Applied Microbiology and Biotechnology
https://doi.org/10.1007/s00253-018-9474-7

MINI-REVIEW

Engineering of the baculovirus expression system for optimized protein production

María Martínez-Solís[1] · Salvador Herrero[1] · Alexandra M. Targovnik[1,2,3]

Received: 17 August 2018 / Revised: 18 October 2018 / Accepted: 19 October 2018

Abstract

Baculoviruses are arthropod-specific large circular double-stranded DNA viruses successfully used for the control of multiple insect pests. In addition to their application in pest control, baculoviruses have become a versatile and powerful eukaryotic vector for the production of large quantities of recombinant proteins for research and biomedical purposes. Since the first recombinant protein was expressed in 1983 using the baculovirus expression system (BEVS), different strategies have been developed for the generation of recombinant viruses and to increase the stability, yield, and posttranslational modifications of recombinant proteins. In this review, we summarize the main methods and elements playing a role in the BEVS emphasizing recent progresses and future developments with respect to the main aspects involved in protein production using the BEVS.

Keywords Nucleopolyhedrovirus · Protein expression · Insect cells · AcMNPV · Insect biofactory

Introduction

Baculoviruses, also referred as nucleopolyhedrovirus (NPVs) due to the typical inclusions they make in the nucleus of infected cells, are among the most important viral entomopathogens used in pest control. So far, more than 1000 species have been described infecting different species of insects, mainly from the order Lepidoptera (Jehle et al. 2006). In addition to their application in pest control, genetic engineering of baculoviruses have promoted their use as a versatile and powerful vector for the production of large quantities of recombinant proteins in research laboratories and biopharmaceutical companies throughout the world.

✉ Salvador Herrero
sherrero@uv.es

[1] Department of Genetics and Estructura de Recerca Interdisciplinar en Biotecnologia i Biomedicina (ERI-BIOTECMED), Universitat de València, Dr Moliner 50, 46100 Burjassot, Valencia, Spain

[2] Facultad de Farmacia y Bioquímica.Departamento de Microbiología, Inmunología y Biotecnología. Cátedra de Biotecnología, Universidad de Buenos Aires, Junín 956, 1113, Buenos Aires, Argentina

[3] Instituto de Nanobiotecnología (NANOBIOTEC), CONICET-Universidad de Buenos Aires, Junín 956, 1113, Buenos Aires, Argentina

In nature, baculoviruses virions are found embedded in a paracrystalline protein matrix mainly formed by a single protein called polyhedrin. Once ingested by the susceptible caterpillars, the matrix is dissolved in the gut of the insect and the virions (ODVs; occluded derived viruses) will merge to the epithelial cells, then nucleocapsids will migrate into the nucleus establishing the primary infection. As part of their biphasic replication, the infected cells produce another type of virion (BV; budded virus) that facilitates viral entrance by endocytosis into other cell types, and the establishment of a systemic infection of the insect larvae (Rohrmann 2013).

The Baculovirus Expression Vector System (BEVS) was originally developed in the early 1980s in the laboratories of Dr. Summer (Smith et al. 1983b) and Dr. Miller (Miller 1981). Recombinant viruses were initially obtained by replacement of the polyhedrin gene from *Autographa californica nucleopolyhedrovirus* (AcMNPV) by co-transfection of Sf21 cells with a mixture of the viral DNA and the donor vector and further isolation of the recombinant BVs from the viral plaques (Smith et al. 1983b). Since then, different changes have been introduced to improve the methods for the generation and selection of recombinant viruses, and to increase the yield and quality of the expressed proteins. Although the methods for the generation of recombinant viruses have been optimized, and recombinant viruses can be generated in only a few working days, there is still room for improvement in the production of recombinant proteins at an industrial scale that

Published online: 29 October 2018

competes in a cost-effective manner with other available protein production systems. In addition, to increase protein production yields by improving protein expression and stability, baculovirus engineering has also been addressed to improve the introduction of complex N-glycan structures that occur in mammals.

In this review, we aim to summarize the main methods and elements playing a role in the BEVS, emphasizing recent progress and future developments in the three main aspects that regulate protein production using the BEVS: (i) the viral properties, (ii) the cellular host, and (iii) the bioprocesses involved in protein production.

Engineering of the viral vector

Methods for the generation of recombinant baculoviruses

Initially, recombinant baculoviruses were obtained by homologous recombination between the viral genome and a donor plasmid into the insect cells (Smith et al. 1983b). However, this approach is not very efficient and the rates of recombinant baculoviruses were lower than 0.1% of the total viruses, necessitating several rounds of plaque assays for the isolation of recombinant viruses. The first improvement was provided by Kitts el al. (Kitts et al. 1990) with a linearized viral genome at the gene insertion site, increasing the percentage of recombinant baculoviruses up to 30%. Shortly after, a vector which interrupted an essential gene (*orf1629*) during the linearization was constructed, and recombination rates in the recovered viruses were close to 100% (Kitts and Possee 1993). The original vector containing these improvements was named as BacPAK6™, and currently, there are other commercially available vectors based on the same principle, such as the BaculoGold™ baculovirus expression system (BD Biosciences).

An important breakthrough was the development of the first bacmid, a vector containing the whole AcMNPV genome that can be propagated in *E. coli* cells (Luckow et al. 1993) and commercialized by Invitrogen as the Bac-to-Bac® system. The introduction of the gene of interest into the bacmid occurs in the bacterial host and is based on the transposition of a shuttle vector containing the gene of interest into the baculovirus genome mediated by Tn7-recombinase. This system has been broadly adopted because it simplifies the generation, selection, and isolation of recombinant bacmids, which are subsequently used for transfection of insect cells, producing recombinant baculoviruses at a rate of 100%. Bacmid systems have also been developed for other viral species such as the *Spodoptera exigua multiple nucleopolyhedrovirus* (SeMNPV) (Choi et al. 1999) and the *Bombyx mori nucleopolyhedrovirus* (BmNPV) (Motohashi et al. 2005).

One of the advantages of the baculovirus system is the possibility to express more than one protein simultaneously. Initially, expression of multiple proteins was achieved by co-infection with multiple recombinant viruses, each of them expressing a single protein (Angelo et al. 1987; Dodson et al. 1989). However, co-infections on a large scale are usually inefficient because it is not guaranteed that the simultaneous infection with all viral vectors will occur in the same cell (van Oers et al. 2015). This limitation was partially solved with the development of shuttle vectors that accept more than one heterologous gene into the genome for the expression of different recombinant proteins simultaneously. Some examples of commercially available vectors are the pFastBacDual (Invitrogen), for the expression of two different proteins, or the MultiBac system (Sari et al. 2016), which allows the expression of multi-protein subunit complexes.

In recent years, other systems have been developed with different improvements and/or applicabilities. The *flash*BAC™ system (Possee et al. 2008) (Oxford Expression Technologies) combines bacmid technology with homologous recombination in insect cells to generate recombinant baculoviruses. In 2014, Thimiri Govinda Raj et al. (2014) designed OmniBac transfer vectors which can be used to generate recombinant baculoviruses, both using bacmid technology as well as homologous recombination, using the same DNA elements. A second generation of the Tn-7-based system, named as Bac-2-the-Future, has been developed to generate recombinant baculoviruses with reduced time and labor in comparison with the Bac-to-Bac system (Mehalko and Esposito 2016). In addition, a method known as biGBac (Weissmann et al. 2016) has been developed to generate recombinant baculoviruses based on the assembly of several linear DNA fragments, using Gibson assembly methodology (Gibson et al. 2009).

Cis and *trans* DNA elements improving protein expression

Expression of recombinant proteins can be modulated by DNA regulatory sequences located at regions neighboring the gene of interest (*cis* elements), promoters and enhancers being the most common. Both types of regulatory sequences have been explored to improve the quantity and quality of recombinant proteins in the BEVS.

