Baculoviruses as biopesticides

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Baculoviruses have been recognized as possessing the ability to develop into potential biopesticides, besides their widespread use as expression vectors. These insect-specific viruses can be developed and used against insect pests of the order Lepidoptera, Hymenoptera and Coleoptera which are a threat to many agricultural crops and forest trees. Attempts to meet the failure of naturally occurring baculoviruses to overcome certain limitations in their commercialization and widespread use as bio-pesticide have been made by genetically modifying the baculoviruses using the baculovirus expression vector system technology. This mini-review attempts to give an overall picture of the development of baculoviruses as eco-friendly potential control agents, in view of the recognition of hazards posed by the more commonly used chemical insecticides in recent years.

INTEREST in the use of baculoviruses as pest-control agents came as early as 1527, when the baculovirus disease of insects was found during studies on the 'jaundice disease' of the silkworm, Bombyx mori. The viral nature of the disease was established by 1947 and it soon became clear that these viruses were widespread in nature among economically important insect pests, and so could be potentially useful in pest-management in agricultural practices.

The widespread use of chemical pesticides for pest control created numerous agricultural and health-related problems and environmental pollution. Efforts were then put into identifying more eco-friendly methods of pest-control which would be effective, more selective and safer (Box 1). Such specialized and effective biological control agents were soon found: Bacillus thuringiensis bacteria that kill larval forms of many insect pests, small wasps parasitizing caterpillars, baculoviruses pathogenic to insects, etc. Out of these, attention was also focused on the use of baculoviruses as bio-insecticides.

Baculoviruses are one of the largest and most diverse group of viruses pathogenic only for insects mostly of the order Lepidoptera, Hymenoptera and Coleoptera and have also been isolated from crustaceans (Box 2). The prototype member is Autographa californica nuclear polyhedrosis virus, AcNPV, isolated from the alfalfa looper, Autographa californica, since it is most intensively studied. In nature, baculoviruses occur as virions that are occluded within proteinaceous crystals known as polyhedra (Figure 1) on plant foliage, plant debris and soil. These virions are rod-shaped (hence the term 'baculo') and measure 40–50 nm in diameter and 200–400 nm in length. Their genome is circular, covalently closed and double-stranded of 88–200 kb (ref. 3). The infection cycle is divided into three phases: early, late and very late. During the early phase, there is transcription of genes whose products are essential for viral DNA replication, and these genes are transcribed by the RNA polymerase encoded by the host. This phase continues

**Box 1. Biopesticides**

Biopesticides are biological agents used to control weeds and pests of agriculturally important crops and plants. They are natural enemies of weeds and pests including parasites, diseases and predators. These can provide lasting, highly selective and effective pest control and are very safe from the environmental point of view. Examples of these are ladybugs, small wasps that parasitize caterpillars, B. thuringiensis bacteria that kill many insect larvae, NPVs (nuclear polyhedrosis viruses) effective against lepidopteran pests, fungi that control both weeds and insect pests. The use of chemical insecticides has a long-standing ill-effect on man and environment and many of these usually target varied species of insects (broad-spectrum pesticides). Also, insect resistance to these chemical insecticides has developed. The safety feature of biopesticides as well as their targeting of specific pests, and their relatively new introduction in IPM (insect pest management) programme provides attractive possibility of their expansion in saving crops from damage and loss.

**Box 2. Baculoviruses**

Baculoviruses or NPVs (nuclear polyhedrosis viruses) are the viruses that infect insects, mostly of the order Lepidoptera (butterflies, moths), Hymenoptera (sawflies) and Coleoptera (beetles) and have also been found to infect crustaceans. They are rod shaped ('baculo' meaning rod-shaped) and have double stranded DNA as their genome. In nature, they are found occluded within proteinaceous crystals known as 'polyhedra' on plant foliage, plant debris and soil. Insect larvae get infected when they feed on plant foliage, and after a few days, show feeding cessation and ultimately die. Being un-infectious for non-arthropod organisms, including man, and being of a restricted host range, they have every potential to be exploited for use as a biopesticide.
up to 5 to 6 h post-infection (h pi). Between 5 and 18 h pi, late phase genes are transcribed which encode structural proteins and budding of the nucleocapsid. The very late phase starts from around 20 h pi and is characterized by the transcription of occlusion-specific genes (polyhedrin gene, p10 gene) involved in viral occlusion process. The promoters of these genes are so strong that these genes continue to be overexpressed such that 50–75% of the total protein in an infected cell is polyhedrin protein. The late phase and very late phase genes are transcribed by virus-encoded or virus-modified host RNA polymerase.

