Yield improvement in Baculovirus biological control

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Introduction

Baculoviruses high specificity towards Lepidoptera species make them potential biocontrol agents (BCA) in agriculture and forestry. Thanks to their genome able to carry up to 50kb of exogenous DNA[1] it is effective to use genetic manipulation to insert new potential genes for a yield improvement in biological control. Baculovirus pest control relies on its restricted host range to one or few species[2]. At this moment, high specificity and low toxicity levels can't be found in pest control by using chemical products.

Ideally, in order to launch a baculovirus product to market with success it would be necessary to increase the virulence 100 to 1000 times from the original wild type virus[3]. The aim of this review is to provide solutions and strategies for the development of efficient and rentable biopesticides based on baculovirus. A compilation of results from many studies is exposed below divided in several keystones.

Virulence

Almost all the current proposals to improve the virulence require of genetic manipulation to insert exogenous genes with new or improved properties. A compilation of results is exposed in Fig 1. containing different types of tested genes. Most popular genes encode for:

➢**Insect specific proteases** that digest the basement membrane of Lepidoptera and cause fragmentation and myelination of the tissues as seen on figure 2. E.g. Scath L[6].

➢**Insect specific toxins** from mites, scorpions, spiders, sea anemones and bacteria act on major ion channels resulting in immediate paralysis. E.g. Bt toxin[7].

➢**Insect specific hormones** overexpressed or early expressed are able to cause abnormalities such as abnormal growth, feeding alterations and death of the insect. E.g. Diuretic hormone [4].

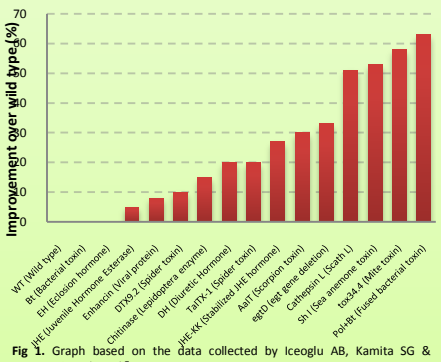


Fig 1. Graph based on the data collected by Iceoglu AB, Kamita SG & Hammock BD (2006)[7].

Production

The main method for baculovirus mass production is the "in vivo" culture using Lepidoptera larvae. Boxes containing 100 individuals are fed with occlusion bodies (OB) mixed with synthetic food. Over $1.4x10^{11}$ OB are obtained from each box. Unfortunately 20% of the larvae are usually lost due to cannibalism[5].

$5x10^{11}$ OB are needed to treat one hectare of crops. Lepidoptera juvenile hormone analogs can be used efficiently to increase three times the number of viral particles per larvae produced.

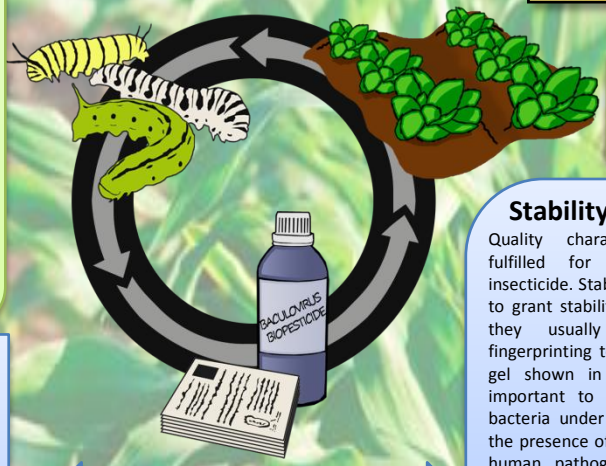
BIOLOGICAL

Host range

The high natural specificity of baculovirus is both good due to low harming of other species and bad because of the limited potential in market.

Specificity is determined by Gene-for-gene relations. Until now it has only been proven how to increase more the specificity by deleting essential genes for viral replication [4].

It is necessary to choose the baculovirus strain previously according to the natural specificity between our virus and the target Lepidoptera species.



ECONOMICAL

Persistence

Stilbene derivates are used to protect the OB and absorb UV radiation. They also increase the insecticidal capacity up to 1000 fold thanks to the interaction with the peritrophic membrane in the insect midgut [5].

Maintaining the polyhedrin matrix is also essential to extend the virus lifespan. Because the polyhedrin gene is usually replaced for other genes to increase the strain virulence, the solution left is to produce the recombinant virus along with the wild type (wt) and thus obtain both viruses enveloped. A wt virus envelope is shown in figure 3.

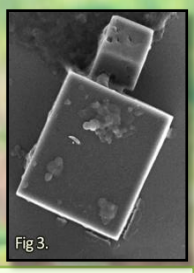
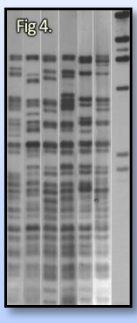


Fig 2. Lymantria dispar NPV by Michael Grove. www.galileonet.it
Fig 3. Scanning electron microscopy of Autographa californica MNPV (Coulibaly F *et al*. 2009).
Fig 4. Restriction profiles of *Orgyia pseudotsugata* SNPV (Williams HL *et al*. 2011).

Stability and registry

Quality characteristics must be fulfilled for any microbiological insecticide. Stability tests are required to grant stability of the genome and they usually consist of DNA fingerprinting techniques as the RFLP gel shown in figure 4. It is also important to maintain the aerobic bacteria under 10^8 cfu/g and avoid the presence of coliform bacteria and human pathogens. Cold storage is recommended between -20 and 4 ºC for a maximum of 18 months [5].



Registry process is one of the greatest obstacles before launching a new BCA product. United States of America is the preferred region because in Europe it takes 3-4 times longer to register the product and it is 2-4 times more expensive.

Conclusions

While promising, baculovirus aren't yet effective enough to challenge any of the chemical products in the market. Its potential remains in the high specificity of the virus and the capacity to deliver selected molecules to the desired Lepidoptera species thanks to genetic recombination. There are many genes to be inserted but only a few replaceable as *polh* (polyhedrin gene), *p10 (cytoskeletal-related)* and *egt (hormonal enzyme)*.

At the moment, using exogenous genes expressed in the baculovirus genome may be one of the fastest ways to improve the virus yield. Although working with GMOs has been proven to slow down the registry process, considering the registry process as non avoidable step, it will be faced up sooner or later. A combination of all the effective modifications should be attempted to see if there's synergy between them. This selection would include a specific virus with inserted genes as the Bt toxin fused with the polyhedrin matrix, some protease replacing the *egt* gene and optical brighteners for UV protection.

Bibliography

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