



Review Article

Baculoviruses: Lethal pathogens of lepidopteran caterpillars

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ABSTRACT: Baculoviruses, a diverse group of arthropod-specific viruses, have long been employed for the biological control of many economically significant insect pests on agricultural and forest crops all over the world. They are primarily pathogens of caterpillars and about 90 per cent are reported to cause diseases in members belonging to the order Lepidoptera. Baculoviruses have been attractive biological control agents because of their safety to vertebrates, other non-target fauna and high pathogenicity with host death being most likely outcome of an infection. They have an ability to persist outside the host insect by producing virions sequestered within the protein matrix and potential to trigger epizootics in insect population, thus being important factor in regulating the size of host insect population. Baculoviruses are relatively quick acting and lethal among the various insect pathogens infecting globally significant pest species. Considering the importance of baculoviruses in insect pest management, a review of the research work carried out is presented.

KEYWORDS: Baculoviruses, biological control, insect pathogenic viruses, lepidopteran pests

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INTRODUCTION

Insect pathogenic viruses from fifteen families were known to cause diseases in insects (Hu *et al.*, 2002; Szewczyk *et al.*, 2006). However, of all the insect viruses known to mankind, only members of family Baculoviridae have been considered as potential pathogen of insects. Baculoviruses were the most intensely studied and major group of arthropod-specific viruses (Herniou and Jehle, 2007), found ubiquitously in the environment. They were predominantly associated with members of arthropod species and reported non-pathogenic in members outside the phylum Arthropoda (Miller, 1997). Baculoviruses were employed in biological control of insect pests of forestry and agriculture since 1900s (Slack and Arif, 2007; Beas-Catena *et al.*, 2014) with more than 600 host-insect species (Martignoni and Iwai, 1986) belonging to the orders Lepidoptera, Hymenoptera, Diptera (Herniou *et al.*, 2012), Orthoptera, Coleoptera, Neuroptera, Thysanura, Trichoptera (Murphy *et al.*, 1995) and crustacean order, Decapoda (Blissard *et al.*, 2000). About 35 insect pathogenic viruses were reported in India (Rao *et al.*, 2015) mostly from economically important pests like *Helicoverpa armigera* (Hubner), *Spodoptera litura* (Fab.), *Spilosoma obliqua* (Walk.), *Amsacta albistriga* (Walk.), *Achaea janata*,

(L.) *Chilo* spp., etc., infesting cotton, pigeonpea, chickpea, tomato, groundnut, castor and sugarcane.

History

The historical accounts of baculovirus mainly the early discoveries were documented by Steinhaus (1949) and reviewed by Arif (2005). The earliest accounts of baculovirus infections were found in ancient Chinese literature describing the disease of silkworms as early as 16th century. The first description of baculovirus disease was credited to Marco Vida, an Italian poet in 1527 (Benz, 1986). Infected silkworm larvae had swollen body and appeared yellow shiny which eventually caused rupture of insect skin and exudation of body fluid. In 19th century, this disease of silkworm was referred as “jaundice” in America and “*grasserie*” in France (Steinhaus, 1949). Subsequently several investigators observed crystal like corpuscles in the tissues and body fluids of diseased larvae of silkworm. Maestri, A. and Cornalia, E. were among the first to describe about the refractive bodies in jaundiced larvae in 1856; later Bolle, J. demonstrated these refractive occlusion bodies as causative agents of infection. These causal agents were observed as tiny granules under dark-field microscope and named as *Borrellina bombycis* by Andre Paillot in honor of Professor Borrel of the Pasteur Institute.

