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The Biotechnological Potential of Baculoviruses: From Insect Viruses to Biotechnology Workhorse

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Review Article

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ABSTRACT

Baculoviruses are a significant group of arthropod viruses widely recognized for their potential as biological control agents against pests in agriculture and forestry. The Baculoviridae is a vast family of viruses that primarily infect various species within the Arthropoda phylum, particularly insects. Baculoviruses are widely used not only as biopesticides in agricultural applications but also as efficient tools for recombinant protein production. The Baculovirus Expression Vector System (BEVS) is particularly effective for expressing complex or difficult-to-produce proteins in mammalian cells. Owing to its high expression capacity and post-translational modification capabilities, BEVS has been successfully employed in various biotechnological fields, including vaccine development, therapeutic protein production, and the synthesis of enzymes and antibodies.
In this review, the BEVS technique, one of the significant areas of use of Baculoviruses, is discussed along with its advantages and practical applications.

Keywords: Baculovirus, BEVS, recombinant protein, vaccine

Baculovirüslerin Biyoteknolojik Potansiyeli: Insekt Viruslerinden Çok Yönlü Biyoteknolojik Araçlara

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ÖZ

Baculovirüsler, tarım ve ormancılıkta pestisitlere karşı biyolojik kontrol ajanları olarak potansiyelleri iyi bilinen artropodların önemli bir virüs grubudur. Baculoviridae, özellikle insektler olmak üzere, başlıca Arthropoda phylum şubesindeki farklı türleri enfekte eden geniş bir virüs ailesidir. Baculovirüsler, yalnızca tarımsal uygulamalarda biyopestisitler olarak değil, aynı zamanda rekombinant protein üretimi için etkili araçlar olarak da yaygın olarak kullanılmaktadır. Baculovirüs Ekspresyon Vektör Sistemi'nin (BEVS), memeli hücrelerinde kompleks veya üretilmesi zor proteinleri eksprese etmede özellikle etkili olduğu gösterilmiştir. BEVS, yüksek ekspresyon kapasitesi ve translasyon sonrası modifikasyon yetenekleri nedeniyle aşı geliştirme, terapötik protein üretimi, enzim ve antikor sentezi dahil olmak üzere çeşitli biyoteknolojik alanlarda başarıyla kullanılmıştır.
Bu derlemede, baculovirüslerin başlıca kullanım alanlarından biri olan BEVS tekniği, sağladığı avantajlar ve çeşitli pratik uygulamaları birlikte ele alınmaktadır

Anahtar Kelimeler: Baculovirus, BEVS, rekombinant protein, aşı

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Introduction

Baculoviruses are arthropod-specific viruses in the family Baculoviridae and infect more than 600 host species (Chambers et al., 2018). There are more than 500 species of baculoviruses in nature, all of which are restricted to a host range limited to invertebrates (Felberbaum, 2015). They are regarded as effective biological control agents for managing pest populations in agricultural and forestry sectors (Grzywacz, 2017). Baculovirus is an enveloped virus characterized by a double-stranded, circular DNA genome (Luckow, 1993). The length of the baculovirus rod-shaped nucleocapsid ranges from 200 to 400 nm, with a diameter of approximately 36 nm. The genome size varies between 80 and 180 kb (Van-Oers, 2011; Chambers et al., 2018). According to phylogenetic analyses, baculoviruses are divided into four genera: Alphabaculovirus, Betabaculovirus, Deltabaculovirus, and Gammabaculovirus. Alphabaculovirus includes all specific lepidopteran nucleopolyhedroviruses, single nucleocapsid type (SNPV) and multiple nucleocapsid type (MNPV). Betabaculovirus includes members of the lepidopteran-specific Granulovirus genus, Deltabaculovirus includes Diptera-specific baculoviruses, and Gammabaculovirus includes Hymenopteran-specific NPVs (Herniou & Jehle, 2007; Miele et al., 2011; Kelly et al., 2016). To date, 103 complete genome sequence data of at least 101 baculovirus species and isolates have been reported and are publicly available in GenBank (GenBank, 2024).

