AAQ88174	*Spodoptera littoralis*	BAA00824
Epinotia aporema	AAO14643	*Spodoptera litura*	ABB96246	*Epiphyas postvittana*	AAC72189	*Spodoptera litura*	CAA64211
Erinnyis ello	AAW49155	*Trichoplusia ni*	AAA43834	*Helicoverpa armigera*	AAB82410	*Thysanoplusia orichalcea*	AAD51629
Euxoa ochrogaster	AAW49156	*Xestia c-nigrum*	AAB42059	*Hyposidra infixaria*	AEK86286	*Wiseana signata*	AAB97154

Viruses, cell lines and insects

The nucleopolyheroviruses and granulovirus tested in this study were as follows: *Autographa californica* (*Ac*MNPV), *Bombyx mori* NPV (*Bm*NPV), *Lymantria dispar* NPV (*Ld*NPV), *Spodoptera littoralis* NPV (*Spli*NPV), *S. littoralis* GV (*Spli*GV), *Pieris rapae* GV (*Pr*GV), and two local GV isolates (GV_{G213} and GV_{F115}). The *Ac*MNPV and *Bm*NPV were propagated in *Sf*9 cells maintained at 27 °C in a TC-100 medium (Gibco-BRL, USA) that was supplemented with 10% fetal bovine serum (Gibco-BRL, USA). The *Spli*NPV, GV_{G213} and GV_{F115} and *Ld*NPV were propagated in *S. littoralis* and *L. dispar* larvae, respctively. Routine cell culture maintenance and virus production procedures were carried out according to O'Reilly *et al.* (1992). Insect colonization and maintenance of the cotton leafworm, *Spodoptera littoralis*, was done in the insectary of Department of Entomology, Faculty of Science, Cairo University under highly controlled conditions from 1995 to date. The colony was maintained in the laboratory according to Seufi (2008). These insects were used for viral propagation and purification assays.

Virus DNA purification

Virus DNA was extracted from purified and semipurified viruses collected from infected cells and from insect larvae, as well. Total genomic DNA was also extracted from insect larvae. The virus isolates were successfully propagated and purified following the method described by Lacey *et al.* (2002). To extract virus DNA, purified or semipurified viruses were resuspended in a 0.1 M sodium carbonate solution (0.1 M Na_2CO_3, 0.17 M NaCl, 0.01 M EDTA, pH 10.9), and incubated at 37 °C overnight with a final concentration of 0.5 mg/ ml of proteinase K (Sigma, USA) and 1% of SDS. A further extraction with phenol and chloroform: isoamylalcohol (24:1) was performed and the DNA was ethanol-precipitated. The DNA was resuspended in a TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0).

PCR amplification

PCR amplification was performed according to Saiki *et al.* (1988) with minor modifications. Total DNA was extracted from the NPV isolates and the DNA segment was amplified using two sets of primers designed based on conserved a.a. sequences of 91 and 73 different polyhedrin/granulin sequences, representing 28 NPV and 28 GV species. Sequence of the forward and reverse primers used in this study, their length, GC content and base counts were shown in Tables (3 and 4). The total reaction volume was 25 µl containing 1× PCR buffer (Promega), 1.5 mM $MgCl_2$, 200 µM dNTPs, 2.5 U *Taq* DNA polymerase (Promega), 50 pmol of each primer and 30 ng of template DNA. The amplification program used was 7 min at 94°C (hot start), 30 sec at 94 °C, 1 min at 53 °C and 1 min at 72 °C for 30 cycles followed by one cycle of 72 °C for 10 min. PCR amplification was carried out in a DNA thermal cycler (Model 380 A, Applied Biosystems, CA, USA).

RESULTS

Selection of candidate conserved region for primer design

Alignment of the selected polyhedrin/granulin sequences were used as a guide to enable identification of conserved regions of the sequence to be used in the design of degenerate primers for PCR. No potentially useful conserved sites were identified in the first complete multiple alignment, utilizing all available sequences in GenBank, EMBL, and DDBJ databases. However, once divergent sequences were removed, seven conserved regions were identified. Two candidate regions (from 51 to 58 and 171 to 191 relative to the *Adoxophyes honmai* NPV polyhedrin (Acc# BAC67252) with relatively low levels of degeneracy were selected to design primers (Table 2). One set of degenerate PCR primers (*PolG*91F and *PolG*91R) was designed from these regions.