The infectious nature of filtrate of diseased silkworm passed through several layers of filter papers was observed by Von Prowazek in 1907. Glaser, R.W. in 1918 documented the transmission of polyhedral disease in gypsy moth by using filtrates of infected larvae (Benz, 1986). Nearly a decade later, French scientist, Andre Paillot discovered disease of *Pieris brassicae* (L.) having etiology different from earlier descriptions and characterized by presence of numerous small granules and later he designated the pierid disease as “*pseudo-grasserie*”, the causative agent of which was subsequently termed as *Granulosis* by Steinhaus. Ishimori (1934) discovered another disease with Occlusion Bodies (OB) in the cytoplasm of infected host insect representing different replication site and etiology which was later referred as cytoplasmic virus (reviewed by Arif, 2005). Bergold (1950) contributed to biochemical properties of virus in 1930s-40s and revealed the rod shaped virions within the polyhedra. Contributions of Steinhaus and other workers from 1950 to 1975 led to recognition of baculoviruses as biological control agent of many insect pests (reviewed by Miller, 1997). The first insect pathogenic baculovirus product, Elcar was registered as insecticide by Sandoz Inc., in the United States in 1975 (Ignoffo, 1981) based on the preparation of nuclear polyhedrosis virus (NPV) of *Heliothis zea*. The two different morphs of baculoviruses, Budded Virus (BV) and Occluded Virus (OV) were recognized during 1970 to 1985 (Miller, 1997) which lead to the understanding of its pathogenesis in insect hosts. The progress on molecular biology and genetics of baculovirus was attempted during last two decades of 20th century. The complete nucleotide sequence of AcNPV (NPV of *Autographa californica*) genome was published in 1994 having genome size of 133,894bp with 148 predicted non-overlapping Open Reading Frames (ORFs) (Ayres *et al.*, 1994). The advances in insect cell line culture paved the way for studying genetics of baculovirus and its use as vector for expression of heterologous genes.

In India, the existence of NPV in *H. armigera* was reported from Gujarat by Patel *et al.* (1968) subsequently, Jacob and Subramanian (1972) reported NPV from *H. armigera* and *A. albistriga* from Coimbatore. The occurrence of Granulosis Virus (GV) was reported on shoot borer, *Chilo infus catellus* Snell (Easwaramoorthy and David, 1979), internode borer, *Chilo sacchariphagus indicus* (Kapur) (Mehta and David, 1980) and top borer, *Scirpophaga excerptalis* Walker (Singaravelu *et al.*, 1999) in sugarcane. Gopinadhan *et al.* (1990) reported Cytoplasmic Polyhedrosis Virus (CPV) infection in all life stages of red palm weevil (*Rhynchophorus ferrugineus* Olivier) in coconut from Kerala.

Baculovirus structure

Baculoviruses are bacilliform, enveloped viruses with covalently closed circular double-stranded DNA genome

(Blissard, 1996) ranging in size from 82 kb to 180 kb in size (Lauzon *et al.*, 2006; Hayakawa *et al.*, 1999) and predicted to encode for about 90 to 180 genes (Okhano *et al.*, 2006). The genome packaged in bacillus-shaped nucleocapsids and hence the name “baculovirus” (Slack and Arif, 2007). Rod shaped nucleocapsid (average 30-60 nm diameter and 250-300 nm length) (Jehle *et al.*, 2006) surrounded by lipoprotein envelope and referred as virion (Occlusion Derived Virus). Two phenotypically distinct but genetically identical forms, Occlusion-Derived Virion (ODV) and Budded Virion (BV) were recognized in baculoviruses (Blissard and Theilmann, 2018). BV differed from ODV in the structure of their envelope (Okano *et al.*, 2006). Virions were occluded either within polyhedral or granular OB's. Polyhedral-shaped Occlusion Bodies (POB's) of NPV's composed of matrix protein called polyhedron (Murphy *et al.*, 1995; Hooft van Iddekinge *et al.*, 1983), while GVs produced smaller ovi-cylindrical occlusions (Williams and Faulkner, 1997) composed of a major matrix protein called granulin (Funk *et al.*, 1997; Winstanley and O'Reilly, 1999). Proteinaceous occlusion bodies contributed to stability and persistence of virus outside the host insect in the environment (Slack and Arif, 2007).

Baculovirus classification

Francis O. Holmes published an account of virus classification titled “Order Virales, The Filterable Viruses” in sixth edition of *Bergey's Manual of Determinative Bacteriology*. Holmes group categorized the insect pathogenic viruses into two genera, *Borrelina* and *Morator* in the family Borrelinaceae, suborder Zoophagineae and order Virales (Holmes, 1948). The genus *Borrelina* included viruses inducing polyhedral, wilt and other diseases of Lepidoptera. Genus *Morator* had only one virus species inducing sacbrood disease in honeybee. Steinhaus (1949) revised the Holmes classification and recognized four genera based on the type of inclusion bodies formed by viruses. Genera, *Borrelina* and *Bergoldia* were characterized by the polyhedral and granular inclusions, respectively, in the infected cells of host-insect. Genus, *Paillotella* showed refringent, polymorphic inclusions of very irregular shape and size. Genus, *Morator* was characterized by no visible pathological inclusions.