The majority baculoviruses have a minimal host range and typically target a single insect species (Clem & Passarelli, 2013). The replication cycle of this virus occurs in two distinct phases, resulting to the formation of two

morphologically different types: budded virus (BV) and occlusion-derived virus (ODV) (Thiem & Cheng, 2009). These two virions differ in the origin and constituents of their envelope structures and their functions in the life cycle. In both forms, the viral DNA associates with multiple copies of the small, positively charged protein p6.9, which neutralizes the DNA's negative charge. This nucleoprotein complex is further stabilized by structural proteins that assemble into the nucleocapsid (Haase et al., 2013). Primary infection and horizontal transmission of baculoviruses in host larvae are initiated orally through ODV. The second stage, secondary infection, which enables the spread of disease between tissues is initiated by virions known as the BV form (Chambers et al., 2018). The preferred virion form in cell culture for BEVS applications is the BV form, which has approximately 1800-fold higher infectivity than ODV (Possee et al., 2010) (Figure 1). Baculovirus vectors frequently used as vectors in studies are Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) and Bombyx mori nucleopolyhedrovirus (BmNPV). Although AcMNPV and BmNPV are genetically close viruses with approximately 90% amino acid sequence similarity in their ORFs, AcMNPV has a broader host range, distinguishing these two viruses from each other. AcMNPV, which forms the basis of baculovirus expression systems and is widely used in biotechnological applications, was identified as the first fully sequenced baculovirus prototype with a double-stranded circular DNA genome of 134,894 bp and 154 ORFs (Kang et al., 1999; Kato et al., 2016).

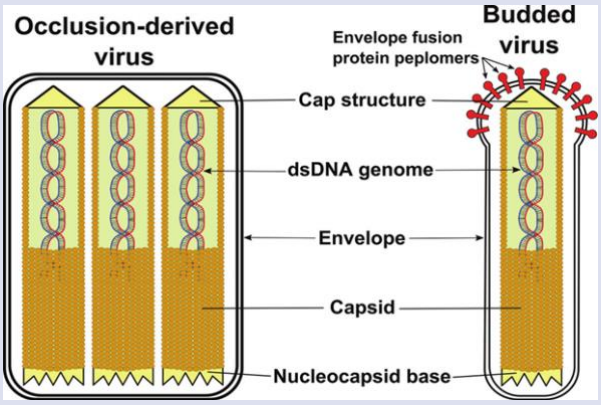

Figure 1. Extracellular Baculovirus virions: BV (budded virus) and ODV (occluded virus) (Harrison et al., 2018)

Baculoviruses are regarded as valuable vectors for producing foreign proteins due to their capacity to generate large amounts of polyhedrin. Polyhedrin is one of the essential genes that promotes viral production in vectors used to express foreign proteins. Recombinant baculoviruses have been engineered to express foreign genes under the control of the polyhedrin promoter, facilitating high-yield protein production (Yu et al., 2012). The ability of these viruses to produce polyhedrin has led to the notion that they can be utilized for the large-scale production of other proteins of significant interest to the research community. The discovery that baculoviruses do not require the polyhedrin protein for replication in insect cells supported the idea that recombinant baculoviruses could be produced by replacing the polyhedrin-encoding gene of these viruses with a targeted foreign gene. This approach enabled the production of independent recombinant baculoviruses that expressed the target proteins with high efficiency by integrating the foreign gene instead of the polyhedrin gene. The theoretical framework was subsequently implemented through the development of the first recombinant baculoviruses, generated via homologous recombination between the polyhedrin locus of the AcMNPV genome and transfer plasmids carrying a foreign gene under the regulatory control of the polyhedrin promoter (Jarvis, 2009; Rychlowska et al., 2011; Hong et al., 2022; Hong et al., 2023).