Since its inception, the viral *polh* and *p10* promoters are routinely used to drive the expression of recombinant proteins in the BEVS. They are strong and very late promoters that control the expression of two structural proteins (polyhedrin and p10, respectively) which are not needed for the in vitro replication of the virus in insect culture cells (Smith et al. 1983a; Williams et al. 1989). Although these promoters provide high transcription levels, they act at very late stages of the infection, when the ability of the cells to express proteins is

already compromised. For that reason, other promoters have been tested to enhance the expression in baculovirus vectors. Late viral promoters, such as *p6.9* or *vp39*, were demonstrated to express different recombinant proteins (Hill-Perkins and Possee 1990; Thiem and Miller 1990; Bonning et al. 1994; Ishiyama and Ikeda 2010), even increasing the expression levels obtained with the standard *p10* and *polh* promoters. More recently, a viral promoter derived from the SeMNPV, *pSeL*, has been proposed as an alternative to the *polh* promoter to increase the expression of recombinant proteins in different insect cell lines (Martínez-Solís et al. 2016). All these promoters naturally drive the expression of structural proteins, and their combination with the standard *p10* and *polh* promoters generally results in higher expression yields. For instance, the combination of the *polh* promoter with the *vp39*or *pSeL* promoters improves the recombinant expression levels obtained with each individual promoter (Thiem and Miller 1990; Martínez-Solís et al. 2016). An alternative strategy to solve the problems related with the use of late promoters has been the introduction of earlier promoters such as *ie1*, *39k*, or *gp64* (Jarvis et al. 1996; Grabherr et al. 1997; Regev et al. 2006) to express high levels of recombinant proteins in the early stages of cell infection. Although the tested promoters are usually derived from baculoviral sequences, Choi et al. (Choi et al. 2009) also reported the use of bracovirus-derived promoters to improve the expression of recombinant proteins in the BEVS. In addition, a Lepidoptera-derived promoter, named *pB2*, has shown higher expression levels than the *polh* promoter at early post-infection stages (López-Vidal et al. 2013).

Enhancers are DNA elements which are able to increase the expression of certain genes and can be combined with the standard promoters to enhance their activity. Baculovirus genomes are characterized by the presence of homologous regions (*hr*) distributed along the genome (Cochran and Faulkner 1983). These non-coding regions act as the origin of viral DNA replication as well as enhancers of gene transcription. It has been reported that the introduction of *hr1* from AcMNPV into different locations of recombinant viruses results in increased production of different recombinant proteins (Lo et al. 2002; Venkaiah et al. 2004; Tiwari et al. 2010). The *hr3* sequence from BmNPV also has been introduced to increase the expression of recombinant proteins and improve the stability of the expressed proteins (Lu and Miller 1997; Chen et al. 2004; Ishiyama and Ikeda 2010). The introduction of an element derived from the 5′-end of the *polyhedrin* gene, the partial polyhedrin homology sequence (PPHS), into the recombinant baculovirus, is also able to improve the expression of foreign genes in cell culture as well as in insect larvae (Gong et al. 2006; Romero et al. 2011). More recently, an improvement in the pFastBac™ vectors has been described that allows similar expression levels of recombinant proteins to those obtained by the wild-type AcMNPV (Shang et al. 2017). The modification consists of the introduction of an 80 pb *cis* element upstream of the *polh* promoter, and the substitution of the polyadenylation signal (pA) SV40 by the polhpA.

In addition to the inclusion of regulatory sequences enhancing the expression of neighboring genes, other strategies to increase protein expression have been focused on the expression of *trans* elements, alone or in combination with cis-acting regulatory sequences. Gómez-Sebastián et al. (2014) developed an expression cassette containing the *hr1* of AcMNPV, and also the transactivation factors IE1 and IE0. The use of this new cassette in recombinant baculoviruses increases the cell viability and integrity of infected cells, which finally results in improved yields of the recombinant proteins. It is known that binding of the transcriptional factor VLF-1 to the burst sequence (BS) enhances the activity of the *polh* promoter. Manohar et al. (2010) increased the level of recombinant gene expression in Hi5 cells and *T. ni* larvae by increasing the number of BS between the *polh* promoter and the target gene, and co-expressing VLF-1. Liu and Zhang (2015) introduced the 3′-untranslated region P10UTR and a synthetic AT-rich 21-bp sequence (Syn21), increasing the yield of the porcine circovirus type 2 (PCV2) VLPs when compared to the original baculovirus vector. In another type of approach, it has been shown that the overexpression of the *lef5* gene from SeMNPV in recombinant AcMNPV baculoviruses results in an increased stability of the transgene, and therefore in an improved expression of recombinant protein after successive replication passages in cultured cells (Martínez-Solís et al. 2017).

Baculoviral genome engineering

The facility to modify the baculovirus genome has allowed the removal of non-essential genes for *in vitro* viral replication, which has negative effects on the production of recombinant proteins as a strategy to improve expression using the BEVS. The first attempts were focused on deletion of the *v-cath* and *chiA* genes. These genes encode for enzymes responsible for the liquefaction of infected larvae, and its proteolytic action has a negative effect on the production of recombinant proteins. Different studies have shown that deletion of these viral genes increases the stability and expression of the secreted recombinant proteins (Suzuki et al. 1997; Kaba et al. 2004; Hiyoshi et al. 2007; Hitchman et al. 2010b). This new viral genome was later improved with the deletion of other non-essential genes for in vitro replication. Removing the *p26*, *p10*, and *p74* genes from the AcMNPV genome enhanced the expression of recombinant proteins (Hitchman et al. 2010a). Nevertheless, not only protein-coding genes can be removed to improve recombinant expression. Pijlman et al. observed the accumulation of *non-hr ori* sequences in the genome of SeMNPV (2002) and

AcMNPV (2003) after several viral multiplication passages in insect cells. Removal of these *non-hr ori* sequences from the recombinant viral genome resulted in an enhanced genetic stability of the viral genome upon passaging, and therefore increased the expression of the recombinant proteins.

Another strategy to enhance the production of recombinant proteins in the BEVS has been the addition of certain genes into the viral genome with beneficial properties for protein production. An example is the introduction of *vankyrin* genes from the insect virus *Campoletis sonorensis ichnovirus*. The function of these genes is related with the suppression of the host cell immune system. Accordingly, their introduction in the system results in delayed lysis of the infected cells, and an increased time for the expression of recombinant proteins (Fath-Goodin et al. 2006). In a similar way, non-lytic vectors have been obtained by random mutagenesis of viral genomes, along with selection of those producing recombinant proteins for a greater period of time and increasing the final yield (Ho et al. 2004). Enhancement of the stability of recombinant protein production has also been reported with the use of bicistronic cassettes, which express in a single transcript the gene of interest coupled to a viral essential gene (*gp64*) using an IRES (internal ribosome entry site) sequence between them (Pijlman et al. 2006).

The secretory pathway is generally affected by the virus infection, resulting, in certain cases, in the production of insoluble proteins. A strategy to overcome this issue and improve the recombinant protein expression has been the introduction of sequences coding for chaperones into the viral vector. Thus, Teng et al. (2013) showed that the secreted alkaline phosphatase (SEAP) activity was increased when it was co-expressed with the calreticulin chaperon in Sf21 cells.

Engineering of the cellular host

The production of recombinant proteins in classical insect cell lines presents two principal limitations. The first is that they produce less complex N-glycan structure than mammals, resulting in a loss of stability, solubility, and bioactivity of therapeutic mammalian recombinant protein (Kato et al. 2017). The second limitation is that cell death and lysis are natural processes that are triggered by baculovirus infection, resulting in the production of immature proteins and increasing the activity of proteases and caspases that negatively affect recombinant protein integrity and yield (Steele et al. 2017). To address these limitations, several new cell lines have been developed, and genetically modified insect cells have been engineered, to produce mammalian recombinant glycoproteins and delay insect cell death after infection.

