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Review Article

BIOEFFICACY AND GENOMIC CHARACTERIZATION OF BACULOVIRUSES

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ABSTRACT
Among microbial insecticides, insect pathogenic viruses known as baculoviruses (BVs) are an important component of Integrated Pest Management (IPM) approach in several field and horticultural crops. BVs are found ubiquitously in the environment and more than 600 have been isolated from insect species so far. Several BVs are commercially produced for biological control of lepidopteran insect pests. BVs are classified based on structure and mode of action into two groups: Nucleopolyhedrovirus (NPVs) and granulovirus (GVs). Bioefficacy studies of granuloviruses include several techniques such as diet contamination, leaf disc bioassay and droplet feeding to estimate median lethal dose and time. Histopathological studies to elucidate invasion pathway in insect host led to categorization of 3 types of GVs based on tissue tropisim and speed of kill. Baculovirus genomes possibly encode for 89 to 183 predicted ORFs in both strands and genome sequencing improved the understanding of the molecular biology of these viruses and gene functions determining host range, virulence and phylogeny. This review focuses on bioefficacy, biochemical and molecular aspects and phylogenetic studies of GVs.

KEY WORDS: Baculovirus, Granulovirus, *Achaea janata*, Semilooper, Bioefficacy, Genome, Pathogenicity.

INTRODUCTION

Baculoviruses (*nucleopolyhedrovirus*, NPVs granulovirus, GVs) are ubiquitous and naturally occurring in the environment. These viruses have been isolated from insect species in the orders: Lepidoptera (604 species), Diptera (27 species), Hymenoptera (31 species), and Coleoptera (5 species) (Adams and McClintock, 1991; Couch, 1974; Martignoni and Iwai, 1986). Most of the BVs especially GVs have been isolated from Lepidoptera (Murphy *et al*., 1995). As a group, NPVs have a wider host range (456 species) than GVs (150 species) (Crook, 1991). The NPVs of *Autographa californica* (*AcMNPV*) and *Anagrapha falcifera* (*AfMNPV*) exhibit a broad host range. *AcMNPV* infects more than 30 species from about 10 insect families, all within the order Lepidoptera, *Mamestra Brassicae* MNPV (*MbMNPV)* was found to infect 32 out of 66 tested Lepidopteran species from 4 different families (Groner, 1986; Doyle *et al*., 1990; Hostetter and Puttler, 1991). In case of GVs, the host range appears to be restricted to a fairly small number of species within the same family as the original host (Groner, 1986). However, at least three GVs isolated from *Cydia pomonella*, *Helicoverpa armigera*, and *Scotogramma trifolii* appear to have a moderately broad host range and cause infection in a number of species from several genera (Crook, 1991). However, homologous viruses are more infective to the host insect than heterologous viruses as demonstrated in the case of *CpGV* to *C. pomonella* neonate larvae which was more infective than the broad-spectrum and cross-infective *AcMNPV* and *AfMNPV* (Lacey *et al*., 2002).

Insect host: *Achaea janata* (Lepidoptera: *Noctuidae*)

Among different pests that attack the castor crop (*Ricinus communis* L.), *Achaea janata* (Linnaeus) popularly known as castor semilooper, is a voracious feeder that causes extensive defoliation (Fig. 1) (Parthasarathy and Rao, 1989). It is widely distributed in the Indian sub-continent, Australia, New Zealand, Thailand, Malaysia, Philippines and Indonesia. At times of severe infestation larvae also feed on developing capsules resulting in massive crop loss (Singh *et al*., 1992). For obvious reasons chemical insecticides have been used extensively to control *Achaea janata*, but now gradually this is becoming difficult due to development of resistance. Therefore, in recent years there is an increased emphasis on developing more effective yet eco-friendly and safer methods of pest control.

Pest status on castor

Semilooper is a polyphagous defoliator and is a major pest on Castor. However, a wide range of hosts have been recorded from a variety of habitats in different parts of India (Nayar *et al*., 1976; Gaikwad and Bilapate, 1992). Many plants including weeds, shrubs and trees in the family Euphorbiaceae, in which castor is included, are hosts of *A. janata* (Ismail and Salim, 1982; Whitten and Damanik, 1986; Gaikwad and Bilapate, 1992). Other secondary hosts for *A. janata* reported are: rose (*Rosa indica*) (Vyas, 1994), tamarind, mustard, Chinese cabbage, sugarcane, tomato as well as some other legumes, teas, other Brassica species (John and *Muraleedharan*, 1989). *A. janata* larvae have been found defoliating ornamental garden plants such as *Quisqualis indica* and *Dodonea viscosa* (Kavadia and Verma, 1973), even if castor was

available. Adult moths of *A. janata* were found to feed on the fruits of guava (Gaikwad and Bilapate, 1992) and pomegranate (Mote *et al*., 1991).

FIGURE 1: (A) Semilooper larva feeding on Castor (B) Damaged castor field.

Semilooper occurs in 5-6 overlapping generations during the crop season. It commonly occurs between August and January, but severe damage to castor crop occurs from July to September. Castor farmers resort to delayed sowings to avoid seedling damage by key pests, semilooper and red hairy caterpillar; even though such a practice results in harvesting lower seed yield. The complete life cycle of castor semilooper from egg to adult takes place in 48-50 days (French, 1950) and its populations fluctuate in abundance and seasonal occurrence from year to year. Its developmental stages include eggs (2-5 days), five to six larval instars (11-15 days), pupa (10-14 days, but may be prolonged to few months under winter conditions) and adult (1-25 days). Females start laying eggs 2 to 5 days after emerging from the pupa and lay an average of 1305 eggs during their lifetime (Karmawati and Tobing, 1988). Eggs are laid during the night since the moths are nocturnal. The second and third generations of *A. janata* on castor are important and cause serious defoliation if unchecked (Prasad *et al*., 2010). The pest causes extensive defoliation in castor during its peak vegetative growth phase (30-75 days after sowing) and also feeds on tender capsules in developing spikes (Parthasarathy and Rao, 1989). The duration of the pupal stage is influenced by temperature with warmer temperatures shortening the development time (Muthukrishman and Pandian, 1984).

Research on insect viruses

Among the various BVs, extensive research attention has been given to nucleopolyhedrvirus (NPVs) of *Spodoptera litura* and *Helicoverpa armigera*. Research on nature of viral diseases, physico-chemical properties, bioassay, production, mammalian pathogenicity tests, field efficacy compatibility to adjuvants for increasing efficacy and UV protectants for increasing field persistence have been carried out on these two BVs. A significant contribution towards the development of *S. litura* NPV as a microbial agent is from the Division of Entomology, IARI, New Delhi (Ramakrishnan and Tiwari, 1969). Tamil Nadu Agricultural University has successfully tested NPVs in field trials against the *H. armigera*, tobacco cutworm and the red hairy caterpillar (Jayaraj 1989; Rabindra *et al*., 1991). Work on granuloviruses (GVs) in India is on GV infecting sugarcane shoot borer, internode borer; castor semilooper GV (Vimala Devi and Prasad, 1997), diamond back moth GV on crucifers. Effective field use of GVs has been reported on castor (Prabhakar *et al*., 2003). At present, the following NPVs have been registered with CIB for commercialization: NPV of *Helicovepa armigera* (Aqueous suspension, AS) and NPV of *Spodoptera litura* (0.5% AS).

Bioefficacy

Quantitative dose-mortality relationships of BVs are measured in a number ways depending on the purpose of the assay. Death of the organism confirmed through microscopic examination to ascertain the cause is the most commonly used response. However, it may not always be easy to determine precisely when the insects have died and hence the virus treated insects are to be examined as frequently as possible (daily time interval or less) (Hunter-Fujita *et al*., 1998). Infectivity of BVs is through the oral route i.e., occlusion bodies need to be ingested by larvae. Susceptible life stages of insects (mostly early larval instars) are exposed to the virus through contaminated food (natural plant part such as leaf or artificial diet) either by surface contamination or diet incorporation technique (Hatem *et al*., 2012; Muthamia *et al*., 2011). Another method which has been used in insect bioassays is the droplet feeding technique where larvae are allowed to 'drink' known volume of virus (Hughes and Wood, 1981; Hilton and winstanely, 2007). Irrespective of the method of virus administration, wherever precise dosage ingestion is determined the end point is expressed as the median lethal dose (LD_{50}) (Table-1) and where it is not possible to determine the precise dosage ingested the end point is the median lethal concentration (LC_{50}) (Table-2).

Another attribute determining the bioefficacy of a BV is the time it takes to kill its host which is expressed as median survival time (ST_{50}) and is estimated by daily monitoring of virus administered larvae until all the larvae are either dead or pupated. LC_{50} and LD_{50} values are determined through probit analysis (Finney, 1971), while ST_{50} value is calculated from life tables (Kalbfleisch and Prentice, 1980; Subramanian *et al*., 2008).

TABLE 1: Estimated median lethal dose (LD_{50}) and survival time (ST_{50}) for wild type BVs

Virus / Strain	Larval stage	Bioassay method	LD_{50} (OBs/larva)	ST_{50} (days)	Reference
CpGV	Neonate	Droplet feeding	1-5	-	Payne, 1986
AsGV	Neonate	Diet contamination	10000	-	Allaway and Payne, 1984
AdorGV	Neonate	Droplet feeding	31	37*	Hilton and winstanley, 2007
AdorGV	Fourth	Droplet feeding	6000	28.8*	Hilton and Winstanley, 2007
SpliGV	1st, 3rd and 5th	Diet contamination	3.74×10^4, 6.86×10^5, and 1×10^7	28-16	Hatem *et al*., 2012
SpliGV	Third	Leaf disc	$5.6x10^5$ and $1.4x10^8$	3-6	Hatem *et al*., 2012
SpliNPV	Third	Leaf disc	2.56 and 63.9	3-5	Hatem *et al*., 2012
PxGV F4 and I5	Second	Diet contamination	7169-7649 OBs/mm^2	5-7	Muthamia *et al*.,2011
PhopGV-CR1	Neonate	Potato tubers	17.9-69.1 OBs/mm^2	-	Gomez-Bonilla *et al*., 2011

*LD_{80} dose, '-' not estimated.