Baculovirus expression vector system (BEVS)

Baculovirus expression vector system (BEVS) is a system that utilizes the insect cell-specific infection ability of baculoviruses (Felberbaum, 2015). The basis of BEVS is to replace non-essential regions in the baculovirus genome with a foreign gene of interest, allowing the recombinant baculovirus to serve as a delivery vector and to transfer the target gene into insect host cells for subsequent replication and protein expression (Hong et al., 2023). The first study describing a baculovirus vector in insect cells was conducted by Smith et al. (1983) to produce recombinant human β-interferon. Since its establishment, BEVS has been widely utilized to produce numerous recombinant proteins, including those used in commercial vaccine development (Hong et al., 2022).

Baculoviruses possess two gene products, polyhedrin and p10, which are not essential for the continued production of viral particles in cell culture. Therefore, a foreign gene encoding the desired protein can be inserted into the coding regions of the genes corresponding to these products (Maeda et al., 1993). The classical BEVS is defined as a recombinant baculovirus engineered to carry a foreign nucleic acid sequence, typically a complementary DNA (mostly cDNA), under the transcriptional regulation of the strong polyhedrin promoter, enabling high-level expression of the target protein (Jarvis, 2009).

BEVS comprises three fundamental elements: a plasmid vector containing the target gene, a baculovirus genome (in the form of a bacmid or linear DNA), and an insect cell line (Hitchman et al., 2011). The BEVS process starts by creating a recombinant baculovirus containing the interest gene. This gene is cloned into a transfer plasmid under the control of a strong promoter, such as polyhedrin or p10, both of which are known to promote high levels of protein expression in insect cells (Felberbaum, 2015; Martínez-Solís et al., 2016).

The many commercially available BEVS can be classified into two main groups based on the mechanism of recombinant virus production. Commercially available BEVS are generally classified into two main categories based on the mechanism of recombinant virus generation.

The first category utilizes transposon-mediated technology, enabling recombinant bacmids to be produced. In this system, the gene of interest is initially cloned into a transfer vector, which is subsequently introduced into an Escherichia coli (E. coli)-based bacmid. Integration of the target gene into the bacmid genome occurs via a site-specific transposition process, facilitating the generation of a recombinant baculoviral genome suitable for transfection into insect cells (Wang et al., 2024). One commercially developed system that implements this method is the Bac-to-Bac™ system, provided by Invitrogen Inc. This method enables the integration of a target gene (GOI) into bacmid DNA using a transfer plasmid through site-specific transposition under the control of a specific promoter. This process occurs in E. coli DH10Bac cells and facilitates the transformation of bacmid DNA into a baculovirus expression vector. These cells include the bacmid and a helper plasmid, which provides the Tn7 transposase enzyme. After transposition, the recombinant bacmid is isolated and transfected into insect cells to generate recombinant baculovirus for expression (Pidre et al., 2023). The baculovirus genome and the transfer vector, which carries the gene of interest, are simultaneously transfected into insect cells. In vivo homologous recombination occurs within the host cells, creating a recombinant virus (Adeniyi and Lua, 2020).

Advantages and Disadvantages of Baculoviral Expression Vector System

The baculovirus expression vector system is a highly efficient and versatile with many advantages for protein expression (Bruder & Aucoin, 2022). BEVS provides several advantages over other widely utilized expression systems (Sandro & Benchaouir, 2019). In general, it is a safe, easy, and effective eukaryotic expression system for the production of recombinant proteins. Protein expression facilitated by the BEVS is considerably more cost-effective than expression in mammalian cell systems. It has many advantages, including high levels of protein expression, expression of large proteins, simultaneous expression of multiple genes, etc. Proteins expressed with BEVS are generally correctly folded and biologically active (Fath-Goodin et al., 2006; Adeniyi & Lua, 2020; Tang et al., 2020).