Cell lines for the BEVS

The most common cell lines susceptible to AcMNPV infection and used in the BEVS for academic or commercial purpose are the IPLB-SF21-AE (marketed as Sf21) (Vaughn et al. 1977) and its subclone Sf9, and the BT1-Tn5B1-4 (marketed as High Five™, Hi5)(Wickham et al. 1992). These cells are derived from pupal ovarian tissue of the fall armyworm *Spodoptera frugiperda* and from the adult ovarian tissue of the cabbage looper *Trichoplusia ni*, respectively. There are substantial differences among the different cells lines that must be considered according to the type of protein and purpose. For instance, Sf21 and Sf9 are comparable in their characteristics. They are highly susceptible to virus infection, can be grown as a monolayer at 27 °C without CO_2, and are adaptable to growth in suspension culture and in a serum-free medium. In addition, these cells also achieve high cell densities, increasing the yield and virus titers (Granados et al. 2007). The Sf9 clone was selected based on the faster growth rate and higher cell densities with respect to Sf21 (Ejiofor 2016), and are also more tolerant to osmotic, pH, and sheer stress (Agathos 2010) than Sf21 cells. On the other hand, Hi5 can also be multiplied as a monolayer as well as a suspension at 27 °C without CO_2. In general, Hi5 cells produce a better expression level of recombinant proteins than Sf9 and Sf21, partially due to its larger size. In contrast, Hi5 cells release three times more proteases than Sf21 and Sf9 cells, which could cause degradation of the target protein (Kwon et al. 2003). Different approaches have been taken to overcome some of the limitations of these cell lines and improve their performance in the BEVS. Cell lines derived from the Hi5 cells, such as Tn-H5CL-B and Tn-H5CL-F, which show more resistance to nutrient stress and higher expression of β-galactosidase and secretory alkaline phosphatase (SEAP) have been developed (Li et al. 2003). Also, a cell line derived from Sf9 cells and marketed under the name of express SF+ (Protein Sciences Corp) was obtained after selection in a serum-free medium supplemented with human insulin and was established to optimize protein production in the BEVS (Granados et al. 2007). Currently, porcine circovirus type 2 (PCV-2) and Influenza (Flublok®) vaccines are produced in these cells (van Oers et al. 2015).Other common cell lines used in combination with BmNPV are Bm5 and BmN4. They were established from ovarian tissue from *Bombyx mori* larvae (Grace 1967; Maeda 1989). The production of viruses and the expression of recombinant proteins in these cell lines seem less effective than in Sf9 and Sf21 (Lee et al. 2012). Other alternative cell lines derived from *B. mori* embryos with better performance such as Bme21 have been developed (Lee et al. 2012), although they are used less extensively than traditional Bm5 and BmN4 cells. Other non-conventional Lepidoptera cell lines have been established such as A7S and DpN1, from *Pseudaletia unipuncta* and

Danaus plexipus larvae, respectively. Those cell lines were capable of producing recombinant proteins with complex *N*-glycosylation sites, although the expression levels achieved were less than in the Hi5 cell line (Palomares et al. 2003).

Improvement of the glycosylation patterns

The baculovirus-insect cell system produces mostly recombinant proteins with paucimannose structure (Geisler et al. 2008). In general, these cells are unable to add terminal galactose and sialic acid residues like mammalian cells, mainly due to the absence or poor expression of glycogen and the great activity of glycosidase (Chavez-Pena and Kamen 2018). In addition, Hi5 cells express glycoprotein with α1–3 fucose, and patients treated with biopharmaceuticals produced in these cells can develop hypersensitivity. Due to that, Sf9 cells are a better host to produce recombinant proteins with therapeutic purposes (Palomares et al. 2018). The first effort to improve the *N*-glycosylation profile in insect cells involved co-infection ofSf9 cells with two recombinant baculovirus, one encoding for a human-glycosyltransferase and the other encoding for a foreign gene (Wagner et al. 1996). Afterwards, due to the reduced efficiency of co-infection, Palmberger and co-workers (Palmberger et al. 2012) developed, based on MultiBac Technology, the SweetBac system for the simultaneous expression of two glycosyltransferases and a target gene using a single virus (Palmberger et al. 2012, 2013). In addition, using the same technology, it has been possible to minimize the fucosylation in Hi5 cells by coexpression of an inhibitor of the GDP-L-fucose *de novo* synthesis pathway along with the protein of interest (Palmberger et al. 2014). Also, efficient strategies involving the modification of the cellular host have been developed. Several insect cell lines expressing recombinant mammalian glycosyltransferases, or enzymes that are involved in sialic acid and CMP-sialic acid biosynthesis and transport, have been established with the aim to produce *N*-glycoproteins with human-type complex (Toth et al. 2014). For instance, SfSWT-1 cells (Hollister et al. 2002) were the first Sf9 cells stably transformed with five mammalian glycogenes. This cell line is commercialized under the name Mimic™ Sf9 insect cells (ThermoFisher).Toth et al. (2014) established a more efficient transgenic insect cell line (Sf39KSWT) by stably transforming the Sf9 insect cells with a set of nine mammalian glycogenes under the control of the *39k*-inducible promoter. This inducible promoter by viral infection is a potent tool, because the expression of foreign genes does not affect the growth and stability of the transgenic insect cell line as is seen with the constitutive promoters (Aumiller et al. 2012). Despite the improvements achieved in terms of glycosylation, the production of recombinant proteins with human-type glycosylation has not reached 100% efficiency, and the paucimannose glycan structures are still present in the recombinant protein produced (Palomares et al. 2018). For example, when recombinant erythropoietin (rEPO) was expressed in Sf39KSWT insect cells, only about 40%was processed with human-type structure (Toth et al. 2014).

In contrast to recombinant expression of mammalian-derived proteins in the cell host, natural host genes have also been silenced or knocked-out to optimize their glycosylation performance. The insect cells present an intrinsic potential to generate more complex chains than the paucimannose structure without reaching the complexity of the human-type structure. For example, the β-*N*-acetylglucosaminidase, known as fused lobed protein (FDL), is an enzyme expressed only in insect cells that antagonize *N*-glycan elongation (Chavez-Pena and Kamen 2018). RNAi technology was applied to silence the *fdl* gene in Sf9 and BmN4-SID1 cells, a derived *B. mori* cell line (Geisler et al. 2008; Nagata et al. 2013). Even though this approach reduced FDL expression, the phenotypic impact on the glycosylation process was slight (Geisler et al. 2008). However, CRISPR-Cas9 technology allowed the development of a new derived Sf9 insect cell line (SfFDLt1), unable to produce FDL. Knock-out of the *fdl* gene increased to 65% the proportion of partially elongated, mammalian-type *N*-glycan complexes, and reduced to 8% the proportion of paucimannose structures on rEPO produced in SfFDLt1 cells (Mabashi-Asazuma and Jarvis 2017). Although this strategy enhances the glycosylation capabilities of insect cells, it is insufficient to achieve the human *N*-glycosylation type, so it is still necessary to associate them with the heterologous expression of other glycosyltransferases (Kim and Cha 2015).