TABLE 2: Estimated median lethal concentration (LC_{50}) and survival time (ST_{50}) for wild type BVs

Virus / Strain	Larval stage	Bioassay method	LC_{50} (OBs/mm$^{2)}$	ST_{50} (days)	Reference
SlGV	Fourth instar	Diet contamination	1740	14	Subramanian *et al*., 2005
PxGV	Early third	Leaf disc	3-6	4.5-5	Subramanian *et al*., 2008
PxGV	Late third	Leaf disc	6-11	4.5-5.5	Subramanian *et al*., 2008
PoGV-FR and CR	Neonates	Potato tubers	17-18	-	Gomez-Bonilla *et al*., 2011
PoGV VG001-5	Neonates	Potato tubers	0.8-26.2	-	Espinel-Correal *et al*., 2010
PapyGV	Neonates	Leaf disc	38-274	7-10	Unruh *et al*., 2012
	Second	Leaf disc	813-139,487	7-10	Unruh *et al*., 2012

'-' not estimated

Bacterial contaminant profile in virus preparations and formulations

In vivo virus propagation invariably leads to contamination with a variety of bacteria and fungi (Podgewaite *et al*., 1983). Since the virus preparation is done by homogenization of the whole infected larva, many chances prevail for the presence of the gut bacteria in the virus sample as observed in the case of *Spodoptera littoralis* NPV, *Lymantria dispar* NPV and *Cydia pomenella* GV (McKinley *et al*., 1989, Podgwaite *et al*., 1983; Huber, 1985). These viruses were produced under temperate conditions and it is reasonably anticipated that production in a tropical situation would involve greater microbiological contamination problems. Recent published data concerning the bacterial flora of microbial pesticides produced from insects are limited, but a number of main subgroups may be distinguished. The gut microflora of insectary-reared host insects comprises a more limited range of species of wild type. Some dominant contaminants in insect virus preparations are the fecal *Enterococci: Enteroccus faecalis, E. faecium,* and *E. faecium var. casseliflavius.* These have been widely reported as common inhabitants in the guts of insects, including a number of other Lepidopteran larvae (Eaves and Mundt, 1960; Martin and Mundt, 1972), gypsy moth (Doane and Redys, 1970), turnip moth (Charpentier *et al*., 1978), as well as the desert locust *Schistocerca gregaria* (Hunt and Charnley, 1981). Opportunistic saprophytes are found on diet, frass and colonizing larval cadavers which include various members of the genera *Bacillus*, *Alcaligens*, *Flavobacterium*, *Micrococcus* and *Pseudomonas*. Guidelines have been developed for the production and marketing of microbial pesticides by the government under the Insecticide act, 1968. According to the Act, human pathogenic strains such as S*almonella, Shigella, Vibrio etc*., should be completely absence and presence of other bacterial is limited up to a load of 1×10^4 ml^{-1} or g^{-1} formulation.

Formulations of microbial biopesticides such as *Bacillus thuringiensis* (Bt) and insect viruses are designed to retain biological activity of the active ingredient, to prevent the in-storage replication of any contaminant microorganisms (largely bacteria and fungi) and to ease handling of the product (Hunter-Fujita, 1998). Formulations of insect viruses are primarily either dry or wet preparations. Dry formulations such as dusts and granules in sealed containers ensure unaltered contamination levels during storage. However, in wet preparations or liquid formulations such as flowable and settling concentrates, maintenance of an acceptable concentration of cells of contaminant microorganisms is more difficult (Boyette *et al*., 1991). This problem is accentuated if virus is produced '*in vivo*' i.e., in insects cultured on plant tissue such as leaf. Selective removal of contaminating bacteria in wet preparations is possible through centrifugation. The microbial load in liquid formulations can also be minimized by lowering the pH ($\leq$4.0) which suppresses replication of many microorganisms without loss of biological activity of virus and also with the addition of food grade, disinfecting chemical substances such as sorbic acid and potassium sorbate (Prasad *et al*., 2010).

Pathogenicity

Several GVs have been discovered since the first discovery in 1936 (Paillot). Most studies are focused on establishing the efficacy of the virus and environmental factors affecting its activity. However, histopathological studies elucidating the invasion pathway have not been carried out for all the known GVs. Broadly, GVs have

been categorized into three types depending on the tissues infected (Federici, 1993; Tanada and Hess, 1991) (Table-3). Type I GVs (*e.g*., *Trichoplusia ni* GV) invade the host larvae through the midgut epithelium, much like the typical NPV, but subsequently only infect the fat body tissue (Federici, 1993). Because other important tissues are not attacked, the larva may live around 10 - 14 days post infection in the fourth instar, which is as much as a week longer than a larva of the same stage infected with a similar amount of NPV. These larvae typically maintain their appetites and grow much larger than healthy larvae, only becoming lethargic within a day or two before death. After infection, larvae become markedly swollen, developing a creamy yellow appearance, due to the accumulation of large number of infected cells packed with viral granules in the fat body. Typically, there is no liquefaction of the body, as the epidermis is not infected. Larvae typically turn dark brown or black, desiccate or disintegrate because the body is invaded by gut flora (Federici, 1997).

In the Type II GVs (*e.g*. *Cydia pomenella* GV), viral infection and gross pathology parallel that of a typical lepidopteran NPV disease. After invasion of the midgut, the virus attacks most of the major body tissues, including the tracheal matrix, epidermis, and fat body (Huger, 1963; Tanada and Kaya, 1993). This disease is more acute than that caused by Type I GV, typically lasting only 5 - 10 days in larvae infected during the fourth instar (Federici, 1997). Infected larvae swell and distend slightly, developing irregular white to yellow patches below the cuticle. After death, the body liquefies.

Type III GVs solely represented by *Harrisina brillians* GV is characterized by tissue tropism restricted to the midgut epithelium, producing virions and OBs in both larvae and adults (Federici and Stern, 1990). The disease is acute and larvae infected in the third or fourth instar usually die within 4 - 7 days. The pathogenic cycle of GVs is very similar to that of NPVs and most of the information obtained for the replication and cytopathology of GVs has been from *in vivo* studies using electron microscopy. The initial stages of host infection appear to be similar for both NPVs and GVs (Fedrici, 1997) and later stages differ in tissue tropism, cytopathology and histopathology. Studies on pathogenesis of GVs which are much smaller in size compared to NPVs are limited mainly due to lag in culturing GVs in cell culture (Federici, 1997). Primarily, infection with GVs takes place by ingestion of virus contaminated food by larvae of the host insect (Crook, 1991). The ingested OB protein matrix of GVs is dissolved by prevailing high alkaline midgut pH of >9.0 (Summers, 1971; Winstanley and O'Reilly, 1999), and the liberated enveloped virions attach and fuse to the plasma membrane of the microvilli of the columnar midgut cells. The virion particles enter the cells, migrate to the nucleus and attach to the nuclear pores within 2 - 6 h after infection (Tanada and Kaya, 1993). The uncoating of the nucleic acid occurs at a nucleopore (or within a nucleus in the case of NPV) and DNA is injected into the nucleus (Summers, 1971).

TABLE 3: GV types based on tissue tropism and speed of kill

Category	Virus	Insect	Reference
Type I	*SpliGV*	*Spodoptera littoralis*	Hatem *et al*., 2012
	XcGV	*Xestia c-nigrum*	Wang *et al*., 2008
	TnGV	*Trichoplusia ni*	Wang *et al*., 2008
	AoGV	*Adoxophyes orana*	Winstanley and O'Reilly, 1999
	HearGV	*Helicoverpa armigera*	Harrison and Popham, 2008
Type II	*PsunGV*	*Pseudaletia unipuncta*	Ferrelli *et al*., 2012
	PhopGV	*Phthorimaea operculella*	Lacey *et al*., 2011
	HycuGV	*Hyphantria cunea*	Watanabe and Kobayashi, 1970
	PiGV	*Plodia interpuctella*	Arnott and Smith, 1968
	CpGV	*Cydia pomonella*	Tanada and Leutenegger, 1968
	EpapGV	*Epinotia aporema*	Goldberg *et al*., 2002
	PxGV	*Plutella xylostella*	Hashimoto *et al*., 2000
	ClGV	*Cryptophlebia leucotreta*	Lange and Jehle, 2003
	PrGV	*Pieris rapae*	Zhang *et al*., 2012
Type III	*HbGV*	*Harrisina brillians*	Bideshi *et al*., 2000

Virogenesis begins in the nucleus with the formation of the virogenic stroma (Tanada and Kaya, 1993). Capsids appear in 6 - 12 hours post infection and are incorporated with the viral nucleoprotein core. Offspring nucleocapsids are formed in 12 - 18 hours post infection in regions of densely aggregated material distinct from host chromatin. Shortly after the appearance of the nucleocapsids, the nuclear envelope breaks down and virogenesis continues in the nucleus and the cytoplasm in case of GVs (Lacey *et al*., 2011) unlike in NPVs where the OBs are localized in the nucleus. The encasement of the nucleocapsids and their occlusion in capsules also occurs in the nucleus and the cytoplasm. About 24 hours post infection, enveloped and unenveloped nucleocapsids may occur in continuous rows in intercellular spaces between midgut cells and near the basement membrane. In the final stage, many nucleocapsids are embedded in the basement membrane or bud through the membrane and thereby acquire envelopes with peplomers. Budded virions move from the midgut epithelium into the hemocoel, where the secondary infection takes place (Tanada and Kaya, 1993). The infection of the fat body is by viropexis of nucleocapsids with peplomer envelopes. OBs are released into the environment when the insect dies and disintegrates. Horizontal transmission (larva to larva) of the virus can now occur, and the replication cycle continues.

