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Expression of a Paralytic Neurotoxin Gene to Improve Plant Protective Efficacy of Baculoviruses as Biopesticides

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Abstract

Due to increased pressures in current years to reduce the use of agro-chemical products and their residues in the environment and food, biological control of agricultural pests has gains in importance. Some families have viruses which infect insects, but only the highly specialized Baculoviridae family has been used as biopesticides. Their specificity is very narrow and secure for people and wildlife. Because of their slow killing and technical difficulties with in vitro commercial production, their use as bioinsecticides was until recently limited. Two methods will be applied in future for the broader application of biopesticides by baculoviruses. The development will be largely at the level of treatment and in vitro output and improvements in formulations of biopesticide in countries with minimal use of genetically modified organisms. The second approach allows for genetic changes of the genome of baculovirus with genes from another natural pathogen to improve the killing ability of baculoviruses. Genetic modifications of baculoviruses are expected to be introduced gradually in countries that have fewer concerns about GMOs.

Key words: Agro-chemical products, Agricultural pests, Biopesticides, Bioinsecticides, GMOs.

Introduction

Since chemical pesticides also present environmental and health hazards, carefully selected biological control agents such as rodents, parasitoids and pathogenic products have become attractive alternatives for insect pest control on the ground. Arthropod-specific pathogens are Baculoviruses (Baculoviridae) that have been used as microbial biopesticides in several decades to control many lepidopteran pests [1]. These pathogens are characterized by large, double-stranded genomes of DNA and rib-shaped virions, which form polyhedra in polyhedrins. While baculoviruses have an insect control potential, infected lepidopteran larvae still cause considerable crop loss, resulting in reduced field efficacy because of the continued feeding for several days after the initial infection.

Several studies have shown that insect pest control viruses have significantly improved their efficacy when foreign genes are inserted into the virus genome [2]. It includes genes encoding the diuretic hormone Manduca sexta (Linnaeus), the young hormone esterase Heliothis virescens, the d-endotoxin Bacillus thuringiensis, mite or spider toxin, protease and scorpion neurotoxins. The major contribution of exogens to the effectiveness of baculovirus in pest management is demonstrated in all of these studies. A polypeptide with 61 residues is the LqhIT2 depressant insect toxin derived from Israel's yellow Leiurus quinquestriatus hebraeus (Ehrenberg). Injecting LqhIT2 toxin into sarcophagus foam larvae (pandella) caused by the induction of long-term flaccidity symptoms typical of transient excitatory effects. The larvae are completely inactive, immobilized and paralyzed.

The insecticide activity of the very late p10 promoter was greater than the early p35 promoter when the toxins of the scorpion were tested using recombinant bacculoviruses. These data suggest that promoting efficiency may play a role in the infection of recombinant baculoviruses by insect pest larvae as an early promoter would have an earlier effect than the late promoter. If the original promoter is good enough, the recombinant virus should be more lethal. The Cytomegalovirus Minima human (CMVm) promoter, connected in a cis with a newly discovered activator, was shown to produce high rates of expression of foreign genes by the upstream polyhedrin (pu) sequence and early p-PCm (pu plus CMVm) promoter. Though the early p-PCm promoter was less luciferase than the late p10 promoter, the former expressed a better overall luciferase activity earlier.

1. Agents used for pest bioregulation:

Biological regulation can be defined as the regulation by another living organism added to the environment of a species that has reached the level of a pest to prevent pesticides [3]. There is, therefore, a natural or genetically modified enemy ecological management of pesticides. Depending on the target species, the natural enemy alternative will differ significantly. Insects contain many natural pathogens, including bacteria, fungi, nematodes and viruses, in addition to predators. Artificially applied, these pathogens can effectively kill pests as microbial pesticides. As the pest is being used as a nutritional source, the population of microbial pathogens and/or predators increases, which in turn leads to gradual population decrease [4]. Biological control may be potentially permanent because the natural enemies provided from the outside are founded in the pest population and can protect themselves against target pest species for a longer period of time.

The ways of applying biological control are divided into three main groups: production, the protection and increase of natural enemies and the application of microbial pesticides, including predators, pests and causative agents. Import can be defined as a foreign biology control agent for populations, while conservation or increase involves actions which preserve an indigenous agent and allow an indigenous agent to increase their number and opportunities to act as a homosexual factor in the treatment of a plague. The entry of foreign enemies is the most vulnerable to ecological equilibrium changes by these approaches. On the other hand, the introduction of a foreign species was one of the most successful attempts to introduce a bio-regulatory drug. In California orange orchards were first introduced at the end of the 19th century to monitor the scale, Icerya purchase cotton cushion, and Australian lady bug species *Rodalia cardinalis*. The majority of the techniques is commonly used and is technically relatively safe. Ultimately, the benefits of biopesticides production outweigh the money lost due to failures that occur often [5]. Although essential, economic benefits are not only biocontrol's advantages. Animal life, as effective biocontrol decreases toxic chemicals emissions, farmers and their families often benefit greatly [6].

