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Applications of insect cell culture and baculoviruse for recombinant protein production: a review

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Abstract
Insect cell cultures are widely used for the production of recombinant proteins, vaccines and viral pesticides. It has become a common expression system for both basic research and large-scale commercial applications. The baculoviruses expression system is based on the replacement of non-essential, viral gene, termed polyhedrin, with a gene of interest. The insertion of foreign DNA at the polyhedrin locus within the viral genome results in incorporation of the foreign DNA into progeny virus particles and subsequent high-level expression of the recombinant protein within eukaryotic insect cell lines. A main factor to the popularity of insect cell expression is the ability of insect cells to produce relatively large quantities eukaryotic proteins in a relatively short period of time.
In this review, we focus on the cell line of insects and baculoviruses that used in BEV system and recombinant proteins production in this system

Keywords: Baculoviruse Expression Vectors system, insect cell, vaccin, recombinant proteins

Introduction

The researches based on insect cell culture have been increasingly growth recently The utility of insect cell lines for protein production has grown from laboratory-scale experimental work to industrial applications (Elias, 2007). The establishment of an in vitro cell line starts with the isolation of cells from a tissue of interest, which once obtained are maintained in a culture dish under conditions closely resembling the in vivo environment of the cells (Jones, 1962). Primary cells are the cultures that initiated directly from tissues and these typically survive in culture for a limited time with limited

rounds of cell division occurring before senescence. Cells which overcome the barrier of senescence are capable of dividing continuously, providing a constant source of cells without needing to harvest new primary cells from tissues. Such cultures are immortalized and represent a continuous cell line (Lundberg & Hahn, 2000).

Cell culture has been well established as an important research tool in both vertebrate and invertebrate systems, and over 500 insect cell lines have been created from different tissues of numerous insect orders. Table 1 shows cell lines from a variety of insect orders (Lynn, 2007 & Smagghe et al., 2009). Insect cell culture has been used to research diverse such as signaling mechanisms, immune pathways, gene expression, cell migration and evaluation of host-pathogen interactions (Smagghe et al., 2009 & Hunter, 2010).

Table 1. cell lines derived from different insect species and tissue origin[5]

Insect species	*Origin (tissue)*
Drosophila melanogaster	Embryo, Hemocytes, Wing disc
Aedes albopictus	Hemocytes
Chironomus tentans	Embryo
Manduca sexta	Embryo
Plodia interpunctella	Pupal imaginal wing discs
Choristoneura fumiferana	Pupal ovaries, Midgut
Malacosoma disstria	Hemocytes
Spodoptera frugiperda Imaginal	wing discs, Pupal ovaries
Ostrinia nubilalis	Embryo
Spodoptera exigua	Embryo
Trichoplusia ni	Imaginal discs
Bombyx mori	Ovaries
Leptinotarsa decemlineata	Embryo, Pupal fat body
Blatella germanica	Embryo
Trichogramma confusum	Muscle-like

Insect cell lines

The most utilized cell lines in the replication are AcMNPV1 that were derived from the fall army worm, Spodoptera frugiperda (Arif et al., 2013). Figure.1 illustrates Electron micrographs of AcMNPV. SF21 and its clonal isolate, SF9 have been exploited extensively in the replication of AcMNPV and later in the generation of successful baculovirus vectors for the expression of exogenous proteins and production of vaccines (Summers, 2006 & Van, 2006). Later, a major development in cell lines for exogenous protein expression was established from a Tricoplusia ni line, BTI-Tn5b1- 4. The new line that commercially known as the High Five cell was a clonal isolate from the parental line Tn5B1 originally derived from T.ni embryos (Granadso et al., 1994). The high five cell has been used for production of recombinant proteins and also production of a vaccine against Human Papilloma virus (Granados et al., 2007). Many of the cell lines support the replication of the AcMNPV. The availability of excellent cell lines permissive to AcMNPV allowed various investigators to elucidate the replication cycle and understand its characteristics that led to developing the virus as a successful system for the expression of foreign proteins. Numerous cell lines were established from the cabbage looper, Trichoplusia ni larvae and tested for permissiveness to GVs. Fifteen cell lines were permissive to this virus but infection either decreased or the cells became totally refractory to the virus by passage 21–25(Granados, 1986). The most common cell lines used for BEVs applications are listed in table 2.