Baculoviruses primarily infect insect larvae and adult insects are not susceptible to them. The insect larvae, while feeding on the plant foliage, accidentally feed upon the polyhedra which then get solubilized in the insect's midgut, thereby releasing the virions. These virions replicate within the nuclei of epithelial cells lining the midgut to produce more virions which are released in a budded form by 10–12 h pi or get occluded within the polyhedra late in the infection process. Tissue liquefication and then rupture of these cells upon death of the infected larvae liberates masses of these polyhedra in the soil environment. From here, they are again ready to be ingested and infect their hosts (Figure 2). These virion-containing polyhedra are highly stable persisting in the soil environment for many years.

Research on the use of baculoviruses as insecticides has focused mostly on baculoviruses isolated from Lepidoptera (butterflies and moths), Hymenoptera (sawflies only) and Coleoptera (beetles) since these present the best options for pest-control. Their use has been favoured in some countries, e.g. Brazil, China and the former Soviet Union. Since they can be produced relatively easily locally and although being labour-intensive, their production requires less capital expenditure, they are particularly suitable for use in developing countries. Being restricted to a few species, baculoviruses do no harm to the beneficial insects, and because of their persistence in the environment, they are suitable for use in long-term, ecologically-sound control programs.

**Natural baculoviruses as bio-insecticides**

Isolation of local strains of baculoviruses allows the selection of a virus better adapted to a particular host or ecosystem. The majority of baculoviruses have been isolated from the order Lepidoptera (over 500). The general approach for the development of most pest-control programs utilizing baculoviruses is as follows:

**Screening**

A number of criteria can be used to determine the insecticidal activity of available isolates. These are – LD$_{50}$, the lethal dose, the dose of the virus at which 50% of the test insects are killed; LC$_{50}$, the lethal concentration, the concentration of the virus that the test insects feed on which results in 50% of the test insects being killed; LT$_{50}$, the lethal time, time at which 50% of the test insects are dead; the effects on feeding ability and plant damage. Several insect bioassay techniques have been developed for the measurement of these parameters. Two most commonly used techniques are: diet plug assay in which the insects are fed with artificial or semi-artificial diet mixed with viral suspension; droplet feeding assay, where the viruses are fed in small droplets placed near the larvae. These criteria help in determining the efficiency of killing of insects by the virus and so their use as potential control agents.

**Virus production**

After the efficacy of a particular baculovirus as control agent has been established, there is the need for producing the isolate in larger quantities. At present, the production process used is *in vivo*, i.e. the insect larvae are fed with the viruses. The infecting viruses replicate inside their hosts to produce many more copies of themselves. Maintenance of temperature between 20°C and 26°C is very important. Minimum quantity of inoculum to produce the maximum yield and quality of the virus is found out as also the time when the virus is harvested from the larva, e.g. in *Lymantria dispar*, the activity of the virus increases up to the fourth instar and then decreases in the fifth. To liberate the viruses from the larval bodies, the larvae are blended with a diluent such as water and then filtered through a muslin cloth.

**Formulation**

Most baculovirus products are produced in the form of concentrated wettable powders. Methods used are spray
or air-drying after dilution with an inert carrier, freeze-drying with a carbohydrate, or acetone precipitation in lactose. A good residual activity on the target site is essential, and so dependance on the nature of the substrate and the effect of environmental factors comes into being. While little is known of the effect of substrate on the virus viability, of the environmental forces that might affect them, UV radiation at a wavelength of 290–320 nm completely inactivates the virus. Therefore, UV protectants such as metallic oxides or anti-evaporants, spreaders/wetting agents, etc. are also added to the virus formulations. Care is also taken to maintain the pH since baculoviruses cannot withstand alkaline conditions.