Baculovirus genome structure and organization

Baculoviruses have circular, ds DNA genomes ranging in size from approximately 80 – 180 kbp in size (Lange and Jehle, 2003). Since the first complete sequence of a baculoviral genome *AcMNPV* was reported (Ayres *et al*., 1994), many BV genomes were sequenced to further

improve the understanding of the molecular biology of these viruses. To date, there are 60 fully sequenced baculoviral genomes available in GenBank. Out of 60, forty one belong to the *Alphabaculovirus* genus, fifteen to the *Betabaculovirus*, three to the *Gammabaculovirus* and one to the *Deltabaculovirus* (Table 4). Baculovirus genomes possibly encode for 89 to 183 predicted ORFs in both strands, apparently with no preferred orientation. In general, BV genomes have low GC content (<50%).

As most of sequenced genomes belong to BVs specific to lepidopteran insects (*Alpha* and *Betabaculovirus*), there is a good deal of information to characterize a set of genes associated with specificity for Lepidoptera. Likewise, there are some *Betabaculovirus* specific genes, not found in NPVs, which may be implicated in the differential pathogenesis displayed by these viruses. It is worth noting that GVs (*Beta*-GVs) are not well studied as *Alpha*- NPVs at the molecular level because of the lack of susceptible insect cell lines. One GV-specific gene characterized at the functional level is a metalloproteinase of *Xestia c-nigrum* GV (*XcGV)* which has orthologs in all GVs (Ko *et al*., 2000). The wide variation in the size of BV genomes indicates that some BVs may lack many of the genes present in other members of the family (Possee and Rohrmann, 1997). The size of *AcMNPV*- C6 isolate of the NPV genus is 133.894 kbp and it codes for 156 open reading frames (ORFs) (Ayres *et al*., 1994), while *CpGV* is 123.5 kbp and codes for 143 ORFs. About 82 % of the *CpGV* ORFs are homologous to genes previously found in other BVs (Luque *et al*., 2001). Complete genome sequences of both *CpGV* and *AcMNPV* have been generated which illustrate the genetic complexity of these virus families. A novel feature of many BV genomes is the presence of homologous regions (*hrs*) that are located throughout the genome. The homologous regions are composed of repeated sequences consisting both direct repeats and imperfect palindromic sequences (Possee and Rhormann, 1997).

Genome size of all BVs share 31 core genes in common (Miele *et al*., 2011). These are essential genes involved in oral infection (*pif-0 (p74)*, *pif-1*, *pif-2*, *pif-3*, *pif-4/19kd/odv-e28*, *pif-5/odv-e56*), cell cycle arrest (*odv-ec27*, *ac81*), replication (*dnapolymerase*, *helicase*, *lef-1*, *lef-2*), late gene transcription (*lef-4*, *lef-5*, *lef-8*, *lef-9*, *p47*) and virus assembly, packaging and release (*38k/ac98*, *alk-exo*, *desmoplakin*, *gp41*, *odv-e18*, *odv-nc42*, *odv-ec43*, *p6.9*, *p33/ac92*, *p49*, *vlf-1*, *vp39*, *vp91*, *vp1054*) (Miele *et al*., 2011).

TABLE 4: Fully sequenced BV genomes

Genus	Name	Abbrevation	Accession Number	Genome (bp)	No. of ORF	Reference
Alphabaculovirus Group I	*Antheraea pernyi* NPV-Z	AnpeNPV	DQ486030	126629	147	Nie *et al*., 2007
	Antheraea pernyi NPV-	AnpeNPV	EF207986	126246	145	Fan *et al*., 2007
	Anticarsia gemmatalis	AgMNPV	DQ813662	132239	152	Oliveira *et al*., 2006
	Autographa californica	AcMNPV	L22858	133894	156	Ayres *et al*., 1994
	Bombyx mandarina NPV	BomanNPV	NC012672	126770	141	Xu *et al*., 2009, (U)
	Bombyx mori NPV T3	BmNPV	L33180	128413	143	Gomi *et al*., 1999
	Choristoneura	CfDefNPV	AY327402	131160	149	Lauzon *et al*., 2005
	Choristoneura	CfMNPV	AF512031	129593	146	de Jong *et al*., 2005
	Epiphyas postvittana	EppoNPV	AY043265	118584	136	Hyink *et al*., 2002
	Hyphantria cunea NPV	HycuNPV	AP009046	132959	148	Ikeda *et al*., 2006
	Maruca vitrata MNPV	MaviMNPV	EF125867	111953	126	Chen *et al*., 2008
	Orgyia pseudotsugata	OpMNPV	U75930	131995	152	Ahrens *et al*., 1997
	Plutella xylostella	PlxyMNPV	DQ457003	134417	152	Harrison and Lynn, 2007
	Rachiplusia ou MNPV	RoMNPV	AY145471	131526	149	Harrison and Bonning, 2003
	Adoxophyes honmai	AdhoNPV	AP006270	113220	125	Nakai *et al*., 2003
	Adoxophyes orana NPV	AdorNPV	EU591746	111724	121	Hilton and Winstanley, 2008
	Agrotis ipsilon MNPV	AgipMNPV	EU839994	155122	163	Harrison, 2009
	Agrotis segetum NPV	AgseNPV	DQ123841	147544	153	Jakubowska *et al*., 2006
	Apocheima cinerarium	ApciNPV	FJ914221	123876	118	Zhang *et al*., (U)
	Chrysodeixis chalcites	ChchNPV	AY864330	149622	151	van Oers *et al*., 2005
	Clanis bilineata NPV	ClbiNPV	DQ504428	135454	139	Zhu *et al*., 2009
	Ecotropis obliqua NPV	EcobNPV	DQ837165	131204	126	Ma *et al*., 2007
	Euproctis	EupsNPV	NC_012639	141291	139	Tang *et al*., 2009
Alphabaculovirus Group II	*Helicoverpa armigera*	HearMNPV	EU730893	154196	162	Tang *et al*., 2008,(U)
	Helicoverpa armigera NPV	HearSNPV	AF303045	130759	137	Zhang *et al*., 2005a
	Helicoverpa armigera NPV	HearSNPV	AF271059	131405	135	Chen *et al*., 2001
	Helicoverpa armigera	HearSNPV	AP010907	132425	143	Ogembo *et al*., 2009
	Helicoverpa zea SNPV	HzSNPV	AF334030	130869	139	Chen *et al*., 2002
	Leucania separata NPV	LeseNPV	AY394490	168041	169	Xiao and Qi, 2007
	Lymantria dispar NPV	LdMNPV	AF081810	161046	164	Kuzio *et al*., 1999
	Lymantria xylina MNPV	LyxyMNPV	GQ202541	156344	157	Nai *et al*., 2010
	Mamestra configurata	MacoNPV A-90-	U59461	155060	169	Li *et al*., 2002b
	Mamestra configurata	MacoNPV A-90-	AF539999	153656	168	Li *et al*., 2005
	Mamestra configurata	MacoNPV B	AY126275	158482	168	Li *et al*., 2002a
	Orgyia leucostigma NPV CSF-77	OrleNPV	EU309041	156179	135	Eveleigh *et al*., 2008, (U) unpublished
	Spodoptera exigua NPV	SeMNPV	AF169823	135611	139	Ijkel *et al*., 1999
	Spodoptera frugiperda	SfMNPV 19	EU258200	132565	141	Wolff *et al*., 2008
	Spodoptera frugiperda	SfMNPV 3AP2	EF035042	131330	142	Harrison *et al*., 2008
	Spodoptera litura NPV	SpltMNPV	AF325155	139342	141	Pang *et al*., 2001
	Spodoptera litura NPV II	SpltNPV-II	EU780426	148634	147	Li *et al*., 2008 (U)
	Trichoplusia ni SNPV	TnSNPV	DQ017380	134394	145	Willis *et al*., 2005