The baculovirus expression system facilitates the possible production of large amounts of recombinant proteins because it uses a baculovirus capable of high-titer replication in insect cells cultured in suspension without the need for a helper virus. Due to the viral genome being large and has a flexible capsid structure, it can accommodate significant foreign genes and large DNA fragments, allowing for the packaging and expression of polyprotein-encoding DNA. A definitive upper limit for inserting foreign DNA into the baculovirus genome has not been established. Baculoviruses are considered safe for vertebrates, as they are non-infectious and have been demonstrated to be inactive in mammalian cells. Therefore, proteins produced by BEVS can be used for functional studies, vaccine preparations, or diagnostic purposes. It also provides an advantage over other systems expressing oncogenes or toxic proteins (Murphy & Piwnica-Worms, 2001; Haase et al., 2013; Mishra, 2020).

Due to their optimal replication at temperatures between 25°C and 30°C, baculoviruses represent an advantageous expression system for producing proteins from ectothermic organisms that require lower temperatures to maintain biological activity. Therefore, this platform has been critical in developing protein or vaccine platforms against Arbovirus infections. As a result of the efficient replication of arboviruses in insect cells, the arboviral proteins produced in baculovirus-infected insect cells typically exhibit correct folding and glycosylation patterns, which are biologically active (Metz & Pijlman, 2011). Baculoviruses naturally infect arthropods and cannot replicate in vertebrates, plants, or microorganisms. Nonetheless, they can enter certain vertebrate cells under specific conditions and deliver their genetic material. Because of this, the BEVS has become a valuable tool for studying gene expression and protein function in vertebrate cells (Rychlowska et al., 2011).

Additionally, the BEVS is capable of simultaneously expressing multiple heterologous proteins. For example, the MultiBac system, derived from the BEVS platform, is suitable for expressing various proteins at the same time, allowing the formation of specific multi-subunit protein complexes. HR-Bac is another MultiBac-based system that simplifies expression screening and high-yield protein production applications (Sari et al., 2016; Hong et al., 2023). BEVS can carry out various post-translational modifications, such as phosphorylation, different types of glycosylation (N-linked and O-linked), ubiquitination, acetylation, and proteolytic processing. These modifications help understand protein functions and are especially useful for structural biology and crystallography studies (Fraser, 1992; Mishra, 2020; Hong et al., 2023).

Despite its advantages, using baculoviruses in gene therapy presents certain limitations. It can trigger immune responses characterized by the release of inflammatory cytokines and chemokines and activation of the complement system. These immune reactions may cause unwanted effects and contribute to the degradation of the viral genome, particularly when baculoviruses are used for therapeutic purposes other than vaccination (Schaly et al., 2021).

Compared to other viral vectors, baculoviral vectors differ in how long the introduced genetic material remains inside the host cell's nucleus. While the DNA delivered by retroviral, lentiviral, and adenoviral vectors can stay in the nucleus for extended periods either by integrating into the host genome or by existing as episomal DNA, baculoviral DNA has been observed to persist in mammalian cell nuclei for only 24 to 48 hours (Tjia et al., 1983). In addition, it has been demonstrated that baculoviral DNA is degraded over time due to a decrease in the transgene

copy number and mRNA transcription level in baculovirus-transduced cells (Hu, 2006).

Similar to other enveloped viruses, baculoviruses are highly fragile. As a result, they are sensitive to mechanical shear forces, contributing to their relatively low stability. Ultracentrifugation is usually used for baculovirus purification; however, this process can damage the viral envelope and significantly decrease infectivity (Pidre et al., 2023).

Application of the Baculoviral Expression Vector Systems

Viral Vaccinology

The baculovirus expression system has become essential for synthesizing structurally complex eukaryotic proteins. Its first notable application involved successfully expressing recombinant human interferon-beta (IFN-β) in insect cell cultures. The successful expression of IFN-β marked the initial establishment of the baculovirus–insect cell protein expression system. Soon after, other recombinant proteins such as E. coli β-galactosidase, human c-Myc, and human interleukin-2 (IL-2) were also expressed at high levels (Murphy & Piwnica-Worms 2001).