Subsequently arthropod specific viruses were classified as baculoviruses, which had three subgroups; subgroup A (NPV), subgroup B (GV) and subgroup C (Non-occluded Viruses) (Moore *et al.*, 1987). Until 1995, family Baculoviridae was subdivided into two subfamilies, *Eubaculovirinae* comprising occluded viruses and *Nudibaculovirinae* with non-occluded viruses (Francki *et al.*, 1991). *Eubaculovirinae* included two genera, NPV and GV. Two subgenera were established within the NPV genus, one comprising viruses with multiple nucleocapsids

per envelope (MNPV) and other comprising viruses with a single nucleocapsid per envelope (SNPV). A genus Non-Occluded Baculoviruses (NOB) had been established within the subfamily *Nudibacillivirinae*. Following reclassification, family *Baculoviridae* was subdivided into two genera, *Nucleopolyhedroviruses* (NPVs) and *Granuloviruses* (GVs) (Murphy *et al.*, 1995; Rohrmann, 1999; Winstanley and O'Reilly, 1999) mainly based on the morphology of occlusion bodies formed in the infected cells. Both genera showed considerable similarities in genome structure, infection cycle, morphology and structure of nucleocapsids (Ikeda *et al.*, 2015), but were clearly distinguished by the type of occlusion bodies produced. *Granuloviruses* were composed of one nucleocapsid per envelope, while NPVs were packaged either as one nucleocapsid per envelope (SNPVs) or one through many nucleocapsids per envelope (MNPVs) (Adams and McClintock, 1991). The GVs were reported solely from lepidopteran hosts (Murphy *et al.*, 1995), whereas NPVs were isolated from a wider range of insects. About 90 per cent of baculoviruses were reported from 34 different families of Lepidoptera (Lange *et al.*, 2004).

Jehle *et al.* (2006) proposed the revision of baculovirus classification on the basis of genome sequence analysis and associated arthropod host, and recognized four genera, *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus* and *Deltabaculovirus* within the family *Baculoviridae*. Genera, *Alphabaculovirus*, *Gammabaculovirus* and *Deltabaculovirus* included NPVs that were specific for members belonging to orders Lepidoptera, Hymenoptera and Diptera, respectively, whereas *Betabaculovirus* included lepidopteran specific GVs (Herniou *et al.*, 2012). The lepidopteran specific NPVs (*Alphabaculovirus*), on the basis of phylogenetic analyses, were subdivided into Group I and II NPVs (Bulachetal., 1999; Zonatto *et al.*, 1993; Herniou *et al.*, 2001). Group I and II NPVs had GP64 and F-proteins, respectively, as envelope fusion proteins of Budded Viruses (BVs), which were essential for cell attachment, membrane fusion during viral entry through endocytosis, and efficient budding of progeny BVs from infected cells (Garcia-Maruniak *et al.*, 2004; Lauzon *et al.*, 2006; Ikeda *et al.*, 2015).

Baculovirus nomenclature

Baculovirus nomenclature was based on the host-insect species from which they were first isolated (Tinsley and Kelly, 1985) thus species name consisted the name of the host insect followed by the abbreviations GV or NPV indicating the type of OB formed. The *Nucleopolyhedrovirus* isolated from insect host, *H. armigera* was originally named as *HaNPV*. However, this kind of nomenclature occasionally created confusion for baculoviruses that had relatively wide host range. Some baculoviruses were completely specific to their host. e.g., SeMNPV infected only *Spodoptera exigua*

(Hubner) (Williams *et al.*, 2016), whereas AcMNPV reported to infect 30 species from about 10 insect families belonging to order Lepidoptera (Groner, 1986). Presently insect pathogenic viruses were named using first two letters of the genus and species of their host, hence NPV species isolated from *Helicoverpa armigera* abbreviated as HearNPV.

Life cycle and pathogenesis of baculoviruses

Baculoviruses are obligate pathogens (Steinhaus, 1949) and require host cells for their replication. Baculovirus infection involved bi-phasic process, life cycle was characterized by production of two genotypically identical but structurally distinct (reviewed by Blissard, 1996; Rohrmann, 1999) virus phenotypes, each playing specific role during baculovirus life cycle. The BV phenotype (also referred as nonoccluded virus/ extra-cellular virus) produced during early stage of infection which was responsible for systemic cell-to-cell propagation of virus within the host insect (Rohrmann, 1999). In contrast, ODV was produced during the terminal stage of virus infection affecting host-to-host transmission of disease by persisting outside the host insect. The infection of NPVs in Diptera (*Deltabaculoviruses*) and Hymenoptera (*Gammabaculoviruses*) reported to be restricted to gut cells (Federici, 1997; Moser *et al.*, 2001; Young *et al.*, 1972) whereas most of the Lepidopteran specific NPVs (*Alphabaculoviruses* and some *Betabaculoviruses*), infection had spread from the midgut cells to a variety of other tissues via BV's (Okano *et al.*, 2006).