**Pest biology**

Knowledge of pest biology and behaviour is also a must in the integrated pest management approach to pest control. A virus will generally fail in the field if it is not applied at the right place and at the right time, however effective it might be in the laboratory. Knowledge of insect behaviour on the crop after hatching, its distribution within the crop in each instar and the area of foliage ingested per instar, etc. allows effective use of the virus. For Lepidopteran pests, the larval stage is damaging to crops, with most of the damage being done in the final two instars, so the baculovirus must be applied at the larval stage.

**Method of application**

Most baculoviruses are applied as sprays, with the spray-droplet size playing a key factor. Smaller droplets give a better surface coverage on the foliage, thus increasing the chances of an insect encountering a virus-containing droplet. Other application techniques are the release of infected insects in the field; or application as baculovirus dusts to stored product pests, where water-based sprays might pose a problem by encouraging the growth of fungi in stores; or application as baits usually based on bran for pests such as cutworms which are hidden in the soil for most of their lives, etc.

Some of the registered or commercialized baculoviruses against forest and agricultural insect pests are listed in Table 1.
Table 1. Registered or commercialized baculoviruses as insecticides

<table>
<thead>
<tr>
<th>Country</th>
<th>Product name</th>
<th>Active against</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>Elcar</td>
<td>Cotton bollworm (Heliothis zea)</td>
</tr>
<tr>
<td>United States</td>
<td>Tm-Bio-Control-1</td>
<td>Douglas-fir tussock moth (Orgyia pseudotsugata)</td>
</tr>
<tr>
<td>United States</td>
<td>Gyphrek</td>
<td>Gypsy-moth (Lymantria dispar)</td>
</tr>
<tr>
<td>Brazil</td>
<td>Multigen</td>
<td>Velvet-bean caterpillar (Anicuaria gennaialis)</td>
</tr>
<tr>
<td>Canada</td>
<td>Leconti-virus</td>
<td>Red-headed sawfly (Neodiprion leconti)</td>
</tr>
<tr>
<td>UK</td>
<td>–</td>
<td>Pine beauty moth (Panolis flammea)</td>
</tr>
<tr>
<td>China</td>
<td>–</td>
<td>Cotton bollworm (Heliothis zea)</td>
</tr>
</tbody>
</table>

Product name not available.

Although numerous naturally occurring baculoviruses have been tested or even commercialized in a few cases, their use has not expanded as greatly as their development. Their global use has been hampered by various constraints such as the slower speed of kill, narrow host range, product stability, registration and patentability, etc. In contrast to the slower speed of killing by the baculoviruses, chemical pesticides provide a rapid knockdown effect. Their specificity to a few species can be visualized as both their advantage and disadvantage. The advantage is of conserving beneficial insects while killing the targeted ones, and the disadvantage is their limited use in cases where a variety of insects may be infesting a particular crop. This also limits the market potential for their use and commercialization by large agro-chemical companies.

Genetically-engineered baculoviruses as bio-insecticides

To address some of the above problems, biotechnological approaches are being explored. A good deal of the molecular biology of baculoviruses has been learnt and their development as expression vectors has facilitated in their improvement for use as bio-insecticides. Foreign genes exhibiting insecticidal activity driven by the promoters of very late baculovirus genes (polyhedrin or p10) can be expressed in this system in order to improve LT50 or LD50 (ref. 8). Host range also has the potential to be either reduced or expanded as desired9 (Box 3).

Improving insecticidal activity by genetic engineering

Natural insecticidal activity of baculoviruses can be improved by inserting foreign genes encoding insect-specific toxins, hormones, enzymes or other gene products exhibiting insecticidal activity, in lieu of baculovirus' strong, very late promoters (polyhedrin or p10). Some examples are listed in Table 2.

Improvement can also be done by the deletion of a gene(s) from the viral genome that reduces virus-mediated killing but is not essential for viral replication. Examples are genes that block apoptosis, e.g. p35 (ref. 10) or genes which lengthen the life of the infected host by blocking molting, e.g. ecysteoid UDP-glucosyltransferase11.