The quality and pure of baculovirus is very important i.e., a pure baculovirus absent of mutants, and healthy cells (i.e., viability >95%) in exponential growth be used. The most important parameter

[1] Autographa californica multiple nucleopolyhedrovirus

involved in baculovirus infection is the multiplicity of infection (MOI), which is the ratio of infectious baculovirus to cells (Elias, 2007).

Table 2. Insect cell lines commonly used in BEVS applications

Insect Species	Cell line
Spodoptera frugiperda	Sf9
Spodoptera frugiperda	Sf-21
Trichoplusia ni	Tn-368
Trichoplusia ni	High-Five Cell

Baculovirus Structure

Baculoviruses are insect-specific viruses, predominantly infecting insect larvae of the order Lepidoptera. At least 35 different baculoviruses have been propagated in established cell lines (Lynn, 2007 & Arif, 2013). The double stranded, supercoiled, circular DNA genome of baculovirus is highly condensed with a size ranging from 80 to over 180 kbp and encoding 90 to 180 genes (Rohrmann, 2011) .The family of Baculoviruses is divided into two genera, the GVs[2] and the nucleopolyhedroviruses (NPVs) (van Regenmortel,2000) [14]. GVs produce small OBs[3] normally containing one or occasionally two virions encapsulated in a protein called granulin. The NPVs produce large, polyhedronshaped OBs, called polyhedra, containing many virions also NPVs pathogenic for members of the order (IJkel, 2007).
Figure.1 illustrates AcMNPVan example of each of these genera.

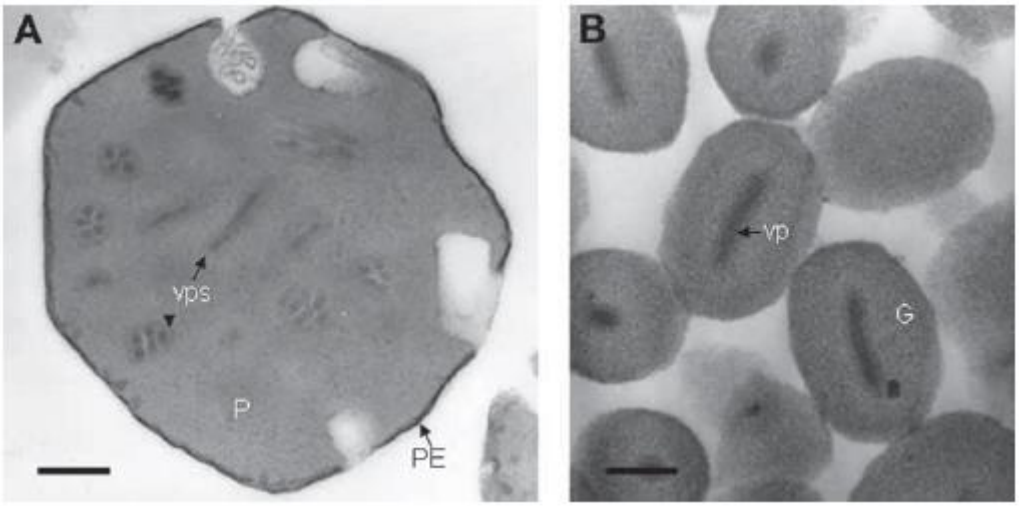

Figure 1. Electron micrographs of (AcMNPV) polyhedron (A) and Plodia interpunctella granulovirus (PiGV) granules (B) (Murhammer, 2007)

The baculovirus and its replication cycle

The baculovirus replication cycle involves two types of virions. ODV4 is present in a protein matrix (polyhedrin or granulin) , responsible for the primary infection of the host and required for

[2] granuloviruses
3 occlusion bodies
4 Occlusion derived virus

environmental dissemination of the virus while the BV5 is released from the infected host cells later during the secondary infection and necessary for systemic spread of the virus in the infected insect. However, there are differences between ODV and BV in morphology, structural proteins, antigenicity, infectivity, timing and cellular site of maturation (Kelly et al., 2007).

Natural infection of baculoviruses occurs when the insect larvae ingest vegetation contaminated with occluded form of the virus. The polyhedra dissolve in the alkaline environment of the host midgut (stomach), releasing ODV, which subsequently enter the midgut cells (Wang, 2008). The natural cycle of infection by baculovirruses in insect larvae is summarized in Figure2 and Figure3

In the most case, viral transcription and replication occur in the cell nucleus and new BV particles are budded out from the basolateral side to spread the infection systemically. In budding stage, BV expressed and displayed viral glycoproteins with a loosely fitting host cell membrane (Kelly et al., 2007). The BVs then infect the other tissue cells or are transmitted through the tracheal matrix to other parts of the body (Federici, 1997).