The most commonly used and cheapest microbial pesticides than any other form of pest bioregulation are possibly. Some species of bacteria can infect insects, but the Bacillus species are the most widely used pesticides. The most successful of these Bacillus thuringiensis [7]. It is a gram positive, parasporal-shaped bacterium. A variety of molecular mechanisms have been developed to generate pesticide toxins, most of which have been programmed by several essential genes.

A variety of insects and other arthropods are associated to a large number of fungi and create various interactions with them including pathogens. Around 750 species of entomopathogens have been identified, belonging to 100 genera, but only around 10 of them are currently established for insect control.

2. Molecular biology of baculoviruses:

Baculoviruses are the largest group of arthropode viruses known for their potential in agriculture and forestry as biological control agents of pests [8]. They are widely used in biotechnology as expression vectors. There are two genera of the Baculoviridae family, Nucleopolyhedrovirus (NPV) and Granulovirus (GV). NPVs can be classified into Group I and Group II phylogenetically [9]. In infected cells (polyhedra and granules, typical polyhedra are shown in Fig. 1) these viruses cause a large number of occlusion bodies, which allow viruses to survive in the environment and spread the disease from insect to insect.

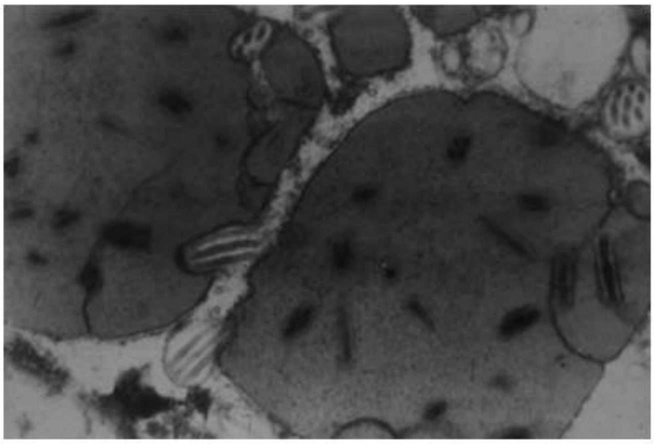

Fig.1: Electron micrograph *Anticarsia gemmatalis* nucleopolyhedrovirus occlusion body formation.

Among vertebrates, the baculoviruses infected arthropods does not replicate in vertebrates, plants and microorganisms [10]. But they cannot reproduce, but may join animal cells under special conditions. Such surprising properties have in these last few years made them a valuable tool in studies of the transience of foreign genes in the genome of baculoviruses. Baculovirus is a large number (more than 600 species described) double-stranded DNA virus; most were insulated from several insect orders: Lepidoptera, Diptera, Hymenoptera and

Coleoptera. The restricted host range is usually limited to several closely related species by individual Baculoviruses. Virions consist of a (SNPV) or higher (MNPV) nucleocapsids embedded in the surrounding membrane. The size of the viral genome is between 80 and 200 kb. The Autographa californica nuclepolyhedrovirus (AcMNPV) is the most widely studied baculovirus [11]. Early work was done on AcMNPV to grow viral pesticides and to construct vector expression based on baculovirus.

Fig. 2 summarizes the natural cycle in AcMNPV infection in insect larvae [12]. As pollutants of their milk, caterpillars eat polyhedra. In the alkaline environment of larval cavities, the paracrystalline polyhedrin matrix is solubilized and the released viruses (occlusion-based virus — ODVs) pass into midgut cells following fusion with microvilli membrane. The virions are discharged into the nucleus, which specifically regulates the expression of viral genes. There are four phases recognized in transcripts: immediate, early, late and very late transcripts. At this stage, host transcription factors are trans-activating immediate early genes and viral proteins are not needed. Delayed early gene transcriptions involve activation by the early gene products. A transcription starts in highly conserved promoter elements: early gene CGT and late gene TAAG, usually upstream from ATG start codon, at 30–90 bps. In the delayed early phase, the DNA and late gene products of the Virus are synthesized. Initiation of viral DNA replication describes the late phase of infection as those cases. Late AcMNPV genes are mainly transcribed at 6–24 hours a day, while very latter genes at about 18 hours a day are transcribed into a rapid explosion and continued until 72 h p.i. The development of infectious BV is significantly reduced during the very late stage. Nucleocapsides interact with nuclear membranes and are typically enclosed in particle groups. Nucleocapsid production is a key first step in the process of nucleocapsid occlusion by the very late polyhedrin protein. The occlusion continues on until the nucleus is completely filled with occlusion.