The first move towards the development of a recombinant baculovirus in order to enhance its insecticidal activity was the introduction of a gene coding for the scorpion, Buthus eurpeus, insect toxin-1 (BeIT) into the AcNPV genome under the control of the polyhedrin promoter12. BeIT is an insect-specific neurotoxin and induces paralysis and halts feeding. Sufficient toxin was not produced for a detectable biological activity. It was thought to be due to either low toxin concentration or instability of the protein because of an improper signal sequence used. Replacement of Bombyx mori nuclear polyhedrosis virus (BmNPV) polyhedrin gene with the diuretic hormone (DH) gene from the tobacco hornworm, Manduca sexta, produced a positive result13. At four days post-infection, all the larvae infected by the recombinant virus died whereas the larvae infected by the wild-type virus died at five days post-infection.

Juvenile hormone esterase (JHE) inactivates juvenile hormone (JH) by hydrolysis in the last instar lepidopterous larvae. A reduction in the titre of JH early in the last larval instar initiates metamorphosis and results in the feeding cessation. The JHE gene from the tobacco hornworm, Heliothis virescens, was inserted into the AcNPV genome under the control of the polyhedrin promoter14. When these recombinant viruses were fed to the first-instar larvae of Trichoplusia ni (T. ni), a profound reduction in feeding and growth was seen as compared to the control or wild-type virus-infected larvae.
Table 2. Foreign gene products exhibiting insecticidal activity and their effects upon the insect host

<table>
<thead>
<tr>
<th>Foreign gene product exhibiting insecticidal activity</th>
<th>Effect on the host insect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scorpion (<em>Buthus euepeus</em>) toxin-1 (BeIT)</td>
<td>Paralysis, halts feeding</td>
<td>2</td>
</tr>
<tr>
<td>Diuretic hormone</td>
<td>Reduction of hemolymph</td>
<td>13</td>
</tr>
<tr>
<td>volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile hormone esterase</td>
<td>Halts feeding</td>
<td>14</td>
</tr>
<tr>
<td>Scorpion (<em>Androctonus australis</em>) toxin (AaIT)</td>
<td>Tremors, feeding cessation, paralysis</td>
<td>15</td>
</tr>
<tr>
<td>Mite (<em>Pyemotes triticum</em>) toxin</td>
<td>Paralysis</td>
<td>16</td>
</tr>
<tr>
<td>Bacillus thuringiensis toxin (Sp-endotoxin)</td>
<td>Halts feeding</td>
<td>17</td>
</tr>
</tbody>
</table>

Infections of the later stage larvae showed no effect. It was attributed to a low level of JHE produced in later stages under the control of a very late promoter which is not sufficient to overcome the level of hormone biosynthesis. It was also speculated that the viral-induced production of ecdysteroid UDP-glucosyltransferase reduced the effects of JHE.

The recombinant viruses carrying DH and JHE did not show a relatively strong increase in potency as compared to wild-type viruses. So, an insect-specific toxin, AaIT, isolated from the venom of scorpion, *Androctonus australis* was tried\textsuperscript{15}. This toxin affects only insects and shows no effect on isopods and mammals even at high doses. It selectively targets the insect sodium channel. AaIT gene preceded by a signal sequence from silkworm neuropeptide bombyxin was inserted in place of polyhedrin gene under the control of polyhedrin gene promoter in BmNPV. When *Bombyx mori* larvae were injected with these viruses, symptoms consistent with sodium channel blocking were seen. Tremors and feeding cessation occurred at 40 h pi followed by paralysis and death at 60 h pi, which occurred in all the larval instars tested. In contrast to the experiment with *Buthus euepeus* toxin, BeIT, where the signal sequence used was from human interferon-α, the use of the signal sequence from silkworm bombyxin allowed the measurement of a detectable biological activity.

Insertion of mite toxin from female mites of the species *Pyemotes triticum* into the AcNPV genome also produced a biological response, i.e. paralysis at a much faster rate compared to that of the wild-type virus\textsuperscript{16}.