Betabaculovirus	*Adoxophyes orana* GV	AdorGV	AF547984	99657	119	Wormleaton *et al*., 2003
	Agrotis segetum GV	AgseGV	AY522332	131680	132	Xiulian *et al*., 2004, (U)
	Choristoneura occidentalis	ChocGV	DQ333351	104710	116	Escasa *et al*., 2006
	Clostera anachoreta GV	ClanGV	HQ116624	101487	123	Liang *et al*., 2011
	Cryptophlebia leucotreta	CrleGV	AY229987	110907	128	Lange and Jehle, 2003
	Cydia pomonella GV	CpGV	U53466	123500	143	Luque *et al*., 2001
	Helicoverpa armigera	HearGV	EU255577	169794	179	Harrison and Popham, 2008
	Phthorimaea operculella	PhopGV	AF499596	119217	130	Croizier *et al*., 2002 (U)
	Pieris rapae GV	PrGV	NC_013797	108592	120	Zhang *et al*., 2010 (U)
	Plutella xylostella GV	PlxyGV K1	AF270937	100999	120	Hashimoto *et al*., 2000
	Pseudelatia unipuncta	PsunGV	EU678671	176677	183	Li *et al*., 2008 (U)
	Spodoptera litura GV K1	SpltGV K1	DQ288858	124121	136	Wang *et al*., 2011
	Xestia c-nigrum GV	XecnGV	AF162221	178733	181	Hayakawa *et al*., 1999
	Epinotia aporemaGV	EpapGV	JN408834	119082	133	Ferrelli *et al*., 2012
	Erinnyis ello GV	ErelGV	KJ406702	102759	130	Ardisson-Araujo *et al*., 2014
Gamma	*Neodiprion abietis* NPV	NeabNPV	DQ317692	84264	93	Duffy *et al*., 2006
	Neodiprion sertifer NPV	NeseNPV	AY430810	86462	90	Garcia-Maruniak *et al*., 2004
	Neodiprion lecontei NPV	NeleNPV	AY349019	81755	89	Lauzon *et al*., 2004
Delta	*Culex nigripalpus* NPV	CuniNPV	AF403738	108252	109	Afonso *et al*., 2001

ORF- open reading frame, U- unpublished

Molecular characterization of baculoviruses

Molecular level identification, characterization and evaluation of phylogenetic status of a particular BV are also important for establishment of purity of seed mother stock. BVs (GVs and NPVs) cannot be distinguished to species level from light or electron microscopic studies. Microscopy and serological tools are not reliable for identification of a given sample or isolate and are not particularly helpful in providing clues about the host range and infectivity (Rovesti *et al*., 2000). To identify the GVs and NPVs we need to look at the DNA sequence using restriction endonuclease analysis or molecular probes, which offer a relatively simple method for identification and differentiation of BVs (Smith and summer, 1978).

Identification and molecular characterization of *granulin* / *polyhedrin* Gene

Granulin and polyhedrin are the major component of the large protein capsule occluding the virions in case of GVs and NPVs. The polyhedrin gene of *AcMNPV* was first reported by Vlak and Smith (1982) after determining its nucleotide sequence. This protein comprises of about 245 to 250 amino acids, and appears to be the most highly conserved BV proteins. These characteristics led to the use of granulin or polyhedrin sequences as the basis for BV phylogenetic studies (Zanotto *et al*., 1993).

A restriction digestion fragment obtained from genome of the *Pieris brassicae* GV (*PbGV*) containing the gene coding for granulin protein, was cloned and sequenced along with some of its 5' and 3' flanking sequences and the primary protein structure of granulin protein predicted from the nucleotide sequence (Chakerian *et al*., 1985). Similarly, the granulin gene of *Cryptophlebia leucotreta* GV (*ClGV*) (Jehle and Backhaus, 1994) and *Harrisina brilliance* (*HbGV)* were identified (Bideshi *et al*. 2000).

A polymerase chain reaction (PCR) based detection system was developed for identification of granulin gene by using degenerate primers generated on the basis of published *Trichiplusia ni* GV (*TnGV*) and *Pieris brassicae* GV (*PbGV*) granulin gene sequences (Bah *et al*., 1997). The granulin gene of *Spodoptera litura* GV (*SlGV*) (750 bp) was identified by using PCR degenerate primers (Wang *et al*., 2008). Similarly, PCR based RFLP was developed for rapid identification and differentiation of *CpGV* isolates from the environment by amplifying the 460 bp fragment from granulin gene using degenerate primers (Karolin *et al*., 2009).

Baculoviruses produce virion occluded within this crystalline matrix of occlusion body (Jacques, 1975; Rohrmann, 1986, 1992). This occlusion body protein stabilizes virions for long time survival outside host after release into the environment. The occlusion body protein gene is highly expressed during the very late phase of virus infection (Rohrmann 1992; Funk *et al*., 1997). Inside the host, the high pH encountered in the midgut of a susceptible insect (Arella *et al*., 1988; Fossiez *et al*., 1989; Yuen *et al*., 1990) is responsible for release of virions after dissolution of the occlusion protein.

***Per os* infectivity factor genes**

Per os infectivity factors (PIFs) are BV proteins essential for oral infection of insect hosts but not relevant in cell culture propagation. Six proteins have been described to play this role and are encoded by 6 core genes *p74*, *pif-1*, *pif-2*, *pif-3*, *pif-4* and *pif-5* (*odv-e56*). PIF-1, PIF-2 and PIF-3 form a stable complex on the surface of *AcMNPV occlusion derived virus* (ODV) in association with P74. It was proposed that these four proteins form an evolutionarily conserved complex on ODV surface that may play an essential role in the initial stage of infection (Peng *et al*., 2010). PIF-4 was found to be essential for oral infection of *AcMNPV* in *Trichoplusia ni* larvae (Fang *et al*., 2009).In recent studies ODV-E56 was demonstrated to be a *PIF* (PIF-5) in *AcMNPV* (Sparks *et al*. 2011) and *mNPV* (Xiang *et al*., 2011). Only few ODV proteins have been shown to be essential for the *AcMNPV per os* infection process (Braunagel *et al*., 1996; Sparks *et al*., 2011; Xiang *et al*., 2011).

Late expression factor genes

In the late stages of the infection, additional genes are implicated in virus transcription. Viral RNA polymerase is made of four subunits coded by four core genes: *lef-4*, *lef-8*, *lef-9* and *p47* (Guarino *et al*., 1998). *Lef-8* and *lef-9* have motifs common to the largest subunits of bacterial and eukaryotic RNA polymerases. *Lef-8* contains the essential C-terminal region conserved in RNA polymerases, while the rest of the polypeptide shows no sequence homology to other known RNA polymerases. *Lef-9* contains the Mg2+ binding site of the catalytic centre found in other RNA polymerases. LEF-4 is an RNA

capping enzyme and P47 does not show homology with other RNA polymerase subunits (Van Oers and Vlak, 2007).

Cathepsin and chitinase

GV cathepsin is a cysteine protease involved in degradation of host tissues and cuticle to facilitate progeny virus release (Kang *et al*., 1998). The *chitinase* gene of BVs is also implicated in virus release from host cadavers for dissemination into the environment (Possee and Rohrmann, 1997). This liquefaction is mediated by two viral-encoded enzymes: cathepsin and chitinase. Cathepsin is a protease that acts together with chitinase disrupting the insect exoskeleton and promotes the release and spread of progeny virus (Hawtin *et al*., 1997). More than 100 sequences of the papain superfamily have been described in a wide range of organisms (Berti and Storer, 1995) including a number of papain-like cysteine proteases in BVs (Rawlings *et al*., 1992; Ohkawa *et al*. 1994; Slack *et al*., 1995). Deletion of either the cathepsin (*cath*) or *chiA* genes results in failure of the virus to cause liquefaction of the host, indicating that the proteins function together to promote degradation of host tissues at the end of the infection process (Ohkawa *et al*., 1994; Slack *et al*., 1995; Hawtin *et al*., 1997; Suzuki *et al*., 1997).

Although many BVs encode a cathepsin-like proteinase, in *AcMNPV* (Ac127) it was most active under acidic (pH 5) conditions (Slack *et al*., 1995). Therefore, the proteinases associated with occlusion bodies are likely to be a combination of enzymes derived from bacteria, the insect gut, and the virus. *SlGV* does not contain either a chitinase or a cathepsin gene. It appears that BVs encode these enzymes to aid breakdown of insect tissues at the end of infection to release OBs into the environment and thereby aid their horizontal spread. *SlGV*-infected larvae do not lyse at the end of infection. The cadavers of *S. litura* larvae infected by *SlGV* appeared smaller than normal larvae, as if they had lost much water, and they were very soft (Wang *et al*., 2011).

Phylogeny of baculoviruses

A BV phylogenetic tree was constructed based on individual gene sequences with the most widely used granulin/ polyhedrin gene (Bideshi *et al*., 2000; Cowan *et al*., 1994). Other genes have also been used for construction of phylogenetic tree such as DNA polymerase (*dpol*), *egt*, *gp41*, *chitinase*, *cathepsin*, *lef* and *gp37*(Herniou *et al*., 2003). Most phylogetic studies agree that the lepidopteran NPVs and GVs constitute distinct well defined groups and similarly NPVs can be subdivided into two subgroups: subgroup-I and subgroup-II NPVs. In particular, polyhedrin phylogenies often disagree with other gene phylogenies (Clarke *et al*., 1996). To avoid the problem with single-gene trees, complete genome sequences have been used for BV phylogenies. Several other methods have been discovered to take advantage of complete genome sequences. Genomes contain multiple levels of phylogenetic information. In addition to the nucleotide and amino acid sequences, complete genomes also contain structural information such as the order of genes or gene composition (Koonin *et al*., 2000). An advantage of evaluating evolution on a genomic scale is that the inferences are based on the complete genetic makeup of species.

Phylogeny construction is based on either nucleotide or amino acid sequences and utility is greatly extended by the ability to use all genes common between the genomes of interests. There are two types of approaches (i) to analyze each gene separately and derive a consensus from the resulting phylogenies (ii) to concatenate the gene sequences and analyze them together. The second approach gives better results because each gene contributes to the overall phylogenetic signal and a synergistic effect is produced by the combination of all the signals. This approach has been proved useful in construction of phylogenetic trees of metazoans based on mitochondrial genomes (Nikaido *et al*., 2001). Phylogenetic trees were constructed based on the translated amino acid sequences of the 30 common genes present in all BVs and also reconstructed based on each single amino acid sequences. However each single gene gave a different tree suggesting weak individual data sets and it is likely that combining data sets would reinforce the signal (Herinou *et al*., 2003).