Improvement of the production and stability

As mentioned above, an efficient strategy to extend the survival of cells after viral infection is the cloning of the antiapoptotic Vankyrin (*Vank-1*) into the baculovirus genome. Alternatively, a similar effect can be obtained by the stable expression of this protein in the cell host. Vankyrin-expressing cell lines derived from Sf9 and Hi5 cells have been obtained and named as VE-CL2 (Fath-Goodin et al. 2009) and VE-High Five (Steele et al. 2017), respectively. The expression of the YFP reporter using the BEVS was significantly increased when infecting these cell lines. Steele and co-workers (Steele et al. 2017) also observed that other proteins expressed in these transformed insect cell lines were subjected to less proteolysis and the secretory pathway remained functional for a longer period of time. The combination of strategies to improve protein expression was also evaluated. For this purpose, Sf9 insect cells were simultaneously transformed with six human glycogenes and the *Vank-1* gene. In this way, it was possible to improve the expression level and the *N*-glycosylation process simultaneously, demonstrating that the *N*-glycosylation pathway is still functioning at the late stages of

infection in Vankyrin-expressing insect cells (Steele et al. 2017).

In order to extend the survival of the insect cell lines, Sf9 cells were stably transformed with the *p35* gene from AcMNPV, an inhibitor of caspase. The resulting cell line was more resistant to apoptosis and nutrient stress, and it was able to produce higher levels of recombinant proteins (Lin et al. 2001).

RNAi technology has also been widely applied to silence pro-apoptotic host genes. The silencing of *Caspase-1* was evaluated in different insect cell lines resulting in extended viability after infection with recombinant baculovirus and increased production of intracellular and extracellular recombinant proteins (Hebert et al. 2009; Lai et al. 2012; Wang et al. 2016). In an alternative approach, RNAi technology was also used to silence a regulatory gene involved in the cell cycle. When cyclin E was silenced in Hi5 insect cells, an increase in GFP expression was seen (Wu et al. 2013).

Protein production bioprocess

In the industrial context, the BEVS is usually used for large-scale production of recombinant proteins (Steele et al. 2017). Even though insect cells are generally grown in a monolayer, they can be adapted to suspension cultivation for large-scale protein expression (van Oers et al. 2015). The scale up in insect cells takes several steps from spinner culture and shake flasks to bioreactor system design, and the process depends on an adequate control of process parameters (oxygen delivery, good mixing, appropriate modes of feeding, nutrient availability) that are crucial for optimal cell growth and baculovirus infection (Agathos 2010). For this purpose, standard bioreactors are used such as the single-use wave bag reactor for medium-scale processes and the stirred tank reactor (STR) for large-scale processes (Zitzmann et al. 2017). Due to the lytic characteristics of the system, the production platform is based principally on batch, and to a lesser extent on fed-batch culture, and a new cell culture must be set in each production cycle (Contreras-Gómez et al. 2014). Moreover, the insect cells are influenced by shear stress due to the turbulence and aeration produced in the bioreactor, affecting the quality of the proteins produced. Although STR produces more shear stress than a wave bag, this technology is considered the most appropriate bioreactor system for the scale-up of the BEVS process due to the larger volume of work it allows. For this reason, the insect cell damage can be controlled by the correct aeration rate and the addition of protective agents such as Pluronic® (Weidner et al. 2017). Currently, other bioreactors which are easy to handle such as the single-use iCellis nanofixed-bed bioreactor are being used with the object to entrap, cultivate, and recover non-adherent cells at high cell viability, and consequently to achieve high-quality recombinant protein production (Ventini-Monteiro et al. 2015).

Culture media have also been improved over time to enhance the production of recombinant proteins. The first culture media developed for insect cell culture were Grace's Insect Medium, TNM-FH, IPL-41, and TC100,which need to be supplemented with fetal bovine serum or insect hemolymph. However, the production of recombinant proteins in a medium free of any animal protein offers great lot-to-lot reproducibility and facilitates the downstream processing and product validation (Zitzmann et al. 2017). Because of this, many serum-free and protein-free mediums that do not need any other additives such as Express Five™, Sf900™ II SFM, Sf900™ III SFM (Thermo Fisher Scientific), HyClone™ SFX-Insect™ (Thermo Scientific), ESF 921 (Expression System), ExCell® 420 (Sigma Aldrich), and Insect-XPRESS™ (Lonza) have been developed (Contreras-Gómez et al. 2014; Zitzmann et al. 2017). Nevertheless, pharmaceutical companies have been able to formulate their own culture media as well as select specific cell clones to produce their proteins (van Oers et al. 2015).

The production of recombinant proteins on an industrial scale has high costs due to tissue culture reagents, sophisticated equipment, and the specialized personnel required (Guijarro-Pardo et al. 2017). The use of insect larvae infected with recombinant baculovirus as a small live bioreactor has appeared as an attractive alternative to scale up the production of a wide variety of recombinant proteins in a cost-effective manner. Targovnik et al. (2016) recently reviewed the use of this system to produce commercial recombinant proteins. In vivo production is considered an attractive platform due to the high level of expression achieved and the low risk of contamination; the manufacturing costs could be reduced up to 400 times, and the total process is less time-consuming compared to the process developed in insect cells (Targovnik et al. 2016). Moreover, the cell diversity that forms the whole larvae can increase the expression level of proteins produced in this system (Guijarro-Pardo et al. 2017). To date, the lepidopteran species that have been successfully exploited to produce recombinant proteins are *B.mori* (Silkworm), *S. frugiperda*, *Spodoptera littoralis*, *T.ni*, *Helicoverpa zea*, *Heliothis virescens*, and *Rachiplusia nu* (Targovnik et al. 2016). Currently, Toray Ind. Inc. (Tokyo, Japan) produces recombinant Feline Interferon (Intercat) and Canine Interferon Gamma (Interdog) for veterinary use in *B. mori* larvae. Other companies like Chesapeake PERL (Savage, MD, USA), Sysmex (Japan), ALGENEX (Spain), Werfen Group (Spain), and Boehringer Ingelheim Vetmedica (Germany) produce recombinant proteins for commercial purposes using insect larvae. In addition to the use of larval stages of the insects, the pupal stage has recently become an interesting bioreactor for protein production using the BEVS (Kato et al. 2010). For instance, influenza virus-like particles and human IgG have been produced in pupae from the silkworm

Table 1 Summary of the main biotechnological strategies for the improvement of the BEVS

Modifications	Characteristics	Ref.
Engineering of the viral vector		
Promoters		
vp39	↑ protein expression	Thiem and Miller (1990); Ishiyama and Ikeda (2010)
p6.9	↑ secreted proteins	Hill-Perkins and Possee (1990); Bonning et al. (1994)
ie1	↑ protein expression	Jarvis et al. (1996)
gp64	↑ protein expression	Grabherr et al. (1997)
39k	↑ protein expression	Regev et al. (2006)
pSeL	↑ protein expression	Martínez-Solís et al. (2016)
pB2	↑ protein expression	López-Vidal et al. (2013)
Enhancers		
hr1 of AcMNPV	↑ protein expression	Lo et al. (2002); Venkaiah et al. (2004); Tiwari et al. (2010)
hr3 of BmNPV	↑ protein expression ↓ protein aggregation	Lu and Miller (1997); Chen et al. (2004); Ishiyama and Ikeda (2010)
PPHS	↑ protein expression	Gong et al. (2006); Romero et al. (2011)
80 pb*cis* element + polhpA	↑ protein expression	Shang et al. (2017)
Trans elements		
hr1 + IE0/IE1	↑ protein expression	Gómez-Sebastián et al. (2014)
BS + VLF-1	↑ protein expression	Manohar et al. (2010)
3′- P10UTR + Syn21	↑ protein expression	Liu and Zhang (2015)
lef5 from SeMNPV	↑ genome stability	Martínez-Solís et al. (2017)
Virus manipulation		
Δc*hi*	↑ secreted proteins ↓ proteolytic degradation	Suzuki et al. (1997); Kaba et al. (2004); Hitchman et al. (2010b)
Δ*cat*	↓ proteolytic degradation	Kaba et al. (2004); Hiyoshi et al. (2007); Hitchman et al. (2010b)
Δ*p26/p10/p74*	↑ protein expression	Hitchman et al. (2010a)
Removal *non-hr*ori	↑ genome stability	Pijlman et al. (2002 and 2003)
vankyrin	↑ protein expression	Fath-Goodin et al. (2006)
Non-lytic vectors	↓ proteolytic degradation	Ho et al. (2004)
IRES sequence	Bicistronic expression ↑ protein stability	Pijlman et al. (2006)
chaperones	↑ protein activity	Teng et al. (2013)
Engineering of the cellular host		
Improve quality		
Mammalian glycogens constitutive expression in Sf9	↑ *N*-glycoproteins with human-type complex	Hollister et al. (2002)
Mammalian glycogens inducible expression in Sf9	↑ *N*-glycoproteins with human-type complex	Toth et al. (2014)
FDL gene editing in Sf9 (sfFDLt1)	↑ partial elongated N-glycoproteins with human-type complex	Mabashi-Asazuma and Jarvis (2017)
Production and stability		
Vankyrin expression in Sf9 and Hi5 (VE-CL2, VE-Hi5)	↑ expression ↓ proteolytic degradation	Fath-Goodin et al. (2009); Steele et al. (2017)
Silencing caspase Hi5, Sf9, Bm51 cells by RNAi	↓ apoptosis ↑ expression	Hebert et al. (2009); Lai et al. (2012); Wang et al. (2016)
p35 expression in Sf9 cells	↓ apoptosis ↓ nutrient stress ↑ expression	Lin et al. (2001)