Table 2: Number, length and location of the identified conserved regions in the polyhedrin/granulin amino acid sequences used in this study. Locations were determined in relation to the *Adoxophyes honmai* NPV polyhedrin gene (Acc# BAC67252). The candidate conserved regions were determined using pairwise and multiple sequence alignments.

Conserved region	Location	Length (a.a.)
1	51-58*	8
2	74-80	7
3	100-107	8
4	137-142	6
5	171-179*	9
6	195-203	9
7	211-217	8

* The chosen candidate conserved regions for primer design.

Primer Selection

The *PolG*91F and *PolG*91R primers were designed to amplify 384 bp within the polyhedrin/granulin sequences. Sequences of the *PolG*91F and *PolG*91R primers and the base count of the respective viral DNAs are shown in Tables (3 and 4). Degenerate sites were considered as low base by base frequency was produced by multiple alignment of the candidate regions. The primers were selected on the basis of having relatively low levels of degeneracy. The bases represented in lower than 5% in the base count were not considered in designing the primers (Tables 3 and 4). To compensate for the primer multiplicity, a slightly higher primer concentration (50 pmol per 25 µl reaction) was used in the PCR.

Table 3: Sequence and base counts of the forward primer based on the first candidate conserved region of polyhedrin/granulin sequences. Base count less than 5% was neglected in primer design.

Base count	Forward primer (5`---3`)																			
	V	G	G	D	C	C	B	A	A	R	A	Y	G	K	R	A	C	G	G	C
C	136	153	152		155	157	60		3		1	91	2	2		1	156	4	4	162
G	13	4	4	71	4	1	89	4	3	86	1	1	158	46	66	1	4	156	156	
A	15	3	4	56	3	3		160	154	78	160	2	1	1	98	161	1	2		2
T		4	4	37	2	3	13		4		2	70	3	115		1	3	2	1	

V= G, A or C, D= A, T or G, B= C, G or T, R= A or G, Y= C or T, K= G or T.

Table 4: Sequence and base counts of the reverse primer based on the fifth candidate conserved region of polyhedrin/granulin sequences. Base count less than 5% was neglected in primer design.

Base count	Reverse primer (5`---3`)																	
	C	D	B	T	T	S	K	G	T	A	Y	M	R	D	A	C	G	G
C	159	4	54	4		90		1	1	2	93	121		4		160	3	
G	1	56	43	2		67	117	158	1	1	3	2	65	66	2	1	161	163
A	2	43	2		1	3		3	1	161	4	41	98	43	159	1		1
T	2	61	65	158	163	4	47	2	159		64		1	51	1			

D= A, T or G, B= C, G or T, S= C or G, K= G or T, Y= C or T, M= C or A, R= A or G.

Experimental verification

Two degenerate primers were designed to anneal within the *orf* of polyhedrin/granulin sequence (*PolG*91F and *PolG*91R). The degenerate PCR primer set successfully amplified the expected polyhedrin/granulin DNA fragment (384 bp) from the *Ac*MNPV, *Bm*NPV, *Ld*NPV, *Spli*NPV as well as from *Spli*GV, *Pr*GV, GV_{G213} and GV_{F115} isolates. Few non-specific amplification products were observed for the tested viruses (Fig. 1). Four randomly chosen PCR products (2 NPV and 2 GV) were cloned into *p*GEM-T vector and sequenced using the universal M_{13} primers and using *PolG*91F and *PolG*91R, as well. The sequencing results showed that the four PCR products were fragments of polyhedrin and granulin genes showing a high percentage of similarity (up to 85 %).

