Genetically Modified Baculoviruses- An Important Tool in Insect Pest Management

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Insect viruses are important pathogens of many arthropod species. They have been recorded from a wide range of insects (Miller and Ball, 1998), and their association with these hosts is long, perhaps for more than 200 million years. Insect viruses are submicroscopic, obligate, intracellular, pathogenic entities. These viruses are simplest living forms, composed of a protein shell (capsid) that surrounds the nucleic acid. The nucleic acid is infectious in nature while capsid provides the morphological properties. Each virus has one type of nucleic acid, either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The nucleic acid may be double or single stranded. The nucleic acid together with capsid forms the nucleocapsid or virion. Insect virus particles may be either *enveloped or nonenveloped.* A bilayer lipid membrane referred as the viral membrane or the viral envelope surrounds the nucleocapsid of many complex viruses. Virion is embedded either singly or in multiple in protective crystalline protein matrix known as occlusion body (OB). As on date, 6590 species of viruses have been defined by the International Committee on Taxonomy of Viruses (ICTV), of which more than 1100 viruses are known to infect insects belonging to over 20 different families of insects. Out of 1100 insect viruses, more than 50% viruses belong to a single family Baculoviridae (Eberle *et al*., 2012).

Baculoviruses makes up the large family of insect viruses and most thoroughly studied insect pathogens, they are widely used both as insect control agent and as protein expression vector. In baculovirus it is a bi-phasic infection process in which genotypically identical, but phenotypically different virus forms are produced *viz*., intracellular occlusion derived virus (ODV), or extracellular viral progeny (budded virus or BV). The ODV´s transmit infections from insect to insect, whereas the BV's spread the infection from cell to cell within an infected insect (Granados, 1980).

Based on the genome sequence analysis, morphological, biological and phylogenetic features and host it infects, Jehle *et al*. (2006) classified the members of Baculoviridae and placed under four genera. The Alpha baculoviruses are all Lepidoptera specific nucleopolyhedroviruses (NPVs), and their OBs are the classic many-sided (polyhedral) shape as seen in the type species *Autographa californica* multiple NPV (AcMNPV). The ODV produced by members of this genus can contain one or many nucleocapsids per enveloped virion, a feature not found among members of the other genera. The Betabaculoviruses include Lepidoptera-specific granuloviruses (GVs) and the type species is *Cydia pomonella* GV (CpGV). The Gamma baculoviruses are Hymenoptera-specific NPVs such as the *Neodiprion lecontei* SNPV (singly enveloped NPV) with polyhedral OBs.

Lastly, the Deltabaculoviruses are Diptera-specific NPVs with crystalline OBs of 0.5–15 μm containing many virions. The viral occlusion matrix protein of this virus is significantly larger than those of the viruses from the other three genera. The type species for Deltabaculovirus is *Culex nigripalpus* nucleopolyhedrovirus (CuniNPV). Most species of baculovirus are found within the alpha and beta baculoviruses.

Infection process of Baculoviruses

Infections occur following ingestion of OBs by a susceptible larva by feeding on OB-contaminated food such as foliage. Once ingested, the OB is carried to the alkaline (pH 8–10) larval midgut region where the OB dissolves as the polyhedrin and solubilizes to release the ODV within minutes. The released ODV then needs to pass through the peritrophic membrane (PM), found in the midgut, to access the midgut epithelial cells in order to establish infection. Once they pass PM, virions then attach to and enter the midgut epithelial cells and fuse with the epithelial membrane, allowing the nucleocapsids to enter the cells. Virions entering host cells, reach the nucleus through nuclear pores and start replicating in host cell nucleus. Viral replication in the nucleus of midgut epithelial cells results in the appearance of many progeny nucleocapsids and emerges as budded virus (BV). As BV are known to infect from one cell to another cell, after epithelial cells they reach tracheal cells and tracheoblasts. By moving through the network of trachea and by translocation within the motile tracheoblasts, the virus is able to rapidly spread through the host tissues colonizing haemolymph cells and most larval tissues including gonad, hypodermis, muscles, nerve ganglia, and pericardial cells. The infection and destruction of these tissues eventually results in larval death.

Baculoviruses have numerous unique features that have generated interest in their use as microbial insecticides *viz*., (i) host specificity, (ii) virulence in host insect (iii) no residual toxicity, (iv)environmental and mammalian safety (v) long shelf life, (vi) easily applied using conventional spray equipments, (vii) causing epizootic and (viii) compatibility with other control agents.

On the other hand, limitations include like restricted host range, costly *in vivo* production, limited market size, and relatively low cost-effective, slow speed of action particularly in the crops with low damage threshold, vulnerable to solar UV light and low virulence against the older instars. These limitations necessitate the need for development of recombinant viruses through genetic engineering techniques either by gene insertion or gene deletion. Through genetic engineering, recombinant baculoviruses have been developed aiming at increased speed of action by inserting insect specific toxin genes, affecting physiological process by over expression or inactivation of hormones and enzymes.