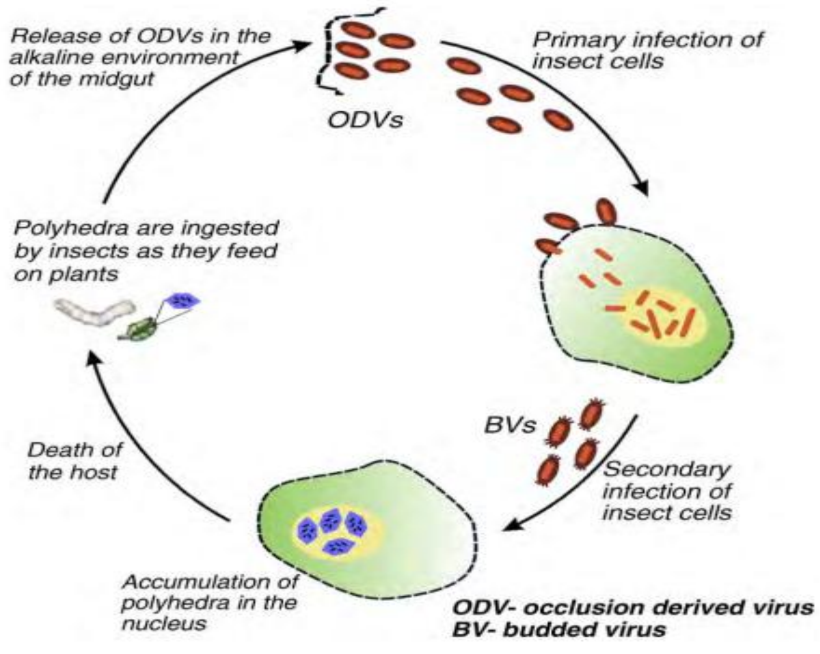

Fig.2: Natural life cycle of baculovirus AcMNPV.

3. Baculovirus pesticides – past and present:

Baculoviruses were attempted to protect European forests in the 19th century, but the first baculovirus was introduced into the world and the disease was effectively controlled in a large area in the 1930s. Although invertebrates are considered safe and selective bioinsecticides, their application has not matched the ability to control pests in crops, forests and pastures. Their application is not compatible with their standard [13].

The exception to this is the A. gemmatalis nucleopolyhedrovirus (AgMNPV) operation of soybean velvet bean caterpillar [14]. The system was initiated in Brazil in the early 1980s and now more than 2,000,000 ha of soya are infected with the virus every year. Though the use of this virus in Brazil is the most remarkable example of viral pesticide bioregulation in the world, the virus is still mainly generated through infection of larva in soy farming in vivo production. A huge increase in the demand for virus production is now the need to protect 4 million hectares of soybean each year. This high demand for AgMNPV allows the study to ensure that the virus is continuously cheap in vitro since broad in vivo baculovirus production encounters a lot of difficulties. However, the generation of mutants, which may lead to loss of virulence, is a major problem in the production of the virus, particularly in in vitro serial passages.

Genomic shifts are a well-documented phenomenon in wild-type isolates of baculoviral populations. The involvement of sub-molar fragments in electrophoretic patterns of endonuclease digestion products is detectable by genotypic variants. Plaque purification of wild isolates indicated that these varieties contain a mixture. Genotypes in the genomes of baculovirus may include both small and big deletions and insertions of point mutations. All regions of the genome are able to change, but certain genome modifications including insertions due to transposable elements or deletions of the hypervariable DA26 gene area have been known to be present at certain hot spots. Wild-type isolates have been reported to contain a mixture of those variants by plating purification. Genotypical variability in genomes of baculoviruses, both small and large deletions and inserts can include point mutations. The existence of certain genomic modifications, such as insertion due to transposable elements or deletion in the hypervariable DA26 gene region, is expected to differ in all genome areas. The genomic variation of AgMNPV has been carefully examined as the selection pressure in the field that lead to alterations in the stability of viruses because of the application of AgMNPV over the following years [15]. The DNA profiles of 11 different seasonal isolates were tested for the genetic stability of this virus. The viral DNA was initially purified in contrast to AGMNPV-79, a wild-like virus used initially and subsequently in this experiment, by diseased larvae collected in several crop seasons. In general it continued in the subsequent years when an alteration was introduced in the viral population, such as a new cleavage site. In evaluating the virulence of these isolates by means of bioassays, the most important finding was that the virus has been held pathogenic during years of use. These findings show that even with certain genetic changes in the DNA restriction profiles, the virus maintains substantial stability.

Conclusion

In our research we obtained all recombinant viruses from linear commercial baculovirus viral DNA and not from the baculovirus genome. Hopefully, as it is already clear in the least stringent attitude of the legislature in numerous countries towards this problem, the negative influence of genetically modified organisms on biotechnological research will gradually decline. This can be seen in two of the world's most populous countries – China and India. Genetically modified biopesticides are therefore highly likely to increasingly increase their market share in pesticides. The public should be properly educated about the dangers and advantages of chemical and biologic pesticides so that it can be promoted. The awareness that biopesticides are focused on baculovirus formulations pose much lower environmental risks than traditional chemical pesticides, as the biology of baculovirus remains uncertain.

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