SUMMARY

The protocols for propagation, extraction, purification and bioefficacy of GV which infects castor semilooper (*Achaea janata*) have been improved. The post inoculation at different time intervals and invasion process through TEM revealed a sequential step in its oral infection pathway. Restriction endonuclease analysis (REN) of *AjGV* DNA profile can serve as an authentic identification tool for *AjGV* mother culture. A PCR protocol was standardized using degenerated primers to amplify and isolate full length granulin gene which encodes for the major occlusion body protein (granulin) of *AjGV*. Thirty core set of genes from *AjGV* whole genome sequence were used for establishing homology and phylogenetic relationship with other GVs. Although a detailed knowledge of the molecular biology of this virus is still required, the present study sets the foundation for genetic improvement of *AjGV* isolate and its successful identification as a biological control agent of *Achaea janata*.

REFERENCES

Adams, J.R. and McClintock, J.T. (1991) Baculoviridae, nuclear polyhedrosis viruses. Part 1: nuclear polyhedrosis viruses of insects; in Atlas of Invertebrate Viruses. Adams JR, Bonami JR (eds). pp 87-180, CRC Press, Boca Raton, FL, USA.

Afonso C.L., Tulman, E.R., Lu, Z., Balinsky, C.A., Moser, B.A., Becnel, J.J., Rock, D.L. and Kutish, G.F. (2001) Genome sequence of a baculovirus pathogenic for *Culex nigripalpus.* J Virol. 75, 11157-11165.

Ahrens, C.H., Russell, R.L., Funk, C.J., Evans, J.T., Harwood, S.H. and Rohrmann, G.F. (1997) The sequence of the *Orgyia pseudotsugata* multi nucleocapsid nuclear polyhedrosis virus genome. Virology 229, 381-399.

Allaway, G.P. and Payne, C.C. (1984) Host range of five baculoviruses for lepidopterous hosts. Ann Appl Biol. 105, 29-37.

Ardisson-Araújo, D.M.P, de Melo, F.L., Souza Andrade, M.S., Sihler, W., Báo, S.N., Ribeiro, B.M., de Souza, M.L. (2014). Genome sequence of *Erinnyis ello* granulovirus (ErelGV), a natural cassava hornworm pesticide and the first sequenced sphingid-infecting betabaculovirus, BMC Genomics 2014, 15:856.

Arella, M., Lavalle, C., Belloncik, S and Furuichi, Y. (1988) Molecular cloning and characterization of cytoplasmic polyhedrosis virus polyhedrin and a viable deletion mutant gene. J Virol 62, 211–217.

Arnott, H.J. and Smith, K.M. (1968) An ultrastructural study of the development of a granulosis virus in the cells of the moth Plodia interpunctella (Hbn.). J Ultrastruc Res. 21, 251-258.

Ayres, M.D., Howard, S.C., Kuzio, J., Lopez-Ferber, M. and Posses, R.D. (1994) The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. Virology 202, 586-605.

Bah, A., Bergeron, J., Arella, M., Lucarotti, C.J. and Guertin, C. (1997) Identification and sequence analyses of the granulin gene of *Choristoneura fumiferana* granulovirus. Arch Virol. 142, 1577-1584.

Berti, P.J. and Storer, A.C. (1995) Alignment / phylogeny of the pepain superfamily of cysteine proteases. J Mol Biol. 246, 273-283.

Bideshi, D.K., Bigot, Y. and Federici, B.A. (2000) Molecular characterization and phylogenetic analysis of the *Harrisina brillians* granulovirus granulin gene. Arch Virol. 145, 1933-1945.

Boytte, C.D., Quimby, P.C., Connick, W.J., Daigle, D.J. and Fulgham, F.E. (1991) Progress in the production, formulation and application of mycoherbicides. In: TeBeest DO (ed) Microbial Control of Weeds, Chapman and Hall, New York, pp 209-224.

Braunagel, S.C., Elton, D.M., Ma, H. and Summers, M.D. (1996) Identification and analysis of an *Autographa californica* nuclear polyhedrosis virus structural protein of the occlusion-derived virus envelope: ODV-E56. J Virol, 217: 97–110.

Chakerian, R., Rohrmann, G.F., Nesson, M.H., Leisy, D.J. and Beaudreau, G.S. (1985) The Nucleotide Sequence of the *Pieris brassicae* Granulosis Virus Granulin Gene. J Gen Virol. 66, 1263-1269.

Charpentier, R., Charpentier, B. and Zethner, O. (1978) The bacterial flora of the midgut of two populations of healthy 5th instar larvae of the turnip moth, *Scotia segetum.* J Invertibr Pathol. 32, 59–63.

Chen, X., WF, I.J., Tarchini, R., Sun, X., Sandbrink, H., Wang, H., Peters, S., Zuidema, D., Lankhorst, R.K., Vlak, J.M. and Hu, Z. (2001) The sequence of the *Helicoverpa armigera* single nucleopolyhedrovirus genome. J Gen Virol. 82, 241-257.

Chen, X., Zhang, W.J., Wong, J., Chun, G., Lu, A., McCutchen, B.F., Presnail, J.K., Herrmann, R., Dolan, M., Tingey, S., Hu, Z.H. and Vlak, J.M. (2002) Comparative analysis of thecomplete genome sequences of *Helicoverpa zea* and *Helicoverpa armigera* single nucleopolyhedroviruses. J Gen Virol. 83, 673-684.

Chen, Y.R., Wu, C.Y., Lee, S.T., Wu, Y.J., Lo, C.F., Tsai, M.F. and Wang, C.H. (2008) Genomic and host range studies of *Maruca vitrata* nucleopolyhedrovirus. J Gen Virol. 9, 2315-2330.

Clarke, E.E., Tristem, M., Cory, J.S. and O'Reilly, D.R. (1996) Characterizationof the ecdysteroid UDP-glucosyltransferase gene from *Mamestra brassicae* nucleopoly hedrosis virus. J Gen Virol. 77, 2865-2871.

Couch, J.A. (1974) Free and occluded virus similar to baculoviruses in hepatopancreas of pink shrimp. Nature 247, 229-231.

Cowan, P., Bulach, D., Goodge, K., Robertson, A. and Tribe, D.E. (1994) Nucleotide sequence of the polyhedrin gene region of *Helicoverpa armigera* nucleocapsid: placement of the virus in lepidopteran nuclear polyhedrosis virus group II. J Gen Virol. 75, 3211-18.

Crook, N.E. (1991) Baculoviridae: Subgroup B. Comparative aspects of granulosis viruses; in Viruses of Invertebrates, Kurstak, E. (ed.), pp 73-110, Marcel Dekker Inc., New York.

de Jong, J.G., Lauzon, H.A., Dominy, C., Poloumienko, A., Carstens, E.B., Arifm B.M. and Krell, P.J. (2005) Analysis of the *Choristoneura fumiferana* nucleopolyhedrovirus genome. J Gen Virol. 86, 929-943.

Doane, C.C. and Redys, J.J. (1970) Characteristics of a motile strain of *Streptococcus faecalis* pathogenic to larvae of the gypsy moth. J Invertebr Pathol. 15, 420–430.

Doyle, C.J., Hirst, M.L., Cory, J.S. and Entwistle, P.F. (1990) Risk assessment studies: detailed host range testing of wild-type cabbage moth, *Mamestra brassicae* (Lepidoptera: Noctuidae), nuclear polyhedrosis virus. Appl Environ Microbiol. 56, 2704–2710.

Duffy, S.P., Young, A.M., Morin, B., Lucarotti, C.J., Koop, B.F. and Levin, D.B. (2006) Sequence analysis and organization of the *Neodiprion abietis* nucleopolyhedrovirus genome. J Virol. 80, 6952-6963.

Eaves, G.E., Mundt, J.O. (1960) Distribution and characterization of *Streptococci* from insects. J Invertibr Pathol. 2, 289–298

Eberle, K.E., Sayed, S., Rezapanah, M., Shojai-Estabragh, S. and Jehle, J.A. (2009) Diversity and evolution of the *Cydia pomonella* granuloviurs. J Gen Virol. 90, 662-671.

Escasa, S.R., Lauzon, H.A., Mathur, A.C., Krell, P.J. and Arif, B.M. (2006) Sequence analysis of the *Choristoneura occidentalis* granulovirus genome. J Gen Virol. 87, 1917-1933.

Espinel-Correal, C., Xavier Lery, Laura Villamizar, Juliana Gomez, Jean Louis Zeddam, Alba Marina Cotes, Miguel Lopez-Ferber (2010) Genetic and Biological Analysis of Colombian *Phthorimaea operculella* Granulovirus Isolated from *Tecia solanivora* (Lepidoptera: Gelechiidae). Appl Environ Microbiol. 7617- 7625.

Fan, Q., Li, S., Wang, L., Zhang, B., Ye, B., Zhao, Z. and Cui, L. (2007) The genome sequence of the multi nucleocapsid nucleopolyhedrovirus of the Chinese oak silkworm *Antheraea pernyi*. Virology 366(2), 304-315.

Fang, M., Nie, Y., Harris. S., Erlandson, M.A. and Theilmann, D.A. (2009) *Autographa californica* multiple nucleopolyhedrovirus core gene ac96 encodes a *per os* infectivity factor (*pif*-4). J Virol. 83, 12569–12578.