Baculovirus infection in insects was initiated by *per os* (Slack and Arif, 2007) when susceptible host-insect larvae inadvertently consumed the OB contaminated foliage. Infectious units of baculovirus, OBs along with food particle passed through foregut of the insect to midgut. The combined action of gut protease (Granados, 1978; Harrap, 1970) and alkaline nature of caterpillar midgut triggered the dissolution of OBs (Federici, 1997) and ODVs reached midgut lumen where they passed through peritrophic membrane and entered the midgut cells, a primary site of infection. In midgut cells, ODV's fused with plasma membrane surrounding microvilli on the apical brush border of columnar epithelial cells (Granados, 1973). The DNA-containing nucleocapsids were transported to the nucleus to initiate infection (Ohkawa *et al.*, 2010) wherein, viral DNA was uncoated followed by transcription and genome replication (reviewed by Slack and Arif, 2007). The newly synthesized genomes were packaged and transported to the basal site of infected midgut cell to produce BVs, which initiated the infection from cell to cell inside the insect body, but in the very late stage of infection, infected cells switched to ODV production and OB formation (Williams *et al.*, 2016). As infection advanced, hundreds of millions of polyhedra were produced and released into the environment upon liquefaction of infected host.

The progression and signs of diseases caused by baculoviruses are influenced by numerous factors including the stage (instar) and physical attributes of the host insect, temperature, inoculum dose and virulence of virus. Ingestion of numerous polyhedra caused the death of early instars (1, 2 and 3) within 72 hours (Federici, 1997). However, the host infected with same amount of virus inoculum during later instars (4th or early 5th) developed the disease between 5-10 days at temperatures of 25-30 °C.

Near the end of infection cycle, the food consumption in NPV infected insects ceased (Aizawa, 1963), larvae turned sluggish and swollen. Cuticle appeared glossy due to accumulation of polyhedra in epidermal and fat body nuclei. The hemolymph of infected host larvae was cloudy owing to the circulation of large numbers of infected hemocytes and polyhedra into the haemolymph upon cell lysis at an advanced stage of disease (Granados and Lawler, 1981). Just prior to death, diseased larvae of many lepidopteran species often found attached themselves to top of the vegetation by their prolegs with the head and rear portion of the abdomen facing downward in an inverted 'V' shape. This characteristic posture of larvae in case of nun-moth caterpillar (*Lymantria monacha* L.) to the tops (*Wipfeln*) of tree was described as 'wipfelkrankheit' in German or tree top disease (Hofmann, 1891; Goulson, 1997). Death of the host insect was accompanied by liquefaction of the internal anatomy, weakening of the cuticle and liberation of progeny OBs into the environment which acted as source of inoculums.

Host range

Baculovirus infection was limited exclusively to arthropods, predominantly holometabolous insects (those undergoing complete metamorphosis). Baculoviruses have been reported to have evolved with their holometabolous insect-hosts beginning around 310 million years ago way back in the Paleozoic Era in the Carboniferous Period (Theze *et al.*, 2011). The host range of GVs was relatively narrow, being pathogenic only to the members of order Lepidoptera, whereas NPVs were reported from Lepidoptera, Diptera, Hymenoptera (Herniou *et al.*, 2012), Coleoptera,

Neuroptera, Thysanura, Trichoptera (Murphy *et al.*, 1995) as well as from the crustacean order Decapoda (Blissard *et al.*, 2000). Although, 90 per cent of NPVs had been isolated from members of Lepidoptera family, most species of baculoviruses were found within the genus *Alphabaculovirus* and *Betabaculovirus*. The NPV species isolated from the family Lymantridae tend to be very narrow in host range (Barber *et al.*, 1993; Grzywacz, 2016), while some of the species isolated from Noctuidae showed extensive host ranges (Vail *et al.*, 1999). Among NPVs, SNPV reported to have fewer hosts than MNPVs (Groner, 1986). The host range of *Autographa californica* MNPV (AcMNPV) was extremely wide and reported to infect at least 32 species of Lepidoptera within 12 families (Volkman, 1997; Vail *et al.*, 1999). The NPVs of *Anagrapha falcifera* (Kirby) and *Mamestra brassicae* (L.) were also reported to have broader host range (Hostetter and Putler, 1991; Groner, 1986). The host range of NPV has been reported to about 300 species of Lepidoptera, 20 species each of Diptera and Hymenoptera while GVs reportedly infected about 150 species of Lepidoptera. (Rohrmann, 1999). Globally several insect pathogenic viruses belonging to the genus *Alphabaculovirus* and *Betabaculovirus* of baculoviridae have either been employed or shown enormous potential in the biological suppression of economically significant caterpillar pests of agriculture and forestry (Table 1).