Inserting the delta-endotoxin gene from *B. thuringiensis* to enhance the insecticidal activity of baculoviruses has also been tried\textsuperscript{17}. Natural isolates of *B. thuringiensis* have been used commercially as insecticides. Crystalline inclusions containing the protoxin are produced by the bacteria, which are ingested by the insects. In the insect midgut, the protoxin is cleaved producing an active toxin which causes an immediate reduction in feeding. The toxin generates pores in the cell membrane leading to the disruption of osmotic balance and cell lysis and eventual death. Production of recombinant AcNPV containing the protoxin sequence was followed by bioassays using second instar *T. ni* larvae. No detectable enhancement in pesticidal properties could be observed as LD\textsubscript{50} and LT\textsubscript{50} with the Bt recombinant and wild-type viruses were not significantly different. However, Bt toxin produced in the larvae when liberated upon death of the larvae could be expected to serve as secondary control. Also the possibility of effecting a faster death of the larvae by the insertion of active toxin sequences in the viral genome cannot be ruled out.

Ecdysteroid-UDP glucosyltransferase (EGT) is produced by AcNPV and maintains the insects in an actively feeding state throughout the infection by blocking molting. The deletion of the gene encoding EGT was thought to accelerate the virus-induced mortality by allowing the infected larvae to begin molting, resulting in feeding cessation. It was indeed so. *Spodoptera frugiperda* larvae infected with egt-negative virus fed less and died more rapidly than those infected with wild-type virus\textsuperscript{18}. An egt-negative baculovirus by itself or an egt-negative baculovirus overexpressing genes such as JHE can be used for the development of enhanced baculovirus insecticides. Wild-type viruses with egt gene cannot be used for overexpressing such genes, since they could not be expected to have any significant effect due to the inhibition of ecdysis by egt.

A new strategy has been devised recently to halt larval growth and feeding much earlier. An antisense gene fragment complementary to the mRNA of a host gene whose protein product is presumed to be essential for larval growth and development is used. As the two transcripts (sense and antisense) would remain bound to each other, there would be a block in the translation of an essential protein and normal insect physiology would be altered. This antisense approach was found to be efficient and larvae stopped feeding almost immediately as the polyhedrin promoter driven transcripts started to appear\textsuperscript{19}.

**Improving host specificity by genetic engineering**

Baculoviruses have very narrow host range. AcNPV has the widest known host specificity among baculoviruses, infecting more than 30 species of Lepidopteran insects\textsuperscript{20}, and so has the greatest potential for development as an insecticide. Whole insects such as the mosquito, and cells of a non-permissive insect cell line have been found to incorporate baculovirus DNA in their nuclei after being inoculated with the baculovirus. On the other hand, in mammalian cells, although viral particles were found to be poorly incorporated into the cytoplasm,
there was no evidence of their DNA being incorporated in their nuclei\textsuperscript{21,22}. This suggested that the host specificity of baculoviruses is tightly controlled at the level of gene expression. For the study of host-range determining mechanisms of baculoviruses, AcNPV and BmNPV have proved to be suitable since these two have a high DNA homology. The general nucleotide sequence similarity between the two is 70% and homology between well-conserved genes is over 90%. Their non-overlapping host specificity has also been advantageous. In-vitro experiments have revealed that AcNPV can be host-range expanded, i.e. made infectious to a BmNPV-specific cell line, BmN, by homologous recombination within a 572 bp-region of a AcNPV gene encoding a DNA helicase\textsuperscript{23}. These experiments also suggested that the AcNPV helicase gene could be one of the many genes controlling species specificity during virus replication. By manipulating this helicase gene, host range could be expanded or reduced as desired. A 79-nucleotide sequence within the p143 helicase gene was identified which was capable of extending the AcNPV host range in-vitro\textsuperscript{24}. In this 79-nucleotide region, AcNPV and BmNPV differ at six positions corresponding to four amino-acid substitutions. Replacement of three AcNPV-specific amino acids in the AcNPV genome by three corresponding BmNPV-specific amino acids of the p143 protein resulted in the extension of AcNPV host range to the Bombyx mori larvae. The limited ability of AcNPV to extend its host range to semipermissive or non-permissive insects or cell lines is surmised to be due to blockage of AcNPV replication cycle at different time points which may be specific to a particular host.