Table 1 (continued)

Modifications	Characteristics	Ref.
Silencing cyclin E in Hi5 cells by RNAi	↑ expression	Wu et al. (2013)
Production Bioprocess		
iCellis nanofixed-bed bioreactor	↑ viability ↑ quality	Ventini-Monteiro et al. (2015)
Medium free of any animal protein	↑ lot to lot reproducibility ↑ downstream processing ↑ product validation	Revised by Zitzmann et al. (2017)
Insect larvae as live bioreactor	↑ expression ↓ contamination risk ↓ manufacturing cost ↓ time-consuming	Revised by Targovnik et al. (2016)
Insect pupae as live bioreactor	↑ expression	Revised by Kato et al. (2010)

(Nerome et al. 2015; Kato et al. 2017). Furthermore, the ALGENEX company has recently developed the CrisBio™ technology to produce recombinant proteins in *T. ni* pupae.

Similar to the expression in insect cell culture, one of the major drawbacks with respect to the expression of recombinant proteins in insect larvae is the *N*-glycosylation pattern. To improve the glycosylation quality, transgenic silkworms constitutively expressing a mammalian glycosyltransferase in the posterior silk glands have been established (Mabashi-Asazuma et al. 2015). However, generation of transgenic insects can be laborious and may require facilities for the rearing and manipulation of genetically modified insects. For this reason, coexpression of glycosyl transferases in silkworm pupae by co-infection with recombinant baculovirus has been successfully used to produce human IgG with the attached complex-type *N*-glycans (Kato et al. 2017). One additional limitation of protein expression using the BEVS, and especially with respect to the production in insects, is related to protein misfolding and formation of aggregates. To partially overcome this problem, Martínez-Alonso and co-workers (Martínez-Alonso et al. 2010) enhanced GFP solubility and extraction yield by co-expression of the recombinant protein along with DnaJ/DnaK chaperones in *T. ni* larvae. Also, in *B. mori* larvae, the expression of chaperones (calreticulin, calnexin, or immunoglobulin heavy-chain binding protein) increased the quality of the recombinant protein (Nakajima et al. 2009; Dojima et al. 2010).

Concluding remarks

Since its first use more than 40 years ago, the BEVS has been consolidated as one of the most effective methods for the production of large quantities of recombinant proteins. Previous and current efforts with respect to the improvement of the BEVS have been addressed to increase protein yield by the identification of novel promoters and other elements enhancing gene expression and protein stability, and by establishing novel approaches for using insect hosts as biofactory platforms (summarized at Table 1). A second front that remains open is focused on the reproduction of the mammalian glycosylation patterns in insect cells to produce more bioactive proteins. It is expected that recent developments in the CRISPR-Cas9 systems for specific genomic edition through mutations, insertions, and deletions (Doudna and Charpentier 2014) will open the door to a new array of strategies for engineering of the viral and host components, to improve the expression and glycosylation pattern of recombinant proteins produced using the BEVS.

Funding This study was partially supported by Generalitat Valenciana (GVPROMETEOII-2015-001), by the Spanish Ministry of Economy, Industry and Competitiveness-European FEDER funds (grant AGL2014-57752-C2-2R), and by ANPCyT-FONCyT (PICT 2015-1992). AMT is a member of the Research Career of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

References

Agathos SN (2010) Insect cell culture. In: Manual of Industrial Microbiology and Biotechnology, 3rd edn. American Society for Microbiology. ASM, pp 212–222

Angelo CS, Smith GE, Summers MD, Krug RM (1987) Two of the three influenza viral polymerase proteins expressed by using baculovirus vectors form a complex in insect cells. J Virol 61:361–365

Aumiller JJ, Mabashi-asazuma H, Hillar A, Shi X, Jarvis DL (2012) A new glycoengineered insect cell line with an inducibly mammalianized protein N-glycosylation pathway. Glycobiology 22:417–428. https://doi.org/10.1093/glycob/cwr160

Bonning BC, Roelvink PW, Vlak JM, Possee RD, Hammock BD (1994) Superior expression of juvenile hormone esterase and p-galactosidase from the basic protein promoter of *Autographa californica* nuclear polyhedrosis virus compared to the p10 protein and polyhedrin promoters. J Gen Virol 75:1551–1556

Chavez-Pena C, Kamen AA (2018) RNA interference technology to improve the baculovirus-insect cell expression system. Biotechnol Adv 36:443–451. https://doi.org/10.1016/j.biotechadv.2018.01.008

Chen Y, Yao B, Zhu Z, Yi Y, Lin X, Zhang Z, Shen G (2004) A constitutive super-enhancer: homologous region 3 of *Bombyx mori* nucleopolyhedrovirus. Biochem Biophys Res Commun 318:1039–1044. https://doi.org/10.1016/j.bbrc.2004.04.136

Choi JY, Woo SD, Je YH, Kang SK (1999) Development of a novel expression vector system using *Spodoptera exigua* nucleopolyhedrovirus. Mol Cells 9:504–509

Choi JY, Kim Y, Wang Y, Kang JN, Roh JY, Shim HJ, Woo S, Jin BR, Je YH (2009) Improved baculovirus vectors expressing barnase using promoters from *Cotesia plutellae* bracovirus. Mol Cells 28:19–24. https://doi.org/10.1007/s10059-009-0096-x

Cochran MA, Faulkner P (1983) Location of homologous DNA sequences interspersed at five regions in the baculovirus AcMNPV genome. J Virol 45:961–970

Contreras-Gómez A, Sánchez-Mirón A, García-Camacho F, Molina-Grima E, Chisti Y (2014) Protein production using the baculovirus-insect cell expression system. Biotechnol Prog 30:1–18. https://doi.org/10.1002/btpr.1842

Dodson MS, Crute JJ, Bruckner RC, Lehman IR (1989) Overexpression and assembly of the herpes simplex virus type 1 helicase-primase in insect cells. J Biol Chem 264:20835–20838

Dojima T, Nishina T, Kato T, Uno T, Yagi H, Kato K, Ueda H, Park EY (2010) Improved secretion of molecular chaperone-assisted human IgG in silkworm , and no alterations in their N-linked glycan structures. Biotechnol Prog 26:232–238. https://doi.org/10.1002/btpr.313