CHARACTERIZATION OF BACULOVIRUSES

Morphological properties

The initial differentiation of virus groups was largely based on light microscopy with phase contrast or bright-field microscope. Baculoviruses were large rod shaped or bacilliform viruses (Blissard, 1996) whose infection was characterized by production of two morphologically distinct virion phenotypes, BV and ODV (Blissard and Theilmann, 2018). Virions of the genera *Alphabaculovirus*, *Gammabaculovirus*, *Deltabaculovirus* involved in infection process composed of crystalline protein matrix (polyhedrin) and polyhedral in shape which measured about 0.5 to 5 µm in diameter. In *Betabaculovirus*, these virions were ovicylindrical composed of granulin protein matrix with a diameter of

Table 1. Baculovirus species associated with economically important lepidopteran caterpillar pests

Sl No	Baculoviruses	Host insect	Crop	References
01	HearNPV	<i>Helicoverpa armigera</i>	Cotton, Pigeonpea, Chickpea, Vegetables	Rabindra and Jayaraj, 1988 and 1995
02	HzNPV	<i>Heliothis zea</i>	Corn, Cotton	Ignoffo, 1973, 1981 and 1999
03	SeMNPV	<i>Spodoptera exigua</i>	Wide variety of vegetables	Kolodny-Hirsch <i>et al.</i> , 1993 and 1997
04	SpltNPV	<i>S. litura</i>	Vegetables	Sun and Peng, 2007

05	SfMNPV	<i>S. frugiperda</i> (J.E. Smith)	Maize, Sorghum	Escribano <i>et al.</i> , 1999
06	SpobNPV	<i>S. obliqua</i>	Pulses, Vegetables	Kumar <i>et al.</i> , 2015
07	AgMNPV	<i>Anticarsia gemmatalis</i> (Hubner)	Soybean	Moscardi, 1999
08	AcMNPV	<i>A. californica</i>	Cotton, Cabbage	Vail <i>et al.</i> , 1999
09	LdMNPV	<i>Lymantria dispar</i> L.	Forest trees	Podgwaite <i>et al.</i> , 1991; Webb <i>et al.</i> , 1999
10	HpNPV	<i>Hyblaea puera</i> (Cramer)	Teak	Nair <i>et al.</i> , 1996
11	TnSNPV	<i>Trichoplusia ni</i> (Hubner)	Cabbage	Vail <i>et al.</i> , 1999; Ignoffo, 1964
12	CpGV	<i>Cydia pomonella</i> L.	Apple, Pear	Vincent <i>et al.</i> , 2007; Moscardi, 1999
13	PlxyGV	<i>Plutella xylostella</i> (L.)	Cabbage	Grzywacz <i>et al.</i> , 2004
14	PbGV	<i>Pieris brassicae</i>	Cabbage	Hostetter <i>et al.</i> , 1973; Tatchell and Paynee, 1984
15	PhopGV	<i>Phthorimaea operculella</i> (Zeller)	Potato	Salah and Aalbu, 1992; Lacey <i>et al.</i> , 2010
16	PiGV	<i>Plodia interpunctella</i> (Hub.)	Stored products	Vail <i>et al.</i> , 1991, Hunter <i>et al.</i> , 1979
17	AmalNPV	<i>Amsacta albistriga</i>	Groundnut	Chandramohan and Kumaraswami, 1979; Rabindra and Balasubramanian, 1980; Veenakumari <i>et al.</i> , 2005
18	AjGV	<i>Achaea janata</i>	Castor	Singaravelu and Ramakrishnan, 1998
19	Gv	<i>Chilo</i> spp.	Sugarcane	Easwaramoorthy and David, 1979; Mehta and David, 1980

about $0.3 \times 0.5 \mu\text{m}$ (Herniou *et al.*, 2012). Occlusion-derived virions consisted of one or more rod-shaped nucleocapsids and enclosed within an envelope. In contrast, virions of the second phenotype, BV- typically had a single nucleocapsid (Jehle *et al.*, 2006). Nucleocapsids measured about 30-60 nm diameter and 250-300 nm in length. (Theilmann *et al.*, 2005).