Federici, B.A. (1993) Viral pathobiology in relation to insect control; in Parasites and pathogens of insects, Vol 2, Pathogens. Beckglage, N.E., Thompson, S.N. and Federici, B.A. (eds.). pp 81-101, Academic Press, San Diego.

Federici, B.A. (1997) Baculovirus pathogenesis; in The baculoviruses. Miller LK (ed), pp 33-35, Plenum Press, New York.

Federici, B.A. and Stern, V.M. (1990) Replication and Occlusion of granulosis virus in larval and adult midgut epithelium of the western grapeleaf skeletionizer, *Harrissina brillians*, J Invertibr Pathol. 56 (3), 401-414.

Ferrelli, M.L., Ricardo Salvador, Marina Elizabeth Biedma, Marcelo Facundo Berretta, Santiago Haase, Alicia Sciocco-Cap, Pablo Daniel Ghiringhelli, Víctor Romanowski (2012) Genome of *Epinotia aporema* granulovirus (EpapGV), a polyorganotropic fast killing betabaculovirus with a novel thymidylate kinase gene. BMC Genomics 13, 548.

Finney, D.J. (1971) Probit analysis, 3rd Eds. Cambridge University Press, Cambridge.

Fossiez, F., Belloncik, S., Arella, M. (1989) Nucleotide sequence of the polyhedrin gene of *Euxoa scandens cytoplasmic polyhedrosis virus* (*EsCPV*). Virology. 169, 462–465.

French, E.W. (1950) A Study of the Biology and Morphology of *Achaea janata* (Linn.), a Cotocaline Moth, as It Occurs in Hawaii. M.S. Thesis. University of Hawaii.

Funk, C.J., Braunagel, S.C. and Rohrmann, G.F. (1997) Baculovirus Structure; in The Baculoviruses, Miller, L.K. (ed.). pp 7-32, Plenum Press, New York.

Gaikwad, B.B. and Bilapate, G.G. (1992) Castor semilooper, *Achaea janata* L. in India - a review. Agricultural Reviews (Karnal) 13, 51-58.

Garcia-Maruniak, A., Maruniak, J.E., Zanotto, P.M., Doumbouya, A.E., Liu, J.C., Merritt, T.M. and Lanoie, J.S. (2004) Sequence analysis of the genome of the *Neodiprion sertifer* nucleopolyhedrovirus. J Virol. 78,7036-7051.

Goldberg, A.V., Romanowski, V., Federici, B.A. and Sciocco de Cap, A. (2002) Effects of the Epap granulovirus on its host, *Epinotia aporema* (Lepidoptera: Tortricidae). J Invertebr Pathol. 80(3), 148–159.

Gomez-Bonilla, Y., Lopez-Ferber, M., Caballero, P., Léry Xavier, Munoz, D. (2011) Characterization of a Costa Rican granulovirus strain highly pathogenic against its indigenous hosts, *Phthorimaea operculella* and *Tecia solanivora*. Entomol Exp Appl. 140 (3), 238-246.

Gomi, S., Majima, K. and Maeda, S. (1999) Sequence analysis of the genome of *Bombyx mori* nucleopolyhedrovirus. J Gen Virol. 80, 1323-1337.

Groner, A. (1986) Specificity and safety of baculoviruses; in The Biology of Baculoviruses. Granados IRR, Federici BA (eds.). pp 177-202, CRC Press, Boca Raton, FL.

Guarino, L.A., Xu, B., Jin, J. and Dong, W. (1998) A virus-encoded RNA polymerase purified from baculovirus-infected cells. J Virol. 72. 7985-7991.

Harrison, R.L. (2009) Genomic sequence analysis of the Illinois strain of the *Agrotis ipsilon* multiple nucleopolyhedrovirus. Virus Genes 38, 155-170.

Harrison, R.L., Bonning, B.C. (2003) Comparative analysis of the genomes of *Rachiplusia ou* and *Autographa californica* multiple nucleopolyhedroviruses. J Gen Virol. 84, 1827-1842.

Harrison, R.L. and Lynn, D.E. (2007) Genomic sequence analysis of a nucleopoly-hedrovirus isolated from the diamondback moth, *Plutella xylostella*. Virus Genes 35, 857-873.

Harrison, R.L. and Popham, H.J. (2008) Genomic sequence analysis of a granulovirus isolated from the Old World bollworm, *Helicoverpa armigera*. Virus Genes 36 (3), 565–581.

Hashimoto, Y., Hayakawa, T., Ueno, Y., Fujita, T. and Sano Y, Matsumoto T (2000) Sequence analysis of the *Plutella xylostella* granulovirus genome. Virology 275(2), 358–372.

Hatem, A.E., Shalaby, H., Fargalla, F.H. and Vargas-Osuna, E. (2012) Combination effects of *Spodoptera littoralis* nuclear polyhedrosis and granulous virus against larvae of the cotton leafworm. World Rural Observations 4(4), 10-16.

Hawtin, R.E., Zarkowska, T., Arnold, K., Thomas, C.J., Gooday, G.W., King, L.A., Kuzio, J.A. and Possee, R.D. (1997) Liquefaction of *Autographa californica* nucleopolyhedrovirus-infected insects is dependent on the integrity of virus-encoded chitinase and cathepsin genes. Virology 238, 243-253.

Hayakawa, T., Ko, R., Okano, K., Seong, S.I., Goto, C. and Maeda, S. (1999) Sequence analysis of the *Xestia c-nigrum* granulovirus genome.Virology 262 (2), 277–297.

Herniou, E.A., Olszewski, J.A., Cory, J.S., O'Reilly, D.R. (2003) The Genome Sequence and Evolution of Baculoviruses. Annu Rev Entomol. 48, 211-234.

Hilton, S. and Winstanley, D. (2007) Identification and functional analysis of the origins of DNA replication in the *Cydia pomonella* granulovirus genome. J Gen Virol. 88, 1496-1504.

Hilton, S. and Winstanley, D. (2008) Genomic sequence and biological characterization of a nucleopolyhedrovirus isolated from the summer fruit tortrix, *Adoxophyes orana*. J Gen Virol. 89, 2898-2908.

Hostetter, D.L. and Puttler, B. (1991) A new broad host spectrum nuclear polyhedrosis virus isolated from a celery looper, *Anagrapha falcifera* (Kirby), (Lepidoptera: Noctuidae). Environ Entomol. 20(5), 1480-1488.

Huber, J. (1985) Progress in the production of codling moth granulosis virus: Comparison of several virus preparations. Production and Application of Bio-Pesticides on a Viral Basis in Orchards and Vegetables, Expert Meeting, Monfavet, France, 5–7 July 1985,Commission of European Communities, General Directorate Agriculture.

Huger, A. (1963) Granules of insects; in Insect pathology ASteinhaus E (ed.). vol.1. p.531-575 Academic Press, Inc., Newyork.

Hughes, P.R. and Wood, H.A. (1981) A synchronous per oral technique for the bioassay of insect viruses. J Invertibr Pathol. 37, 154-159.

Hunt, J. and Charnley, A.K. (1981) Abundance and distribution of the gut flora of the desert locust, *Schistocerca gregaria*. J Invertibr Pathol. 38, 378–385.

Hunter-Fujita, F.R., Entwistle, P.F., Evans, H.F. and Crook NE (1998) Insect Viruses and Pest Management. Chichester: Wiley.

Hyink, O., Dellow, R.A., Olsen, M.J., Caradoc-Davies, K.M., Drake, K., Herniou, E.A., Cory, J.S., O'Reilly, D.R. and Ward, V.K. (2002) Whole genome analysis of the *Epiphyas postvittana* nucleopolyhedrovirus. J Gen Virol. 83, 957-971.

Ijkel, W.F.J., van Strien, E.A., Heldens, J.G., Broer, R., Zuidema, D., Goldbach, R.W. and Vlak, J.M. (1999) Sequence and organization of the *Spodoptera exigua* multicapsid nucleopolyhedrovirus genome. J Gen Virol 80 (12): 3289-3304.

Ikeda, M., Shikata, M., Shirata, N., Chaeychomsri, S., Kobayashi, M. (2006) Gene organization and complete sequence of the *Hyphantria cunea* nucleopolyhedrovirus genome. J Gen Virol. 87, 2549-2562.

Ismail, A. and Salim, J. An outbreak and some notes of the noctuid *Achaea janata* L., on *Exoecaria agallocha* in Hutan Melintang, Perak. MAPPS Newsletter, 1982, 6(2), 2-3.

Jacques, R.P. (1975) Persistence, accumulation and denaturation of Nuclear polyhedrosis and granulosis viruses; in Baculoviruses for insect pest control: safety considerations. Summers, M.D., Engler, R., Falcon, L.A., Vail, P.V. (eds.). pp 90–99, American Society of Microbiology, Washington, DC.

Jakubowska, A.K., Peters, S.A., Ziemnicka, J., Vlak, J.M. and van Oers, M.M. (2006) Genome sequence of an enhancin gene-rich nucleopolyhedrovirus (NPV) from *Agrotis segetum*: collinearity with *Spodoptera exigua* multiple NPV. J Gen Virol. 87, 537-551.

Jayaraj, S. Pest Control: Advances in Biological means. The Hindu Survey of Indian Agriculture Special Issue. Published by the Hindu, Madras, India, 1989, pp 181-187

Jehle, J.A. and Backhaus, H. (1994) The granulin gene region of *Cryptophlebia leucotreta* granulosis virus: sequence analysis and phylogenetic considerations. J Gen Virol. 75, 3667-3671.

John, A. and Muraleedharan, D. (1989) Biology and Morphometrics of Castor Semilooper, *Achoea janata* Linn. (Lepidoptera: Noctuidae). Uttar Pradesh J Zool. 9(1), 48-55.