Expression of insect hormone genes:

Insect growth and development is majorly regulated by hormones. Disruption, over expression or inactivation of one or more insect hormones results in abnormal growth, feeding cessation and/or death. So, the insertion of genes that encode insect hormones were the first strategies used to generate genetically modified baculovirus. A recombinant virus of *Bombyx mori* MNPV (BmNPV) that encodes an active

diuretic hormone (DH) found to be 20% faster in killing larvae than wild type virus (Maeda *et al*., 1989). Some of other hormones that have been expressed in the recombinant NPVs include eclosion hormone, prothoracicotropic hormone, juvenile hormone, but over expression of these hormones did not brought any significant improvement in the speed of kill as compared to wild type.

Expression of insect-selective toxin:

Expression of insect selective toxin *AaIT* gene from *Androctonus australis* scorpion in recombinant baculovirus resulted in increased speed of kill. A recombinant virus containing this gene showed 40% faster in killing larvae than the wild type and a reduction of host feeding by 60% (Inceoglu *et al*., 2001), this is due to inability of infected larvae to control muscle coordination. The site of action of this neurotoxic polypeptide is on insect sodium channel. Lepidopterous larvae infected with an AaIT-expressing baculovirus reveal symptoms of paralysis identical to those induced by injection of the native toxin (Elasar *et al.*, 2001) and many of the physiological effects are very similar to those of pyrethroid insecticides which also act at the same target (Gordon *et al*., 1992).

Other useful insect-selective neurotoxins are SFI1 (obtained from a European spider, *Segestria florentina*) and ButaIT (derived from the South Indian red scorpion *Mesobuthus tamulus* (Wudayagiri *et al*., 2001). Some toxins could exert a cooperative effect when they are co-expressed, such as LqhIT1 and LqhIt2, obtained from *Leiurus quinquestriatus* scorpion (Regev *et al.*, 2003).

Deletion of an endogenous baculovirus gene:

Baculovirus-encoded ecdysteroid UDP-glucosyltransferases (egt) inactivate ecdysteroid hormones in infected insect larvae by conjugating these compounds with glucose or galactose. As a result of this inactivation, normal development of the insect, such as moulting, is arrested, thereby prolonging the larval stage. Larvae continue to grow and feed and ultimately produce large numbers of polyhedra. Thus, egt functions to prolong the length of time the insect feeds after infection, with a resultant increase in the weight gain of the insect. This gene is found in many viruses belonging to the two baculovirus genera that infect Lepidoptera, *Alpha baculovirus* and *Beta baculovirus.* Deletion of the egt gene from viral genome shows 10-20% reduction in lethal time relative to virus having egt gene and 40% reduction in feeding damage. The egt negative AcNPV is likely to be the first recombinant baculovirus approved for commercial use as a pesticide (O'Reily, 1995).

Genetic engineering for increased virulence:

Enhancin is a metalloprotease commonly expressed by baculoviruses that degrades insect intestinal mucin in the peritrophic membrane. Insertion of the *Enhancin* gene derived from *Trichoplusia ni* GV enhanced AcMNPV virulence by 2 to 14-fold in various insect species. Conversely, deletion of two *Enhancin* genes from *Lymantria dispar* MNPV reduced viral potency 12-fold. (Kroemer *et al*., 2015). AcMNPV has been genetically engineered to express an algal virus pyrimidine dimer-specific glycosylase, cv-PDG, so that it is less susceptible to UV inactivation. Additional

benefit of such a recombinant was that its virulence also increased 16-fold while killing *Spodoptera frugiperda.*

Recent development in the baculovirus genetic engineering is the development of baculovirus genomes capable of replicating in a bacterial host as bacterial artificial chromosomes, these recombinant baculoviruses are called bacmids. The principal advantage bacmids have over other high insert capacity vectors like yeast artificial chromosomes (YAC) and mammalian artificial chromosomes is stability of insert propagation over multiple generations. Once transferred into the bacterial host, the baculovirus genome can be manipulated easily through site-specific recombination, Rec-A mediated homologous recombination or transposition (Hasse *et al*., 2013). The first bacmid developed contained the AcMNPV genome, later bacmid systems are also being developed for *Bombyx mor*i NPV, *Helicoverpa armigera* single-nucleocapsid nucleopolyhedrovirus (HearSNPV) and *Cydia pomonella* granulovirus (CpGV) (Hilton *et al*., 2008; Wang *et al*., 2003)

Conclusion

One of the common factors associated with genetic optimization for increased speed of kill, is that the faster the virus kills the host insect, the fewer OBs are produced. Hence, large scale production of these recombinant baculoviruses *in vivo* becomes a challenge. Hence biosafety, commercialisation and resistance from pests are concerns. Another scope is regarding co-expression of two different neurotoxins encoded by a single recombinant baculovirus which could sometimes exhibit a synergistic increase in the degree of reduction in host survival time as well as broadening the host range.

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