An AcNPV gene which blocks apoptosis, i.e. p35, has also shown its role in determining the host-range. Infectivities of wild-type virus and p35 mutant virus were similar in T. ni larvae, but in S. frugiperda larvae, p35 mutants were significantly less infectious. This result was observed when either the budded virus is injected\textsuperscript{25} or when an occluded virus is given as a contaminant of food, i.e. per os\textsuperscript{26}. These studies have opened up the possibility of tackling with one of the main constraints faced in the direction of commercialization of baculoviruses for use as bio-insecticides.

**Engineering strategies**

In the baculovirus expression vector system, a commonly-used strategy is the replacement of polyhedrin gene-coding region by foreign gene(s) exhibiting the desirable trait which then comes under the control of strong polyhedrin promoter. As a result, polyhedrin is not expressed at all and the progeny virions thereafter produced are non-occluded. Although this feature is desirable from the point of view of biological contain-ment, it is undesirable since non-occluded forms of virions are highly unstable and therefore they cannot be delivered to the field in an active state. So, the main issue is the production of genetically-engineered baculovirus in an occluded form. A few of the strategies to deal with this problem are:

(i) Host cells are co-infected with both the wild-type, polyhedrin-positive and genetically-engineered, polyhedrin-negative viruses\textsuperscript{27}. All the progeny virions thus produced are occluded by the polyhedrin protein produced by the wild-type viruses. This co-occlusion process permits the delivery of genetically modified, polyhedrin-negative baculoviruses to the field in a stable, infectious form. Persistence of such a virus in a virus population is determined by the probability of co-infection of individual larvae and cells with both virus types as the virus is passed from insect to insect.

(ii) Replacement of another very late gene, the p10 gene, with foreign gene inserts has also been proposed\textsuperscript{28}. Recombinant viruses lacking the p10 gene produce polyhedra lacking a polyhedral envelope and exhibit significantly reduced LD\textsubscript{50} values as compared to the wild-type viruses. So, polyhedrin-positive viruses with insecticidal gene inserts in the p10 region would be sufficiently stable for commercial use and at the same time, environmentally unstable, desirable in the case of use of recombinant viruses.

(iii) Both the polyhedrin and p10 promoters are very late. The foreign genes under these promoters will be expressed approximately 10 h p.i. So, it will be useful to shorten the time. The use of early viral gene promoters to control the expression of foreign genes and to retain polyhedrin expression has been suggested\textsuperscript{29}. The use of immediate-early promoters may help in the expression of foreign genes during semi-permissive or non-permissive conditions, so that the host-range specificity may be lost\textsuperscript{30}.

**Field-release testing of recombinant baculoviruses**

The first field-release testing of a genetically altered form of AcNPV was carried out in England in 1986 (ref. 7). The modified virus had an 80 bp non-coding insert downstream of the polyhedrin gene-coding region. Subsequent trials with these polyhedrin-negative viruses revealed their lower persistence level. Field release testing in USA in 1989 of modified viruses lacking a functional polyhedrin gene occluded through a co-occlusion process revealed their persistent nature\textsuperscript{4}. By the second and third year, polyhedrin-negative AcNPV accounted for roughly 9% and 6%, respectively, of the population. In 1993 and 1994, field-release trials of a recombinant AcNPV carrying an insect-specific scor-
pion-toxin gene (AaHIT) derived from the scorpion Androctonus australis Hector were conducted in England. The genetically modified virus (AcST3) and the wild-type virus (AcNPV C6) were compared after spraying at low, medium, and high doses against third instar T. ni larvae on cabbage. Virus treatment significantly reduced insect damage compared to untreated controls. Larvae infected with the recombinant virus died by paralysis 10–15% earlier than the larvae infected with wild-type virus. A 22% reduction in leaf area was caused by untreated larvae, while the virus-treated plots showed a loss of only 12.5%, resulting in a reduced crop damage. It was interesting that secondary transmission of modified virus was found to be lower than that of the wild-type virus. While in the initial samplings, there was no difference in the mortality between the two virus treatments; on the last samplings, the modified virus exhibited significantly less mortality. Wild type NPV-infected insects usually remained on the plant after death where they liquified, thus releasing a large body of inocula for further infections. Recombinant virus-infected larvae fell onto the soil and did not lyse reducing the virus yield and thus the reduction in secondary transmission. These results highlighted the importance of speed of action in achieving reduced crop damage.