Kalbfleisch, J.D. and Prentice, R.L. (1980). The Statistical Analysis of Failure Time Data. Wiley, New York, NY.

Kang, W., Tristem, M., Maeda, S., Crook, N.E. and O'Reilly, D.R. (1998) Identification and characterization of the *Cydia pomonella* granulovirus cathepsin and chitinase genes. J GenVirol. 79, 2283–2292.

Karmawati, E. and Tobing, S.L. (1988) Laboratory Biology of *Achaea janata* L. Castor Large Semi-Loopers. Industrial Crops Res J. 1(1), 37-42.

Kavadia, V.S. and Verma, S.K. (1973) Quisqualis-indica and Dodonea-viscosa as new hosts of castor semilooper Achoea-janata. JBNHS 70, 226-227.

Ko, R., Okano, K. and Maeda, S. (2000) Structural and functional analysis of the *Xestia cnigrum* granulovirus matrix metalloproteinase. J Virol. 74, 11240-11246

Koonin, E.V., Aravind, L. and Kondrashov, A.S. (2000) The impact of comparative genomics on our understanding of evolution. Cell 101, 573-576.

Kuzio, J., Pearson, M.N., Harwood, S.H., Funk, C.J., Evans, J.T., Slavicek, J.M. and Rohrmann, G.F. (1999) Sequence and analysis of the genome of a baculovirus pathogenic for *Lymantria dispar*. Virology 253, 17-34.

Lacey, L.A., Hoffmann, D.F. and Federici, B.A. (2011) Histopathology and effect on development of the PhopGV on larvae of the potato tubermoth, *Phthorimaea*

operculella (Lepidoptera: Gelechiidae). J Invertibr Pathlo. 108(1), 52-55.

Lacey, L.A., Vail, P.V. and Hoffmann, D.F. (2002) Comparative activity of baculoviruses against the codling moth *Cydia pomonella* and three other tortricid pests of tree fruit. J Invertebr Pathol. 80, 64–68.

Lange, M. and Jehle JA (2003) The genome of the *Cryptophlebia leucotreta* granulovirus. Virology 317(2), 220–236.

Lauzon, H.A., Jamieson, P.B., Krell, P.J. and Arif, B.M. (2005) Gene organization and sequencing of the *Choristoneura fumiferana* defective nucleopolyhedrovirus genome. J Gen Virol. 86, 945-961.

Lauzon, H.A., Lucarotti, C.J., Krell, P.J., Feng, Q., Retnakaran, A. and Arif, B.M. (2004) Sequence and organization of the *Neodiprion lecontei* nucleopolyhedrovirus genome. J Virol. 78, 7023-7035.

Li, L., Donly, C., Li, Q., Willis, L.G., Keddie, B.A., Erlandson, M.A. and Theilmann, D.A. (2002a) Identification and genomic analysis of a second species of nucleopolyhedrovirus isolated from *Mamestra configurata*. Virology 297, 226-244.

Li, L., Li, Q., Willis, L.G., Erlandson, M., Theilmann, D.A. and Donly, C. (2005) Complete comparative genomic analysis of two field isolates of *Mamestra configurata* nucleopolyhedrovirus-A. J Gen Virol. 86, 91-105.

Li, Q., Donly, C., Li, L., Willis, L.G., Theilmann, D.A. and Erlandson, M. (2002b) Sequence and organization of the *Mamestra configurata* nucleopolyhedrovirus genome. Virology. 294, 106-121.

Liang, Z., Zhang, X., Yin, X., Cao, S. and Xu, F. (2011) Genomic sequencing and analysis of *Clostera anachoreta* granulovirus. Arch Virol. 156, 1185-1198.

Luque, T., Finch, R., Crook, N., O'Reilly, D.R. and Wistanely, D. (2001) The complete sequence of *Cydia pomonella granulovirus* genome. J Gen Virol. 82, 2531-2547.

Ma, X.C., Shang, J.Y., Yang, Z.N., Bao, Y.Y., Xiao, Q. and Zhang, C.X. (2007) Genome sequence and organization of a nucleopolyhedrovirus that infects the tea looper caterpillar, *Ectropis obliqua*. Virology 360, 235-246.

Martignoni, M.E., Iwai, P.J. (1986) A catalogue of Viral Diseases of Insects, Mites and Ticks, (4th ed.) General technical Report PNW-195 US Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR.

Martin, J.D. and Mundt, J.O. (1972) *Enterococci* in insects. Appl Microbiol. 24, 575–580.

McKinely, D.J., Moawad, G., Jones, K., Grzywacz, D. and Turner, C. (1989) The development of nuclear polyhedrosis virus for control of *Spodoptera littoralis* (Boisd.) in cotton; in Pest Management in Cotton. Green, M.B., de, B. and Lyon, D.J. (eds.). pp 93-100, Chichester: Ellis Horwood.

Miele, S.A.B., Garavaglia, M.J., Belaich, M.N. and Ghiringhelli, P.D. (2011) Baculovirus: molecular insights on their diversity and conservation. Int J Evolutio Biol. 15 pages.

Mote, U.N., Tambe, A.B. and Patil, C.S. (1991) Observation on incidence and extent of damage of fruit sucking moths on pomegranate fruits. Journal of Maharashtra Agriculture University 16 (3), 438-439.

Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A., Summers, M.D. (eds) (1995) Virus Taxonomy, Sixth Report of the International Committee on Taxonomy of Viruses, New York

Muthamia, E.K., Ogada, P.A., Mukunzu, M.J., Baya, J.M., van Beek NAM, W., esonga, J.M. and Ateka, E.M. 2011. Chacterization of *Plutella xylostella* granulovirus (PlxyGV) Isolates for the management of diamond back moth in Kenya. Afr. J. Horticult. Sci. 4, 19-23.

Muthukrishman, J. and Pandian, T.J. (1984) Effects of Interaction of Ration and Temperature on growth and Bioenergetics of *Achaea janata* Linnaeus (Lepidoptera: Noctuidae). Oecologia. 62(2), 272-278.

Nai, Y.S., Wu, C.Y., Wang, T.C., Chen, Y.R., Lau, W.H., Lo, C.F., Tsai, M.F. and Wang, C.H. (2010) Genomic sequencing and analyses of *Lymantria xylina* multiple nucleopolyhedrovirus. BMC Genomics. 11, 116.

Nakai, M., Goto, C., Kang, W., Shikata, M., Luque, T. and Kunimi, Y. (2003) Genome sequence and organization of a nucleopolyhedrovirus isolated from the smaller tea totrix, *Adoxophyes honmai.* Virology 316, 171-183.

Nayar, K.K., Ananthakrishnan, T.N. and David, B.V. (1976) General and applied entomology. Tata McGraw Hill Company Ltd., New Delhi. pp 590.

Nie, Z.M., Zhang, Z.F., Wang, D., He, P.A., Jiang, C.Y., Song, L., Chen, F., Xu, J., Yang, L., Yu, L.L., Chen, J., Lv, Z.B., Lu, J.J., Wu, X.F. and Zhang, Y.Z. (2007) Complete sequence and organization of *Antheraea pernyi* nucleopolyhedrovirus, a dr-rich baculovirus. BMC Genomics. 8, 248.

Nikaido, M., Kawai, K., Cao, Y., Harada, M. and Tomita, S. (2001) Maximum likelihood analysis of the complete mitochondrial genomes of eutherians and a reevaluation of the phylogeny of bats and insectivores. J Mol Evol. 53, 508-516.

Ogembo, J.G., Caoili, B.L., Shikata, M., Chaeychomsri, S., Kobayashi, M. and Ikeda, M. (2009) Comparative genomic sequence analysis of novel *Helicoverpa armigera*

nucleopolyhedrovirus (NPV) isolated from Kenya and three other previously sequenced *Helicoverpa* spp. NPVs. Virus Genes 39, 261-272.

Ohkawa, T., Majima, K. and Maeda, S. (1994) A Cysteine protease encoded by the baculovirus *Bombyx mori* nuclear polyhedrosis virus. J Virol. 68, 6619-6625.

Oliveira, J.V., Wolff, J.L., Garcia-Maruniak, A., Ribeiro, B.M., de Castro, M.E., de Souza, M.L., Moscardi, F., Maruniak, J.E., Zanotto, P.M. (2006) Genome of the most widely used viral biopesticide: *Anticarsia gemmatalis* multiple nucleopolyhedrovirus. J Gen Virol. 87, 3233-3250.

Pang, Y., Yu, J., Wang, L., Hu, X., Bao, W., Li, G., Chen, C., Han, H., Hu, S. and Yang, H. (2001) Sequence analysis of the *Spodoptera litura* multicapsid nucleopolyhedrovirus genome. Virology 287: 391-404.

Parthasarathy, S. and Rao, A.P. (1989) Chemical control of castor semilooper *Achaea janata.* Journal of Oilseeds Research 6, 158-161.

Payne, C.C. (1986) Insect pathogenic viruses as pest control agents. Forts Zool. 32, 183-200.

Peng, K., van Oers, M.M., Hu, Z., van Lent, J.W. and Vlak, J.M. (2010) Baculovirus per os infectivity factors form a complex on the surface of occlusion-derived virus. J Virol. 84, 9497-9504.

Podgewaite, J.D., Bruen, R.B. and Shapiro, M. (1983) Micro-organisms associated with production lots of the nuclear polyhedrosis virus of the gyspy moth *Lymantria dispar*. Entomophaga. 28, 9-16.