Biological activity

The biological activity of virus was expressed in terms of pathogenicity and virulence. Virulence of insect pathogenic virus measured in terms of dose-response or time-response relationships through bioassay technique using its host insect (Eberle *et al.*, 2012) and expressed as median lethal concentration (LC) and median lethal time (LT) or median survival time (ST). Several workers assessed the biological activity of virus isolates from members of same host species collected from different geographic locations. The results indicated significant variation in LC values with overlapping fiducial limits; hence use of native isolate was advocated for the biological suppression of respective insect pests (Rao *et al.*, 2015).

Baculovirus genome

Baculovirus genome consisted double-stranded, covalently closed circular DNA molecules of 82 kbp to 180 kbp in size and encodes between 90 to 180 putative genes

(Lauzon *et al.*, 2006; Hayakawa *et al.*, 1999). *Autographa californica* MNPV was the first baculovirus with whole genome sequenced and designated as the type species of the genus *Alphabaculovirus* (Herniou *et al.*, 2012; Chateigner *et al.*, 2015). To date, about 85 baculovirus whole genome sequences had been reported according to National Centre for Biotechnology Information (NCBI, 2019). *Polyhedrin* gene was the first baculovirus gene ever sequenced (van Oers and Vlak, 2007) and was one of the most conserved genes of lepidopteran-specific baculoviruses (Lange *et al.*, 2004). Available whole genome sequence analysis had shown that about 38 genes were conserved among all baculoviruses sequenced and they have been assigned as core genes (Garavaglia *et al.*, 2012; Williams *et al.*, 2016; Javed *et al.*, 2017; Blissard and Theilmann, 2018; Herniou *et al.*, 2012). The core genes encode factors that are crucial for the life cycle such as viral DNA replication, viral gene transcription, production of structural proteins, DNA packaging, virion assembly, virion architecture and *per os* infection (Garavaglia *et al.*, 2012; Herniou *et al.*, 2003; Herniou *et al.*, 2012; van Oers and Vlak, 2007) and for uniqueness of baculoviruses (Ikeda *et al.*, 2015). These baculovirus core genes were grouped into five functional categories: RNA transcription, DNA replication, structural proteins, auxiliary proteins and proteins of unknown function (Herniou *et al.*, 2003). Whole genome sequences provided a wealth of data about

the complement of genes present in individual virus, and comparative genomic analyses of baculoviruses provided valuable insights into baculovirus evolution and biology (Herniou *et al.*, 2003).

Baculovirus genomics provided novel approaches for identification and classification of insect pathogenic viruses (Lange *et al.*, 2004), genetic modification of baculoviruses for improving their insecticidal activities (Kamita *et al.*, 2017; Inceoglu *et al.*, 2006), baculovirus expression vectors for gene therapy (Hu *et al.*, 2006); vaccine production (van Oers, 2006) and drug screening (Condreay *et al.* 2006). On the basis of comparative analyses of baculovirus genome sequences, degenerate oligonucleotides were developed for specific amplification of conserved regions using Polymerase Chain Reaction (PCR) followed by DNA sequencing, which enabled phylogenetic inference from amplified sequence (Rowley *et al.*, 2011). Oligonucleotides that amplify partial sequences of the baculovirus core genes (three highly conserved genes), namely *polyhedrin/granulin* (*polh/gran*), *late expression factor-8* (*lef-8*) and *late expression factor-9* (*lef-9*) had been largely used to characterize several baculoviruses (Harrison *et al.*, 2012; Lange *et al.*, 2004); and that led to the proposition of criteria for species demarcation and new classification of baculovirus (Jehle *et al.*, 2006).