The use of baculovirus as a vector for vaccination was first described by Aoki et al. (1999). The study demonstrated that recombinant baculovirus carrying the pseudorabies viral gB gene induced specific antibody responses in mice. It has subsequently been widely used in many recombinant vaccine development platforms. This system has been used to investigate capsid formation (VLP) of many viruses, including rotavirus, picornavirus, orbivirus, calicivirus, papillomavirus, herpesvirus, and parvoviruses (Ju et al., 2011). In the study conducted by Li et al. (2016), the N and G protein genes of Rift Valley fever virus (RVFV) were inserted into the pFastBacDual baculovirus expression vector under the control of the pP10 and pPH promoters. The resulting RVFV VLPs were concluded to provide a foundation for developing future VLP-based RVFV vaccines. In the other study by Dai et al. (2018), the Zika virus (ZIKV) premembrane (prM) and envelope (E) proteins were co-expressed in insect cells. ZIKV VLPs were efficiently and rapidly produced in large quantities using this system. The findings demonstrated that these VLPs exhibited strong immunogenicity in immunized mice by eliciting high virus-neutralizing antibodies, ZIKV-specific IgG responses, and robust memory T cell activation. Yang et al. (2022) reported that H5N6 VLPs, a highly pathogenic avian influenza (HPAI) strain, induced higher neutralizing antibody titers as well as higher levels of IL-2, IL-4, IL-5, IFN-γ, and TNF, suggesting that H5N6 VLPs may be a potential vaccine candidate for broad-spectrum H5Nx avian influenza vaccines. In a study producing Getah virus (GETV) VLPs in insect cells using BEVS (Miao et al., 2024), it was reported that adjuvant-free vaccination with GETV VLP protected wild-type C57/BL6 mice against GETV viremia and arthritic disease. In a study establishing a VLP-based vaccine platform for the recently pandemic SARS-CoV-2 virus (Nguyen et al., 2024), envelope and membrane proteins of the SARS-CoV-2 Wuhan strain were expressed by the recombinant baculovirus BacMam. In a mouse trial, two intramuscular immunizations of the VLP BacMam elicited specific antibodies in sera and bronchoalveolar lavage fluids. In recent years, various virus-like particle (VLP)-based vaccine studies have been conducted, including HIV-1 Gag VLPs (Puente-Massaguer et al., 2020), Simian virus 40 (SV40) VLPs (Saika et al., 2020), Bovine viral diarrhea virus (BVDV) E2 and Erns VLPs (Wang et al., 2021), human papillomavirus (HPV) L1 protein (Razavi-Nikoo et al., 2023), Porcine deltacoronavirus (PDCoV) VLPs (Liu et al., 2023), novel goose parvovirus (NGPV) VP2 protein (Zhang et al., 2024), Rabbit hemorrhagic disease virus (RHDV) VP60 gene (Hu et al., 2025), and Feline panleukopenia (FPL) VP2 protein (Feng et al., 2025).

In vaccine development, BEVS is one of the most commonly used systems for producing the recombinant proteins needed for different types of vaccines. In the study conducted by Skoberne et al. (2013), the herpes simplex virus (HSV) glycoprotein D2-expressing subunit vaccine elicited humoral immune responses in immunized mice and induced $CD4^+$ and $CD8^+$ T cells characterized by multiple cytokine secretion and cytolytic antigen-specific T cell responses. Yin et al. (2013) demonstrated that the rabies virus's nucleoprotein (N) gene can be highly expressed using a silkworm-baculovirus expression system. The N antigen vaccine is a promising approach for preventing the rabies virus. Respiratory syncytial virus (RSV) fusion glycoprotein (F) was modified and expressed as an antigen in BEVS by Blanco et al (2014). Ge et al., 2016, reported that recombinant baculovirus vaccines expressing Newcastle disease virus (NDV) F or HN genes induced a strong cellular and humoral response in chickens. In the study by Hu et al. (2019), a baculovirus-based vaccine was developed to express the hemagglutinin (HA) protein of highly pathogenic avian influenza A (H7N9). The recombinant baculovirus stably expressed the HA protein in insect cells, and immunization with the candidate vaccine effectively reduced both viral shedding and viral replication in chickens. The study by Zhang et al., 2023 explored the development of recombinant vaccine candidates for Crimean-Congo hemorrhagic fever virus (CCHFV) using the BEVS. They designed and constructed three vaccine candidates that encoded the Gn glycoprotein and the nucleocapsid protein (Np) of CCHFV. Experimental results reported that all three recombinant baculoviruses exhibited significant humoral immunity in BALB/c mice. In a study conducted by Caillava et al. (2024), Chikungunya virus CHIKV E1 and E2 envelope proteins expressed on the surface of budded baculovirus virions induced IgG antibodies, neutralizing antibodies, and a specific IFN-γ CD8 + T cell response in C57BL/6 mice. In a comparative analysis between a baculoviral vector vaccine (AcHERV-gE-gB) encoding varicella-zoster virus (VZV) gE and gB glycoproteins and a live attenuated vaccine strain, vOka (Lee et al., 2024), in a mouse model, AcHERV-gE-gB elicited similar or higher levels of IgG, IgG2a and neutralizing antibodies than vOka.