Possee, R.D. and Rohrmann, G.F. (1997) Baculovirus genome Organisation and Evolution; in The Baculoviruses. Miller, L. (ed.). Plenum Press, New York.

Prabhakar, M., Srinivasa Rao, M. and Prasad, Y.G. (2003) Evalution of bio-intensive integrated pest management modules for castor semilooper. *Achaea janata* Lin. Indian J Plant Prot. 31(1), 56-58.

Prasad, Y.G., Prabhakar, M., Phanidhara, A., Naveen Kumar, P. (2010) Development of *Achaea janata* granulosis virus formulation for use a viral biopesticide in the management of semilooper on castor. Journal of Oilseeds Research 27 (special issue), 352-354.

Prasad, Y.G., Srinivas, L. and Vimala Devi, P.S., 2001. A note of granuloviurs infection in *Achaea janata* Linnaeus. *Journal oilseeds Research* (short communication). 18(2), 285-286.

Rabindra, R.J., Balasaraswathy, S. and Jayaraj, S. (1991) Combined use of NPV with certain botanicals for the control of *Heliothis armigera* on chickpea. J Biol Control. 5, 85-87.

Ramakrishnan, N. and Tiwari, L.D. (1969) Polyhedrosis of *Prodenia litura* Fabr, (Noctuidae : Lepidopteran). Indian Journal of Entomology 31(2), 191.

Rawlings, N.D., Pearl, L.H. and Buttle, D.J. (1992) The baculovirus *Autographa californica* nuclear polyhedrosis virus genome includes a papin-like sequence. Biol Chem H-S 373, 1211-1215.

Rohrmann, G.F. (1986) Evolution of occluded baculoviruses; in The biology of baculoviruses. Granados, R. and Federici, R. (ed.). 1, 203-215, CRC Press, Inc. Boca Raton, Florida.

Rohrmann, G.R. (1992) Baculovirus structural proteins. J Gen Virol. 73, 749–761.

Rovesti, L., Crook, N.E. and Winstanely, D. (2000). Biological and Biochemical Relationships between the Nucleopolyhedroviruses of *Mamesta brassicae* and *Heliothis armigera.* J Invertibr Pathol. 75, 2-8.

Singh, V., Lakhminarayana, M. and Ranga Rao, V. (1992) Changing scenario of insect pest problems in annual oilseed crops. National Seminar on the Changing Scenario in Pest and Pest Management in India . CPPTI

Slack, J.M., Kuzio, J. and Faulkner, P. (1995) Characterization of vcath, a cathepsin L-like proteinase expressed by the baculovirus *Autographa californica* multiple nuclear polyhedrosis virus. J Gen Virol. 76, 1091-1098.

Smith, G.E. and Summers, M.D. (1978) Analysis of baculovirus genomes with restriction endonucleases. Virology 89, 517-527.

Sparks, W.O., Harrison, R.L. and Bonning, B.C. (2011) *Autographa californica* multiple nucleopolyhedrovirus ODV-E56 is a per os infectivity factor, but is not essential for binding and fusion of occlusion-derived virus to the host midgut. Virology. 409, 69-76.

Subramanian, S., Rabindra, R.J., Palaniswamy, S., Sathiah, N. and Rajasekaran B (2005) Impact of granulovirus infection on susceptibility of *Spodoptera litura* to insecticides. Biol Cont. 33, 165-172.

Subramanian, S., Rabindra, R.J. and Sithanatham, S. (2008) Genetic and Biological Variations among *Plutella xylostella* Granulovirus Isolates. Phytoparasitica, 36(3), 220-230.

Summers, M.D. (1971) Electron microscopic observations on granulosis virus entry, uncoating, and replication process during infection of the midgut cells of *Trichoplusia ni*. *Journal of* Ultrastructural Research 35, 606-612.

Suzuki, Y.G., Forman, H.J. and Sevanian, A. (1997) Free Radical. Bio Med. 22, 269-285.

Tanada Y, Hess RT (1991) Baculoviridae. Granulosis virus. Pages 227-257. In: Adams JR, Bonami JR (ed.)

Atlas of Invertebrate Viruses, CRC Press, Boca Raton, FL., pp 684.

Tanada, Y. and Kaya, H.K. (1993) Insect Pathology. Academic Press, Inc., San Diego.

Tanada, Y., Leutenegger, R. (1968) Histopathology of a granulosis-virus disease of the codling moth, *Carpocapsa pomonella*. J Invertibr Pathol. 10, 39-47.

Tang, X.D., Xiao, Q., Ma, X.C., Zhu, Z.R. and Zhang, C.X. (2009) Morphology and genome of *Euproctis pseudoconspersa* nucleopolyhedrovirus. Virus Genes 38, 495-506.

Unruh, T.R., Lacey, L.A., Headrick, H.L. and Pfannenstiel, R.S. (2012) The effect of the granulovirus (PapyGV) on larval mortality and feeding behaviour of the *Pandemis leafroller, Pandemis pyrusana* (Lepidoptera: Tortricidae). Biocontrol Sci Techn. 22(9), 981-990.

Van Oers, M.M., Abma-Henkens, M.H., Herniou, E.A., de Groot, J.C., Peters, S. and Vlak, J.M. (2005) Genome sequence of *Chrysodeixis chalcites* nucleopolyhedrovirus, a baculovirus with two DNA photolyase genes J Gen Virol 86, 2069-2080.

Van Oers, M.M. and Vlak, J.M. (2007) Baculovirus genomics. Curr Drug Targets. 8, 1051-1068.

Vimala Devi, P.S. and Prasad, Y.G. (1997) The entomofungal pathogens Nomuraea rileyi. In: Information Bulletin, Directorate of Oilseeds Research Rajendranagar. Hyderabad, pp. 5

Vlak, J.M. and Smith, G.E. (1982) Orientation of the genome of *Autographa californica* nuclear polyhedrosis virus: a proposal. J Virol. 41(3), 1118-1121.

Vyas, H.N. (*1994)* Biology of castor semilooper, *Achaea janata* L. on rose, *Rosa indica*. Crop Research (Hisar) 7(1), 109-111.

Wang, Y., Choi, J.Y., Roh, J.Y., Liu, Q., Tao, X.Y., *et al*. (2011) Genomic Sequence Analysis of Granulovirus Isolated from the Tobacco Cutworm, *Spodoptera litura.* PLoS ONE 6(11), e28163.

Wang, Y., Choi, J.Y., Roh, J.Y., Woo, S.D., Jin, B.R. and Je, Y.H. (2008) Molecular and phylogenetic characterization of *Spodoptera litura* granulovirus. J Microbiol. 46(6), 704–708.

Watanabe, H. and Kobayashi, M. (1970). Histopathology of a granulosis in the larvae of the fall webworm, *Hyphantria cunea*, J Invertebr Pathol. 16, 71-79.

Whitten, A.J. and Damanik, S.J. (1986) Mass defoliation of mangroves in Sumatra, Indonesia. Biotropica 18, 176.

Willis, L.G., Seipp, R., Stewart, T.M., Erlandson, M.A. and Theilmann, D.A. (2005) Sequence analysis of the complete genome of *Trichoplusia ni* single nucleopolyhedrovirus and the identification of a baculoviral photolyase gene. Virology 338, 209-226.

Winstanley, D. and O'Reilly, D.R. (1999) Granuloviruses; in The Encyclopedia of Virology. Webster, R. and Granoff, A. (eds.). 2nd edn, vol 1, pp 140–146, Academic Press, London

Wolff, J.L., Valicente, F.H., Martins, R., Oliveira, J.V. and Zanotto, P.M. (2008) Analysis of the genome of *Spodoptera frugiperda* nucleopolyhedrovirus (SfMNPV-19) and of the high genomic heterogeneity in group II nucleopolyhedroviruses. J Gen Virol. 89, 1202-1211.

Wormleaton, S., Kuzio, J. and Winstanley, D. (2003) The complete sequence of the *Adoxophyes orana* granulovirus genome. Virology 311(2), 350–365.

Xiang, X., Chen, L., Guo, A., Yu, S., Yang, R. and Wu, X. (2011) The *Bombyx mori* nucleopolyhedrovirus (BmNPV) ODV-E56 envelope protein is also a per os infectivity factor. Virus Res. 155, 69-75.

Xiao, H. and Qi, Y. (2007) Genome sequence of *Leucania seperata* nucleopolyhedrovirus. Virus Genes 35, 845-856.

Yuen, L., Dionne, J., Arif, B., Richardson. (1990) Identification and sequencing of the spherodin gene *Choristneura fumiferana* entomopoxvirus. Virology. 175, 427-433.

Zanotto, P.M., Kessing, B.D. and Maruniak, J.E. (1993) Phylogenetic interrelationships among baculoviruses: evolutionary rates and host associations. J Invertibr Pathol. 62, 147–164.

Zhang, B-Q., Cheng, R-L., Wang, X-F. and Zhang, C-X. (2012) The Genome of *Pieris rapae* Granulovirus. J Virol. 86(17), 9544.

Zhang, C.X., Ma, X.C. and Guo, Z.J. (2005a) Comparison of the complete genome sequence between C1 and G4 isolates of the *Helicoverpa armigera* single nucleocapsid nucleopolyhedro virus, Virology. 333, 190-199.

Zhu, S.Y., Yi, J.P., Shen, W.D., Wang, L.Q., He, H.G., Wang, Y., Li, B. and Wang, W.B. (2009) Genomic sequence, organization and characteristics of a new nucleopolyhedrovirus isolated from *Clanis bilineata* larva. BMC Genomics. 10, 91.