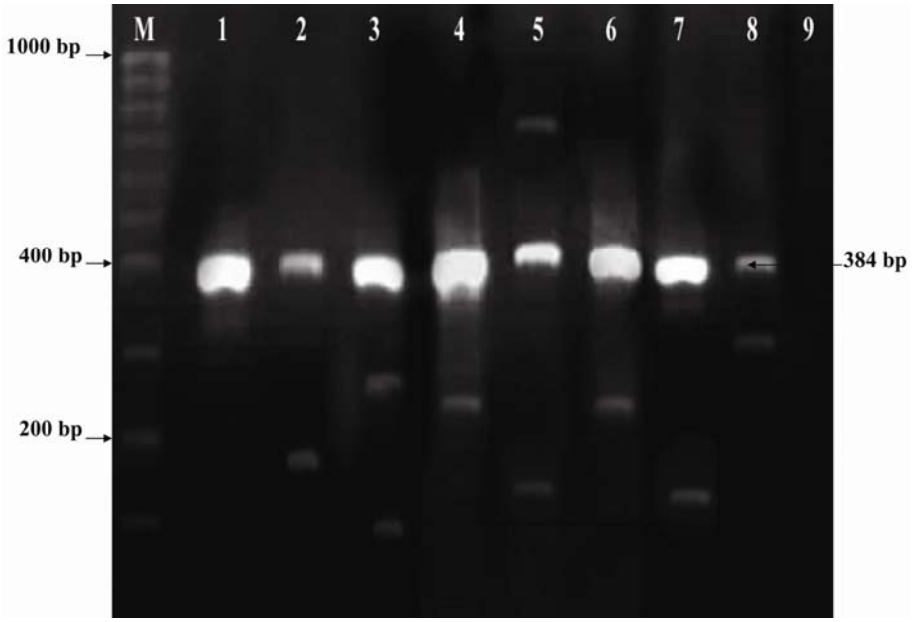

Fig. (1): PCR results showing the amplification of ~384 bp fragment in the polyhedrin/ granulin genes coding region of eight baculovirus isolates. M: 100 bp ladder DNA marker, lanes 1: *Ac*MNPV, 2: *Bm*NPV, 3: *Ld*NPV, 4: *Spli*NPV. Lanes 5-8: *Spli*GV, *Pr*GV, GV_{G213} and GV_{F115}, respectively. Lane 9: Negative control (PCR mix without template DNA). The size of the bands is shown in bp.

DISCUSSION AND CONCLUSION

Polyhedrin and granulin genes of NPVs and GVs encode for the matrix protein of the virus occlusion body and are considered of the most conserved baculovirus genes (Jehle, 2004). These genes were proved to be the most suitable genes in baculoviruses for developing generic amplification techniques (Woo, 2001). Seufi (2008) characterized a highly conserved polyhedrin region of 405 bp molecular size. He reported significant alignment with 11 GV granulins. In addition, lepidopteran polyhedrin genes show about a 50% amino acid identity with granulovirus granulins, and a 40% identity with hymenopteran NPV polyhedrin (Rohrmann, 1992). These high similarities could enhance the strategy to design universal primers for detection of both NPV and GV-infections. The advantage of such approach is that it utilized all NPV and GV polyhedrin/granulin sequences available in the international databases (91 and 73 sequences) and simple public software programs to select optimal candidate regions for PCR amplification. Such approach is unlikely to produce significant bias towards any one species, especially if there is no bias in the multiple sequence alignment, on which the approach was based. These primers make it possible to efficiently amplify DNA from many NPV and GV species. It also allowed further search for novel NPV and GV isolates. Many published reports that investigated polyhedrin gene depended primarily on a Southern hybridization using probes of the polyhedrin gene of other previously identified viruses. However, this technique is efficient only if the similarity between polyhedrin genes of the target NPV and probe NPV is high. Therefore, many limitations will arise when the study based mainly on Southern technique. One major limitation is that this technique requires multiple probes of various NPVs for detection of baculovirus. Also, traditional serological methods based on neutralization and fixed cell ELISA have proven to be effective for identifying baculoviruses (Brown *et al*., 1982). However, difficulties in interpreting antigenic cross reactivity and/ or failure to identify

relatively close antigenic relationships were common complains in this technology. Moreover, serological techniques are time consuming, require highly experienced personnel and are less precise than nucleotide sequence determination. Generally, the use of PCR technology for virus detection, identification and characterization is a basic tool in many virology laboratories (Moraes and Maruniak, 1997, Moraes *et al*., 1999, Wang *et al*., 2000, Christian *et al*., 2001, Moraes and Maruniak, 2001, Woo, 2001, Ikuno *et al*., 2004, Lange *et al*., 2004, Jehle *et al*., 2006, Murillo *et al*., 2006, Kundu *et al.*, 2008, Manzán *et al.*, 2008, Galal, 2009, Hewson *et al.*, 2011, Ravikumar *et al.*, 2011, Arneodo *et al.*, 2012). Indeed, a good set of primers for simultaneous NPV and GV detection is a powerful tool for large *Baculovirus* sample screening. PCR technique is preferred because it is easy, fast, sensitive and reliable, as well. In addition, very small amount of DNA is needed, and it does not utilize radioactive materials. Many attempts to detect NPV from soil and insects have been made using PCR techniques, but it was limited to narrow NPV species (Webb *et al*., 1991, Moraes and Maruniak, 1997, Moraes *et al*., 1999). Woo (2001) designed a pair of degenerate primers to detect multiple NPVs using PCR. One major problem with degenerate primers is that the concentration of some permutations in the mixture is so small that amplification is effectively inhibited (due to their great multiplicity). It was believed that the redundancy of *PolG*91F and *PolG*91R primers was insufficient to cause this problem. In the progress of this issue, Ikuno *et al.* (2004) applied new PCR protocol for evaluating and monitoring *Bombyx mori* NPV-infection, and Ravikumar *et al.* (2011) developed a multiplex polymerase chain reaction for the simultaneous detection of microsporidians, nucleopolyhedrovirus, and densovirus infecting silkworms. In the same time, Hewson *et al.* (2011) reported the detection of NPV in terrestrial and marine habitats using PCR. On the other hand, Kundu *et al.* (2003) and Kundu *et al.* (2008) developed a PCR protocol to detect the GVs of *Adoxophyes orana* and *Cydia pomonella* in their hosts. Manzán *et al.* (2008) developed a multiplex PCR protocol for the quality control of *Epinotia aporema* granulovirus production. Recently, Arneodo *et al.* (2012) developed a real-time PCR approach for detection and and kinetic analysis of *Epinotia aporema* granulovirus in its host.