Genetic improvement of baculoviruses

Despite the high degree of host specificity and pathogenicity of baculoviruses, there were also some limitations for their successful use as biological control agents. The use of baculoviruses for pest control was generally limiting due to their slow speed of killing the host insect and partially due to their relatively narrow host specificity (i.e., more target specificity). Slower acting attribute allows the baculovirus to replicate and multiply in large numbers while allowing the host insect to survive for several days, however this 'slow acting' attribute had severe limitation for successful use of baculoviruses in insect pest management. These drawbacks were addressed by genetic modification of baculovirus using recombinant DNA technology and activity of baculoviruses against their natural hosts had been enhanced. The approaches to improve the speed of kill and activity of baculoviruses included introduction of foreign gene into the baculovirus genome to alters target host-insect physiology, deletion of an endogenous gene from the baculovirus genome, etc. (Kamita *et al.*, 2017). Recombinant baculoviruses could express host-insect specific toxins, hormones or enzymes (Inceoglu *et al.*, 2006; Elvira *et al.*, 2010; Gramkow *et al.*, 2010) affecting paralysis/death, or disturbing the physiology and behaviour of target insects (Ikeda *et al.*, 2015).

Carbonell *et al.* (1988) reported recombinant baculovirus expressing biologically active insectotoxin-1 (*BeIt*) of

scorpion, *Buthus eupeus*. *BeIt* was an insect-specific paralytic neurotoxin, however, paralytic activity was not detected during bioassay on insects. A neurotoxin, *AaIT* from the venom of North African (Algerian) scorpion, *Androctonus australis* Hector which selectively targets the insect sodium channel (Maeda *et al.*, 1991) was incorporated into *Autographa californica* NPV (AcNPV) genome (McCutchen *et al.*, 1991) and recombinant baculovirus showed the reduction in time required to kill the host insect (Stewart *et al.*, 1991). *AaIT* gene was successfully engineered in *Helicoverpa armigera* single-nucleocapsid NPV (HearNPV) which reduced the foliage damage and improved the speed of kill compared to wild type virus (Chen *et al.*, 2000; Sun *et al.*, 2002). Tomalski and Miller (1991) reported recombinant baculovirus expressing *tox34* gene encoding a neurotoxin (TxP-I) associated with venom apparatus of female predatory straw itch mite, *Pyemotes tritici* that induced the rapid muscle-contractive paralysis in the larvae of greater wax moth *Galleria mellonella* (Tomalski and Miller (1992). Insecticidal crystal protein gene, *cryIA(b)* derived from *Bacillus thuringiensis* was engineered into AcNPV (Martens *et al.*, 1995), however, the median lethal time for recombinant viruses did not significantly reduced than wild-type virus (Ribeiro and Crook, 1993). Genes encoding insect Juvenile Hormone Esterase (Hammock *et al.*, 1990), Ecdysion Hormone (Eldridge *et al.*, 1992) and Diuretic Hormone (Maeda, 1989) were expressed in recombinant baculoviruses with an aim of enhancing their efficacy. An *egt* deletion mutant of AcMNPV exhibited reduced feeding and earlier mortality of infected larvae than wild type AcMNPV (O'Reilly and Miller, 1991), hence deletion of *egt* gene that encodes the enzyme, ecdysteroid UDP-glucosyl-transferase reported as a simple approach to improve the activity of baculoviruses. Thus, limiting traits of baculoviruses like slow acting and narrow host range could be addressed by genetically modifying the baculoviruses through recombinant DNA technology.

Baculovirus production

Production of baculoviruses at commercial-scale was done exclusively *in-vivo* by culturing large number of larvae of host insect and subsequently feeding them with semi-synthetic diet contaminated with virus inoculums in laboratory. Mass culturing of host insect under optimal rearing conditions in a synchronized manner for *in-vivo* virus production is found to be labour intensive which accounted for high production cost and potential contamination of viral preparations by microorganisms (Rao *et al.*, 2015). Baculovirus production *in-vitro* by culturing insect cells in bioreactors was a substitute for labour intensive maintenance of the massive host-insect colony. This approach is more promising in terms of process scalability, however, the success of the baculovirus *in-vitro* production strongly influenced by cost of growth medium, media composition

(Huynh *et al.*, 2012) and passage effect. Passage effect or repeated serial passage of baculovirus *in-vitro* resulted in loss of virulence of POB due to the lack of selection pressure for virus in tissue culture to produce infectious polyhedra (Krell, 1996). The process of baculovirus production adopted should be efficient, inexpensive and resulting in production of highly pathogenic virus.