Baculovirus are used to infect insect cells or larvae where high levels of recombinant proteins are produced. They are found in nature commonly on vegetables and, therefore, baculoviruses are the main part of the insect diet and also cause fatal disease in specific insect species. Although baculoviruses are not able to replicate in mammalian cells, they have been shown to efficiently transduce a variety of mammalian cells (Safdar, 2007).

Baculoviruse Expression Vectors system

The recombinant BEVs6 normally involves mixing infectious virus DNA with a plasmid based transfer vector and then cotransfecting insect cells to initiate virus infection (Elias, 2007 & Lynn, 2007). Insect cells to be used in the baculovirus expression system are derived from lepidopteran insects and are relatively easy to grow without any control of oxygen atmosphere (Fig2 and Fig 3). Moreover, insect cells can be adapted to serum free media and production of recombinant protein can be scaled up to pilot plant or larger bioreactors (Inlow, 1989 & Chen, 1998).

Comparison to other viral vectors, e.g. adenovirus, baculovirus is capable of incorporating extremely large DNA inserts. In fact, the successful development of a recombinant baculovirus containing a 38 kb DNA insert producing adenoviral proteins in mammalian cells has been demonstrated (Cheshenko, 2001). Other properties which make baculoviruses excellent for use in expressing foreign proteins include the simplicity and speed in which recombinant baculoviruses can be generated, their ability to concurrently incorporate numerous genes, and their capacity to transduce a wide variety of cell types, including even mammalian cells. Further, because recombinant baculoviruses replicate in insect cells, post-translational modifications critical for the production of biologically active protein from eukaryotic sources still occur (Hu, 2005& Kost, 2002).

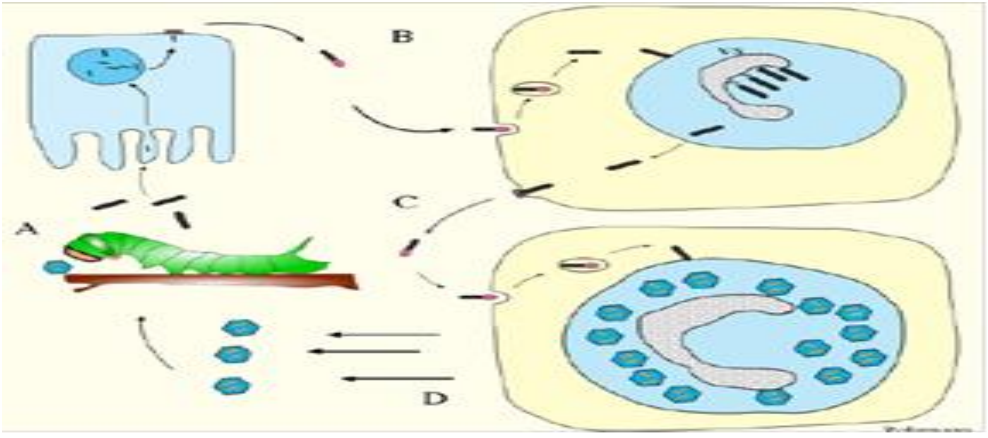

Figure 2. A life cycle of a baculovirus causing systemic infection [13].

5 budded virus

6 baculovirus expression vector system

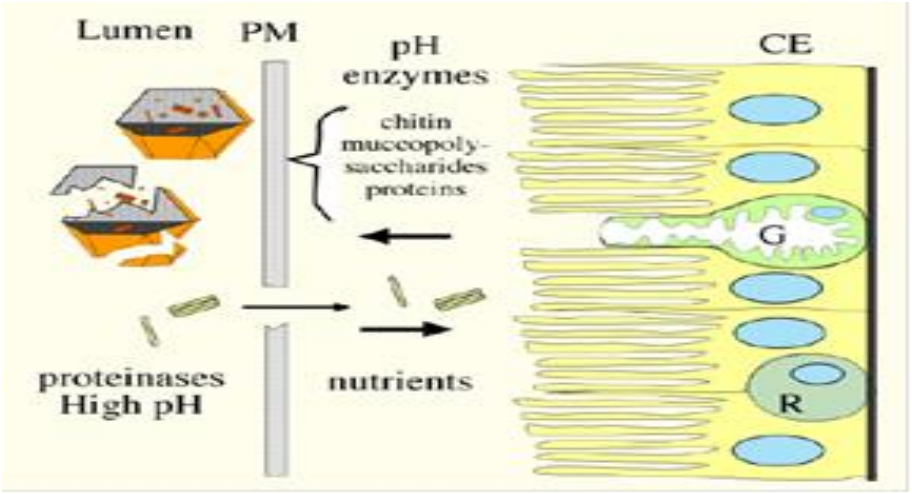

Figure 3. The insect midgut and virus infection [13].