Up to date, no reports were published on the development of PCR protocol that can detect NPV and GV simultaneously. The capacity of *PolG*91F and *PolG*91R primers to efficiently amplify all tested NPVs and GVs made them an invaluable diagnostic and taxonomic tool for virology. The ability of these primers to amplify DNA from local isolates of GVs may demonstrate their capacity to define novel NPVs and GVs species. **In summary**, the PCR primer set employed in this study was chosen from highly conserved regions within the polyhedrin/granulin region. Therefore, the possibility of amplification of multiple *Baculovirus* species was more enhanced. The present study introduced a highly sensitive method for multiple and simultaneous *Baculovirus* detection. Higher sensitivity and cost-efficiency enabled the researcher to identify the structure of the polyhedrin and granulin genes rapidly. The amplification of highly specific and abundant products obtained in this study suggested that this method might be useful to detect both NPVs and GVs with very low amounts of DNA in the environment.

Conclusively, the method described in this paper is considered universal, powerful, and could be used in the future to study the environmental fate of wild type or genetically modified recombinant *Baculovirus*. It may be useful in quality

control studies of *Baculovirus* insecticides, as well.

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ARABIC SUMMARY

تصميم زوج من البادئات الانحلالية للكشف المتزامن عن الفيروس النووى البوليهدروسى و الفيروس الحبيبى باستخدام تفاعل البلمرة المتسلسل

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استهدفت هذه الدراسة تصميم زوج من البادئات الانحلالية للكشف المتزامن عن الفيروس النووى البوليهدروسى والفيروس الحبيبى باستخدام تفاعل البلمرة المتسلسل ، لقد تم تطوير تقنية تفاعل البلمرة المتسلسل وتمت مقارنة 91 و 73 تتابعا للأحماض الأمينية لجينات البوليهيدرين و الجرانيولين المنشورة في قواعد البيانات العالمية باستخدام برامج كمبيوتر متخصصة .وتم تحديد سبع مناطق جينية تمثل تتابعات نيوكلبوتيدية شديدة التشابه فى منطقة الترميز من جينات البوليهيدرين و الجرانيولين موضع الدراسة. وبناء عليه فقد تم اختيار اثنتين من المناطق الجينية المرشحة لتصميم زوج من البادئات لإنتاج قطعة من الدنا طولها حوالى 384 زوجا من القواعد النيتروجينية .وقد تم اختبار ثمانية أنواع مختلفة من الباكيولوفيروسات المعرفة عالميا (4 فيروسات نووية بوليهيدرسية و 4 فيروسات حبيبية)، و علاوة على ذلك، فقد تم اختيار أربعة من نواتج تفاعل البلمرة المتسلسل للكشف عما إذا كانت تتابعاتها النيتروجينية تشبه جينى البوليهيدرين و الجرانيولين أم لا ، وأظهرت النتائج أن التتابعات المختارة هى أجزاء من تتابعات جينى البوليهيدرين و الجرانيولين موضع الاختبار .و قد خلصت الدراسة إلى أن هذه التقنية ستكون مفيدة في رصد انتشار و توزيع الباكيلوفيروسات فى الطبيعة ، وذلك فى حالات إطلاق هذه الفيرروسات ، سواء أنواعها البرية أو تلك المهندسة وراثيا ، كما يمكن الاستفادة منها فى اختبارات الجودة للمبيدات الحيوية الفيروسة.