Doudna JA, Charpentier E (2014) The new frontier of genome engineering with CRISPR-Cas9. Science 346:1258096. https://doi.org/10.1126/science.1258096

Ejiofor AO (2016) Insect Biotechnology. In: Raman C, Goldsmith MR, Agunbiade TA (eds) Short views on insect genomics and proteomics: insect proteomics, vol 2. Springer, Switzerland, pp 194–197

Fath-Goodin A, Kroemer J, Martin S, Reeves K, Webb BA (2006) Polydnavirus genes that enhance the baculovirus expression vector system. Adv Virus Res 68:75–90. https://doi.org/10.1016/S0065-3527(06)68002-0

Fath-Goodin A, Kroemer JA, Webb BA (2009) The *Campoletis sonorensis* ichnovirus vankyrin protein P-vank-1 inhibits apoptosis in insect Sf9 cells. Insect Mol Biol 18:497–506. https://doi.org/10.1111/j.1365-2583.2009.00892.x

Geisler C, Aumiller JJ, Jarvis DL (2008) A *fused lobes* gene encodes the processing β-N-acetylglucosaminidase in Sf9 cells. J Biol Chem 283:11330–11339. https://doi.org/10.1074/jbc.M710279200

Gibson DG, Young L, Chuang R-Y, Venter JC, Hutchison CA, Smith HO (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods 6:343–345. https://doi.org/10.1038/nmeth.1318

Gómez-Sebastián S, López-Vidal J, Escribano JM (2014) Significant productivity improvement of the baculovirus expression vector system by engineering a novel expression cassette. PLoS One 9:e96562. https://doi.org/10.1371/journal.pone.0096562

Gong Z, Jin Y, Zhang Y (2006) Incorporation of partial polyhedrin homology sequences (PPHS) enhances the production of cloned foreign genes in a baculovirus expression system. Biotechnol Appl Biochem 46:165–170. https://doi.org/10.1042/BA20050163

Grabherr R, Ernst W, Doblhoff-Dier O, Sara M, Katinger H (1997) Expression of foreign proteins on the surface of *Autographa californica* nuclear polyhedrosis virus. Biotechniques 22:730–735

Grace TDC (1967) Establishment of a line of cells from the silkworm *Bombyx mori*. Nature 216:613. https://doi.org/10.1038/216613a0

Granados RR, Li G, Blissard GW (2007) Insect cell culture and biotechnology. Virol Sin 22:83–93

Guijarro-Pardo E, Gómez-sebastián S, Escribano JM (2017) In vivo production of recombinant proteins using occluded recombinant *Ac*MNPV-derived baculovirus vectors. J Virol Methods 250:17–24. https://doi.org/10.1016/j.jviromet.2017.09.017

Hebert CG, Valdes JJ, Bentley WE (2009) In vitro and in vivo RNA interference mediated suppression of Tn-caspase-1 for improved recombinant protein production in High Five™ cell culture with the baculovirus expression vector system. Biotechnol Bioeng 104:390–399. https://doi.org/10.1002/bit.22411

Hill-Perkins MS, Possee RD (1990) A baculovirus expression vector derived from the basic protein promoter of *Autographa californica* nuclear polyhedrosis virus. J Gen Virol 71:971–976

Hitchman RB, Possee RD, Crombie AT, Chambers A, Ho K, Siaterli E, Lissina O, Sternard H, Novy R, Loomis K, Bird LE, Owens RJ, King LA (2010a) Genetic modification of a baculovirus vector for increased expression in insect cells. Cell Biol Toxicol 26:57–68. https://doi.org/10.1007/s10565-009-9133-y

Hitchman RB, Possee RD, Siaterli E, Richards KS, Clayton AJ, Bird LE, Owens RJ, Carpentier DCJ, King FL, Danquah JO, Spink KG, King LA (2010b) Improved expression of secreted and membrane-targeted proteins in insect cells. Biotechnol Appl Biochem 56:85–93. https://doi.org/10.1042/BA20090130

Hiyoshi M, Kageshima A, Kato T, Park EY (2007) Construction of a cysteine protease deficient *Bombyx mori* multiple nucleopolyhedrovirus bacmid and its application to improve expression of a fusion protein. J Virol Methods 144:91–97. https://doi.org/10.1016/j.jviromet.2007.04.005

Ho Y, Lo H-R, Lee T-C, Wu CPY, Chao Y-C (2004) Enhancement of correct protein folding in vivo by a non-lytic baculovirus. Biochem J 382:695–702. https://doi.org/10.1042/BJ20040007

Hollister J, Grabenhorst E, Nimtz M, Conradt H, Jarvis DL (2002) Engineering the protein N-glycosylation pathway in insect cells for production of biantennary, complex N-glycans. Biochemistry 41:15093–15104

Ishiyama S, Ikeda M (2010) High-level expression and improved folding of proteins by using the vp39 late promoter enhanced with homologous DNA regions. Biotechnol Lett 32:1637–1647. https://doi.org/10.1007/s10529-010-0340-7

Jarvis DL, Weinkauf C, Guarino LA (1996) Immediate-early baculovirus vectors for foreign gene expression in transformed or infected insect cells. Protein Expr Purif 8:191–203. https://doi.org/10.1006/prep.1996.0092

Jehle JA, Blissard GW, Bonning BC, Cory JS, Herniou EA, Rohrmann GF, Theilmann DA, Thiem SM, Vlak JM (2006) On the classification and nomenclature of baculoviruses: a proposal for revision. Arch Virol 151:1257–1266. https://doi.org/10.1007/s00705-006-0763-6

Kaba SA, Salcedo AM, Wafula PO, Vlak JM, Van Oers MM (2004) Development of a chitinase and v-cathepsin negative bacmid for improved integrity of secreted recombinant proteins. J Virol Methods 122:113–118. https://doi.org/10.1016/j.jviromet.2004.07.006

Kato T, Kajikawa M, Maenaka K, Park EY (2010) Silkworm expression system as a platform technology in life science. Appl Microbiol

Biotechnol 85:459–470. https://doi.org/10.1007/s00253-009-2267-2

Kato T, Kako N, Kotaro K, Miyazaki T, Kondo S, Yagi H, Kato K, Park EY (2017) *N*-glycan modification of a recombinant protein via coexpression of human glycosyltransferases in silkworm pupae. Sci Rep 7:1409. https://doi.org/10.1038/s41598-017-01630-6

Kim YK, Cha HJ (2015) Engineering N-glycosylation pathway in insect cells: suppression of β-N-acetylglucosaminidase and expression of β-1,4-galactosyltransferase. In: Castilho A (ed) Glyco-Engineering. Methods in Molecular Biology. Humana Press, New York, pp 179–191

Kitts PA, Possee RD (1993) A method for producing recombinant baculovirus expression vectors at high frequency. Biotechniques 14:810–817

Kitts PA, Ayres MD, Possee RD (1990) Linearization of baculovirus DNA enhances the recovery of recombinant virus expression vectors. Nucleic Acids Res 18:5667–5672. https://doi.org/10.1093/nar/18.19.5667

Kwon M, Dojima T, Park E (2003) Comparative characterization of growth and recombinant protein production among three insect cell lines with four kinds of serum free media. Biotechnol Bioprocess Eng 8:142–146

Lai Y, Hsu JT, Chu C, Chang T, Pan K, Lin C (2012) Enhanced recombinant protein production and differential expression of molecular chaperones in *sf*-caspase-1-repressed stable cells after baculovirus infection. BMC Biotechnol 12

Lee JM, Kawakami N, Mon H, Mitsunobu H, Iiyama K, Ninaki S, Maenaka K, Park EY, Kusakabe T (2012) Establishment of a *Bombyx mori* nucleopolyhedrovirus (BmNPV) hyper-sensitive cell line from the silkworm e21 strain. Biotechnol Lett 34:1773–1779. https://doi.org/10.1007/s10529-012-0971-y