An AcNPV lacking its ecdysteroid UDP-glucosyl transferase gene had also been field-released in USA in 1994 and the speed of insect killing was found to be faster by about one day as compared to that of the wild-type virus.

How safe is baculovirus as a bio-insecticide?

In terms of the effect on vertebrates (including humans) and beneficial insects and at the molecular level, baculoviruses have been confirmed to be safe. Being a natural agent, they pose relatively little danger to the environment as compared to the persistent, dangerous, broad-spectrum chemical insecticides that leave their residues even after their application has long been withdrawn and also harm non-target species. Safety of the recombinant baculoviruses has also been examined in the field trials described above.

There has always been much skepticism and concern regarding the general safety of any genetically-modified organism, be it plant, animal or a microbe. Genetically modified baculoviruses have been no exception. Although potential benefits have been well-acknowledged, significant risks associated have also been pointed out: whether the modified virus might attack non-target species; whether it will cause ecological imbalance by displacing the wild-type population due to its selective advantage with respect to persistence or infection efficiency; whether it might exchange genes with other wild-type viruses, or frequently resort to mutations, so that new, dangerous strains of viruses might evolve; whether it might be so persistent that when identified as unsafe, it would be difficult to remove it from the environment as rapidly as possible, etc. In fact, the field-release of scorpion-toxin containing recombinant baculovirus in order to study its efficacy, potentiality and safety in natural surroundings had sparked Britain's biggest row over biotechnology.

However, attempts have been made at answering most of these questions. For example, secondary transmission of recombinant viruses was found to be lower as compared to wild-type viruses in the above field-release testing, dispelling the fear of spread to other ecological settings. The theoretical possibility of swapping genes may not be there in the actual situation, since recombination must occur between two closely-related compatible viruses. Recombination between distantly-related viruses would be a remote possibility. A more careful approach to the construction of genetically-engineered baculoviruses, rigorous laboratory assessments and suitability testing, and open discussions between the scientists and the public could go a long way towards clearing many of these doubts. The use of engineered viruses having stable genetic properties that do not resort frequently to mutations and gene transfer will also be favourable.

In an interesting study, beneficial insects such as social wasps, Polistes metricus, were infected with a recombinant baculovirus expressing a scorpion toxin or mite toxin under the control of hsp70 promoter which is active in a variety of insect cells. Although detectable levels of toxin were found, there was no evidence of their effect on the growth or social behaviour of these wasps. When recombinant baculoviruses carrying a marker gene under the control of baculovirus very late promoters were used to infect the wasps, no expression of reporter gene was found (except in one case), indicating that baculovirus promoters (especially of very late genes) are not active in other insects.

Also, pest resistance to chemical insecticides and even to B. thuringiensis endotoxin has been shown. The US National Academy of Sciences has noted a 64-fold increase in pest resistance to pesticides in less than 50 years. Insect resistance to baculoviruses, although being reported, is relatively less frequent and so they present a more effective approach to pest control.

Conclusions

Baculoviruses have a great potential for development as pest-control agents considering their safety, specificity and efficacy; and considering the dangers posed by chemical insecticides and development of insect resistance to these and to other bio-pesticides like B. thuringiensis toxin. It is another matter that they have their limitations as well.
The development of baculovirus expression vector system technology has been advantageous, in an attempt to overcome some of these limitations. Recombinant baculoviruses constructed and produced in laboratories, have been tested in field situations to confirm their safety and efficacy. Results have been encouraging, but there is much scope for improvement as well. Further basic and applied studies are required too, since knowledge about the ecology of baculoviruses has been regarded as a black box. Open discussions between scientists and public will go a long way in creating awareness and for dispelling fears arising out of the use of newly-created baculoviruses.

Scientists studying baculoviruses are enthusiastic that their efforts of today would pave the way for the baculoviruses achieving their well-deserved place as bioinsecticides in the twenty-first century. There is hope that it would not be long before the baculoviruses prove a boon to agriculture.


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