Baculovirus formulation

The baculoviruses were formulated in the same way as other biopesticides to enhance its shelf life, delivery, efficacy and persistence under field condition. In India, several products based on insect pathogenic viruses belonging to the genus *Alphabaculovirus* and *Betabaculovirus* have been developed against economically significant caterpillar pests (DPPQS, 2019) (Table 2). The most common preparation of a baculovirus was liquid formulation, invariably as a suspension concentrate. Dry formulations were generally prepared through air-drying, freeze-drying or lyophilisation, spray drying, co-precipitation with lactose, or microencapsulation (Grzywacz and Moore, 2017). Dust formulations were uncommon but generally employed for post-harvest management of potato tuber moth (*Phthorimaea operculella* Zeller) on stored potatoes with PhopGV (Lagnaoui *et al.*, 1997). Liquid formulations (suspension concentrates) were preferred due to their greater ease of application. Survival or virulence of virus largely influenced by virus isolate, duration and conditions of storage, temperature, additives and pH of the formulation. The most detrimental effect on virus activity was attributed to its sensitivity to solar radiation under field condition. Several additives including spreaders, wetting agents, surfactants, phagostimulants, UV protectants, etc. had been used in the formulations to enhance the efficacy of baculovirus under field conditions. Optical brighteners were used to protect the insect viruses against sunlight exposures (Shapiro, 1992). The synergistic activity of boric acid was demonstrated on *HearNPV* (Bijjur *et al.*, 1991), *SplitNPV*

(Chaudhari, 1992), *LdNPV* (Shapiro and Bell, 1982) and *SfMNPV* (Cisneros *et al.*, 2002). Recently microencapsulation of insect pathogenic viruses using polymers like lignin, sodium alginate (Gifani *et al.*, 2015), starch (Ignoffo *et al.*, 1991), etc. have been attempted to protect the infectivity of viruses under natural conditions.

Conclusion

Baculoviruses have long been recognized as potential and attractive candidates for biological control of several economically important pests due to their high pathogenicity, host specificity, safety to non-target arthropod fauna and ability to persist outside the host for a considerable period of time and induce epizootics in insect population. The potential of baculovirus for biological suppression of economically significant lepidopteran pests has been demonstrated worldwide. In India, under specific instances like pest outbreak, use of baculoviruses appears to be better biological control option to bring down the pests population to the level that would not cause economic damage to the crops. Large scale adoption of baculoviruses as commercial insecticides is, however, hindered by their slow killing action, narrow spectrum as compared to most of the synthetic insecticides and technical difficulties associated with large scale commercial and *in-vitro* production. The success and reliability of insect pathogenic viral products depends on efficacy under field condition, ease and cost of commercial production and quality. The stability of baculovirus formulation is influenced by temperature, moisture, presence of additives, plant surface chemistry and pH but ultraviolet light is probably most detrimental factor affecting the survival of virus particle under field condition. The additives like surfactants, spreaders, optical brighteners, feeding stimulants, etc., are used to improve the efficacy of final product. Notwithstanding these factors, baculoviruses are regarded as one of the lethal microbial biocontrol agents of lepidopteran caterpillars devastating many economically significant agricultural and horticultural crops of the world.

Table 2. Baculovirus based commercial formulations/products developed in India

Sl. No.	Baculovirus species	Product	Formulation	Agency/company	Target pest
01	HearNPV	Helicide	Aqueous Suspension 0.50 %	Pest Control India Ltd.	<i>Helicoverpa armigera</i>
02	HearNPV	Helimar	Aqueous Suspension 0.50%	Multiplex group of companies	<i>H. armigera</i>
03	HearNPV	Biovirus-H	Aqueous Suspension	Biotech International Ltd.	<i>H. armigera</i>
04	HearNPV	Biokill-H	Aqueous Suspension 2 %	Ganesh Bio Control System	<i>H. armigera</i>
05	HearNPV	Helicop	Aqueous Suspension 2 %	Indore Biotech Inputs and Research Pvt Ltd	<i>H. armigera</i>
06	HearNPV	Helistop	Aqueous Suspension 0.50 %	Harit Bio-control Labs	<i>H. armigera</i>

07	SpltNPV	Spodocide	Aqueous Suspension 0.50 %	Pest control India Ltd.	<i>Spodoptera litura</i>
08	SpltNPV	Spodomar	Aqueous Suspension 0.90 %	Multiplex group of companies	<i>S. litura</i>
09	SpltNPV	Biovirus-S	Aqueous Suspension	Biotech International Ltd	<i>S. litura</i>
10	SpltNPV	Biokill-S	Aqueous Suspension 2 %	Ganesh Bio Control System	<i>S. litura</i>

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