Li G-X, Hashimoto Y, Granados R (2003) Growth characteristics and expression of recombinant proteins by new cell clones derived from *Trichoplusia ni* (BTI Tn5B1–4) High Five™ cells. Bioprocess J 2:35–38

Lin G, Li G, Granados RR, Blissard GW (2001) Stable cell lines expressing baculovirus P35: resistance to apoptosis and nutrient stress, and increased glycoprotein secretion. Vitr Cell Dev Biol 37:293–302. https://doi.org/10.1290/1071-2690(2001)037<0293:SCLEBP>2.0.CO;2

Liu Y, Zhang Y (2015) Enhanced production of porcine circovirus type 2 (PCV2) virus-like particles in Sf9 cells by translational enhancers. Biotechnol Lett 37:1765–1771. https://doi.org/10.1007/s10529-015-1856-7

Lo H-R, Chou C-C, Wu T-Y, Yuen JP-Y, Chao Y-C (2002) Novel baculovirus DNA elements strongly stimulate activities of exogenous and endogenous promoters. J Biol Chem 277:5256–5264. https://doi.org/10.1074/jbc.M108895200

López-Vidal J, Gómez-Sebastián S, Sánchez-Ramos I, Escribano JM (2013) Characterization of a *Trichoplusia ni* hexamerin-derived promoter in the AcMNPV baculovirus vector. J Biotechnol 165:201–208. https://doi.org/10.1016/j.jbiotec.2013.03.012

Lu A, Miller LK (1997) Regulation of baculovirus late and very late gene expression. In: The Baculoviruses. pp 193–216

Luckow VA, Lee SC, Barry GF, Olins PO (1993) Efficient generation of infectious recombinant baculoviruses by site-specific transposon-mediated insertion of foreign genes into a baculovirus genome propagated in *Escherichia coli*. J Virol 67:4566–4579

Mabashi-Asazuma H, Jarvis DL (2017) CRISPR-Cas9 vectors for genome editing and host engineering in the baculovirus–insect cell system. PNAS 114:9068–9073. https://doi.org/10.1073/pnas.1705836114

Mabashi-Asazuma H, Sohn B-H, Kim Y-S, Kuo C-W, Khoo K-H, Kucharski CA, Fraser MJ Jr, Jarvis DL (2015) Targeted glycoengineering extends the protein *N*-glycosylation pathway in the silkworm silk gland. Insect Biochem Mol Biol 65:20–27. https://doi.org/10.1016/j.ibmb.2015.07.004.Targeted

Maeda S (1989) Gene transfer vectors of a baculovirus, *Bombyx mori* nuclear polyhedrosis virus, and their use for expression of foreign genes. In: Mitsuhashi J (ed) Invertebrate Cell Systems and Applications, vol 1. CRC Press, Boca Raton, pp 167–181

Manohar SL, Kanamasa S, Nishina T, Kato T, Park EY (2010) Enhanced gene expression in insect cells and silkworm larva by modified polyhedrin promoter using repeated burst sequence and very late transcriptional factor-1. Biotechnol Bioeng 107:909–916. https://doi.org/10.1002/bit.22896

Martínez-Alonso M, Gómez-Sebastián S, Escribano JM, Saiz J-C, Ferrer-Miralles N, Villaverde A (2010) DnaK/DnaJ-assisted recombinant protein production in *Trichoplusia ni* larvae. Appl Microbiol Biotechnol 86:633–639. https://doi.org/10.1007/s00253-009-2305-0

Martínez-Solís M, Gómez-Sebastián S, Escribano JM, Jakubowska AK, Herrero S (2016) A novel baculovirus-derived promoter with high activity in the baculovirus expression system. PeerJ 4:e2183. https://doi.org/10.7717/peerj.2183

Martínez-Solís M, Jakubowska AK, Herrero S (2017) Expression of the *lef5* gene from *Spodoptera exigua multiple nucleopolyhedrovirus* contributes to the baculovirus stability in cell culture. Appl Microbiol Biotechnol 101:7579–7588. https://doi.org/10.1007/s00253-017-8495-y

Mehalko JL, Esposito D (2016) Engineering the transposition-based baculovirus expression vector system for higher efficiency protein production from insect cells. J Biotechnol 238:1–8. https://doi.org/10.1016/j.jbiotec.2016.09.002

Miller LK (1981) A virus vector for genetic engineering in eukaryotes. In: Panopoulos NJ (ed) Genetic Engineering in the Plant Sciences. Praeger Publishers, New York

Motohashi T, Shimojima T, Fukagawa T, Maenaka K, Park EY (2005) Efficient large-scale protein production of larvae and pupae of silkworm by *Bombyx mori* nuclear polyhedrosis virus bacmid system. Biochem Biophys Res Commun 326:564–569. https://doi.org/10.1016/j.bbrc.2004.11.060

Nagata Y, Jae Man L, Hiroaki M, Imanishi S, Hong SM, Komatsu S, Oshima Y, Kusakabe T (2013) RNAi suppression of β-*N*-acetylglucosaminidase (BmFDL) for complex-type N-linked glycan synthesis in cultured silkworm cells. Biotechnol Lett 35:1009–1016. https://doi.org/10.1007/s10529-013-1183-9

Nakajima M, Kato T, Kanamasa S, Park EY (2009) Molecular chaperone-assisted production of human α-1,4-*N*-acetylglucosaminyltransferase in silkworm larvae using recombinant BmNPV bacmids. Mol Biotechnol 43:67–75. https://doi.org/10.1007/s12033-009-9174-8

Nerome K, Sugita S, Kuroda K, Hirose T, Nadia O, Soejoedono RD, Ika NLP, Agungpriyono S, Nerome R (2015) The large-scale production of an artificial influenza virus-like particle vaccine in silkworm pupae. Vaccine 33:117–125

Palmberger D, Wilson IBH, Berger I, Grabherr R, Rendic D (2012) Sweetbac: a new approach for the production of mammalianised glycoproteins in insect cells. PLoS One 7:e342226. https://doi.org/10.1371/journal.pone.0034226

Palmberger D, Klausberger M, Berger I, Grabherr R (2013) MultiBac turns sweet. Bioengineered 4:78–83. https://doi.org/10.4161/bioe.22327

Palmberger D, Ashjaei K, Strell S, Hoffmann-Sommergruber K, Grabherr R (2014) Minimizing fucosylation in insect cell-derived glycoproteins reduces binding to IgE antibodies from the sera of patients with allergy. Biotechnol J 9:1206–1214. https://doi.org/10.1002/biot.201400061

Palomares LA, Joosten CE, Hughes PR, Granados RR, Shuler ML (2003) Novel insect cell line capable of complex Nglycosylation and sialylation of recombinant proteins. Biotechnol Prog 19:185–192

Palomares LA, Srivastava IK, Ramírez OT, Cox MMJ (2018) Glycobiotechnology of the insect cell-baculovirus expression system technology. Adv Biochem Eng Biotechnol Jun 10. https://doi.org/10.1007/10_2018_61

Pijlman GP, Dortmans JCFM, Vermeesch AMG, Yang K, Martens DE, Goldbach RW, Vlak JM (2002) Pivotal role of the non-*hr* origin of DNA replication in the genesis of defective interfering baculoviruses. J Virol 76:5605–5611. https://doi.org/10.1128/JVI.76.11.5605

Pijlman GP, Vermeesch AMG, Vlak JM (2003) Cell line-specific accumulation of the baculovirus non-hr origin of DNA replication in infected insect cells. J Invertebr Pathol 84:214–219. https://doi.org/10.1016/j.jip.2003.10.005