Baculoviruses are attractive vectors for the expression of foreign proteins for a few key reasons. First, in recombinant proteins can be produced at levels ranging between 0.1% and 50% of the total insect cell protein .In this system DNA encoding polyhedrin was unnecessary for the survival of the virus in a laboratory and could be exchanged for genes encoding useful proteins. The protein of interest is usually produced under the control of the polyhedrin promoter which is a very strong promoter belonging to AcNP virus (Elias, 2007 & Lynn, 2007). The gene of interest will be inserted downstream of the polyhedron. Infection of SF9 insect cells with the recombinant virus leads to expression of the recombinant protein at high levels. Therefore, the recombinant protein usually maintains its normal function.

In general Baculovirus expression system has four features: 1) Baculovirus expression levels of the recombinant gene are higher than other eukaryotic expression methods(Fraser, 1992), 2) by infecting the cells with multiple recombinant viruses, this system can be used to express hetero-oligomer protein complexes (Harris 1997), 3) Baculoviruses use specific insect species as host and therefore they are not infectious to human or domestic animals (Huser, 2003), 4)The cell lines that used in Baculovirus expression system can be adapted to grow in suspension and therefore the recombinant protein can be produced in large scales using bio-reactors (Shuler, 1990), 5)Insect cells have the capability of performing many of the post-translational modifications that required for the biological activity of many complex proteins such as glycosylation, disulfide bond formation and phosphorylation (Lynn, 2007, Kelly, 2007). Baculoviruses have been used very successfully as vectors for foreign genes both in vitro and more recently, in vivo particularly in the development of pharmaceuticals, gene therapies, diagnostic reagents and vaccines (Bia, 2008).

Baculoviruses as novel bioinsecticides

The basis of modern baculovirology was stimulated by the potential utility of baculoviruses to control insect pests. Baculoviruses are highly infectious, selective pathogens, very safe to people and long term crop protection can be established (Fuxa, 2002). Although the use of baculovirus bioinsecticides was hampered by their slow speed of action when compared with fast killing chemical insecticides, they gained increasing acceptance as they were considered for long term protection of crops, in the framework of integrated pest management the most successful project was implemented in Brazil where over two million hectares of soybean were controlled by baculovirus AgMNPV (Romanowski, 2013).

Vaccines

The Baculovirus expression vectors system is being increasingly used for the development of vaccine candidates based on the production of virus-like particles (VLPs) and vaccine antigens. Co-expression of protein-modifying enzymes using multiple recombinant BVs can be used to enhance the production

of functional recombinant proteins produced by infected insect cells. Some notable vaccines include anti-malaria vaccines, the human papillomavirus (HPV)-like-particle vaccine, newcastle disease virus (NDV) and also a SARS-like corona virus vaccine (Strauss, 2007, Yoshida, 2010, Strauss, 2007, Nagy, 1994, Bai, 2008).

The transient nature of BEV System technology makes it particularly attractive for the production of an influenza vaccine, requiring annual adjustments because a single well-characterized cell line is used for the production of all HA proteins. Hemagglutinin (HA) is a major viral antigen of Influenza virus A (H1N1), an important subtype of the influenza respiratory viruses. This may offer an improved alternative to conventional influenza vaccines currently produced in embryonated chicken eggs, because of lack of egg-protein or preservatives and high purity of the antigen(Sehgal, 2003).

Conclusions

BEVS has been widely accepted for biotechnological applications. It is a powerful recombinant protein system because of high level of gene expression and capacity of heterooligomer protein complexes production with post-traslational modifications. It can provide large scale commercial production with rapid and low-cost method. It is attractive for biopesticide synthesis with no effect on human or domestic animals and low environmental effect. This system can provide health care solutions to pandemic outbreak of diseases and biodefense. It has been used successfully for producing a number of vaccines with low side effect because of pure antigen and no preservations.

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