Pijlman GP, Roode EC, Fan X, Roberts LO, Belsham GJ, Vlak JM, van Oers MM (2006) Stabilized baculovirus vector expressing a heterologous gene and GP64 from a single bicistronic transcript. J Biotechnol 123:13–21. https://doi.org/10.1016/j.jbiotec.2005.10.022

Possee RD, Hitchman RB, Richards KS, Mann SG, Siaterli E, Nixon CP, Irving H, Assenberg R, Alderton D, Owens RJ, King LA (2008) Generation of baculovirus vectors for the high-throughput production of proteins in insect cells. Biotechnol Bioeng 101:1115–1122. https://doi.org/10.1002/bit.22002

Regev A, Rivkin H, Gurevitz M, Chejanovsky N (2006) New measures of insecticidal efficacy and safety obtained with the 39K promoter of a recombinant baculovirus. FEBS Lett 580:6777–6782. https://doi.org/10.1016/j.febslet.2006.11.037

Rohrmann GF (2013) Baculovirus molecular biology, 3rd edn. National Center for Biotechnology Information (US), Bethesda

Romero LV, Targovnik AM, Wolman FJ, Cascone O, Miranda MV (2011) *Rachiplusia nu* larva as a biofactory to achieve high level expression of horseradish peroxidase. Biotechnol Lett 33:947–956. https://doi.org/10.1007/s10529-011-0540-9

Sari D, Gupta K, Raj DBTG, Aubert A, Drncová P, Garzoni F, Fitzgerald D, Berger I (2016) The MultiBac baculovirus/insect cell expression vector system for producing complex protein biologics. In: Advanced Technologies for Protein Complex Production and Characterization. pp 199–215

Shang H, Garretson TA, Senthil Kumar CM, Dieter RF, Cheng X-W (2017) Improved pFastBac™ donor plasmid vectors for higher protein production using the Bac-to-Bac® baculovirus expression vector system. J Biotechnol 255:37–46. https://doi.org/10.1016/j.jbiotec.2017.06.397

Smith GE, Fraser MJ, Summers MD (1983a) Molecular engineering of the *Autographa californica* nuclear polyhedrosis virus genome: deletion mutations within the polyhedrin gene. J Virol 46:584–593

Smith GE, Summers MD, Fraser MJ (1983b) Production of human beta interferon in insect cells infected with a baculovirus expression vector. Mol Cell Biol 3:2156–2165. https://doi.org/10.1128/MCB.3.12.2156.Updated

Steele KH, Stone BJ, Franklin KM, Fath-goodin A, Zhang X, Webb BA, Geisler C (2017) Improving the baculovirus expression vector system with vankyrin-enhanced technology. Am Inst Chem Eng 1496–1507. https://doi.org/10.1002/btpr.2516

Suzuki T, Kanaya T, Okazaki H, Ogawa K, Usami A, Watanabe H, Kadono-Okuda K, Yamakawa M, Sato H, Mori H, Takahashi S, Oda K (1997) Efficient protein production using a *Bombyx mori* nuclear polyhedrosis virus lacking the cysteine proteinase gene. J Gen Virol 78:3073–3080. https://doi.org/10.1099/0022-1317-78-12-3073

Targovnik AM, Arregui MB, Bracco LF, Urtasun N, Baieli MF, Segura MM, Simonella MA, Fogar M, Wolman FJ, Cascone O, Miranda M V (2016) Insect larvae : a new platform to produce commercial recombinant proteins. Curr Pharm Biotechnol 17

Teng C-Y, Chang S-L, van Oers MM, Wu T-Y (2013) Enhanced protein secretion from insect cells by co-expression of the chaperone calreticulin and translation initiation factor eIF4E. Mol Biotechnol 54:68–78. https://doi.org/10.1007/s12033-012-9545-4

Thiem SM, Miller LK (1990) Differential gene expression mediated by late, very late and hybrid baculovirus promoters. Gene 91:87–94. https://doi.org/10.1016/0378-1119(90)90166-O

Thimiri Govinda Raj DB, Vijayachandran LS, Berger I (2014) OmniBac: universal multigene transfer plasmids for baculovirus expression vector systems. Methods Mol Biol 1091:123–130. https://doi.org/10.1007/978-1-62703-691-7_7

Tiwari P, Saini S, Upmanyu S, Benjamin B, Tandon R, Saini KS, Sahdev S (2010) Enhanced expression of recombinant proteins utilizing a modified baculovirus expression vector. Mol Biotechnol 46:80–89. https://doi.org/10.1007/s12033-010-9284-3

Toth AM, Kuo C-W, Khoo K-H, Jarvis DL (2014) A new insect cell glycoengineering approach provides baculovirus-inducible glycogene expression and increases human-type glycosylation efficiency. J Biotechnol 20:19–29. https://doi.org/10.1016/j.jbiotec.2014.04.011.A

van Oers MM, Pijlman GP, Vlak JM (2015) Thirty years of baculovirus-insect cell protein expression: from dark horse to mainstream technology. J Gen Virol 96:6–23. https://doi.org/10.1099/vir.0.067108-0

Vaughn JL, Goodwin RH, Tompkins GJ, Mccawley P (1977) The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera; Noctuidae). In Vitro 13:213–217

Venkaiah B, Viswanathan P, Habib S, Hasnain SE (2004) An additional copy of the homologous region (hr1) sequence in the *Autographa californica* multinucleocapsid polyhedrosis virus genome promotes hyperexpression of foreign genes. Biochemistry 43:8143–8151. https://doi.org/10.1021/bi049953q

Ventini-Monteiro D, Dubois S, Astray RM, Castillo J, Pereira CA (2015) Insect cell entrapment, growth and recovering using a single-use fixed-bed bioreactor. Scaling up and recombinant protein production. J Biotechnol 216:110–115. https://doi.org/10.1016/j.jbiotec.2015.10.013

Wagner R, Liedtke S, Kretzschmar E, Geyer H, Geyer R, Klenk H-D (1996) Elongation of the *N*-glycans of fowl plague virus hemagglutinin expressed in *Spodoptera frugiperda* (Sf9) cells by coexpression of β1,2-*N*-acetylglucosaminyltransferase I. Glycobiology 6:165–175

Wang Q, Zhou Y, Chen K, Ju X (2016) Suppression of Bm-Caspase-1 expression in BmN cells enhances recombinant protein production in a baculovirus expression vector system. Mol Biotechnol. https://doi.org/10.1007/s12033-016-9931-4

Weidner T, Druzinec D, Buchholz R, Czermak P (2017) The components of shear stress affecting insect cells used with the baculovirus expression vector system. Zeitschrift für Naturforschñ 72:429–439

Weissmann F, Petzold G, Vanderlinden R, Huis in't Veld PJ, Brown NG, Lampert F, Westermann S, Stark H, Schulman BA, Peters J-M (2016) biGBac enables rapid gene assembly for the expression of large multisubunit protein complexes. PNAS E2566. https://doi.org/10.1073/pnas.1604935113

Wickham TJ, Davis T, Shuler RRGML, Woods HA (1992) Screening of insect cell lines for the production of recombinant proteins and infectious virus in the baculovirus expression system. Biotechnol Prog 8:391–396

Williams GV, Rohel DZ, Kuzio J, Faulkner P (1989) A cytopathological investigation of *Autographa californica* nuclear polyhedrosis virus p10 gene function using insertion/deletion mutants. J gen Virol 70:187–202

Wu H, Hebert CG, Hung C, Quan DN, Carter KK, Bentley WE (2013) Tuning cell cycle of insect cells for enhanced protein production. J Biotechnol 168:55–61. https://doi.org/10.1016/j.jbiotec.2013.08.017

Zitzmann J, Sprick G, Weidner T, Schreiber C, Czermak P (2017) Process optimization for recombinant protein expression in insect cells. In: New Insights into Cell Culture Technology. INTECH, pp 43–97