Several studies have shown that baculoviruses exhibit potent adjuvant effects that boost immune responses in vaccination strategies. Blazevic et al., 2016 demonstrated that recombinant polymeric Rotavirus (RV) VP6 protein produced in a baculovirus-insect cell expression system acts as an adjuvant in combination with NoV VLPs and the vaccine candidate induced strong potential protective immune responses in BALB/c mice. According to Heinimäki et al. (2017), baculovirus enhanced the adaptive immune response to monomeric ovalbumin and oligomeric norovirus VLPs, indicating its potential as an effective adjuvant in vaccine development.

The BEVS has facilitated the FDA approval of eight vaccines for human use, including Cervarix™ for the prevention of cervical cancer, Flublok® and Flublok Quadrivalent® for influenza, and the COVID-19 vaccines NVX-CoV2373 and Weikexin, as well as five additional vaccines approved for veterinary purposes. The FDA approved Cervarix, the first therapeutic protein produced in insect cells using baculoviruses as expression vectors 2009. Cervarix is a virus-like particle (VLP) vaccine formulated with the L1 capsid proteins of HPV types 16 and 18 and protects against human papillomavirus (HPV). Flublok, developed against influenza in 2013, was also approved by the FDA. Flublok is a recombinant hemagglutinin (rHA) vaccine developed using the BEVS, derived from three influenza virus strains. Vaccines against SARS-CoV-2, recently synthesized using BEVS, are also based on 3 different VLP-based technologies. In addition to these vaccines, many BEVS-derived vaccines are in clinical trials against Norovirus, Parvovirus B19, human respiratory syncytial virus (RSV) and Ebola (EBOV) viruses. Not only has the BEVS been used in human vaccines, but it has also significantly contributed to veterinary medicine, enabling the development of two protective vaccines against classical swine fever virus and three vaccines targeting porcine circovirus type 2 (Sokolenko et al., 2012; Hong et al., 2023).

Pesticide

Historically, a primary focus of baculovirus-based product development has been controlling Heliothis/Helicoverpa species in cotton, as these insects have long represented the most significant pests of this globally important agricultural commodity. The first baculovirus used commercially was a nucleopolyhedrovirus (NPV) obtained from Helicoverpa zea, and it was developed for use in cotton farming. It became a promising alternative for pest control, mainly due to the growing resistance of cotton pests to synthetic pyrethroids (Cory & Bishop, 1997; Grzywacz, 2017). Similarly, the gypsy moth virus (Lymantria dispar nucleopolyhedrovirus) is considered a key natural agent in regulating gypsy moth populations. In the same way, baculoviruses infecting the Douglas fir tussock moth (Orgyia pseudotsugata) have also been important in regulating this insect's population (Rohrmann, 2019).

According to the OECD's 2023 report (OECD, 2023), approximately 60 baculovirus-based pesticides have been marketed to control pest insects worldwide.

Gene Therapy

With the advancement of baculovirus research, it has been demonstrated that recombinant baculoviruses can enter not only insect host cells but also a variety of mammalian cells through the envelope glycoprotein GP64, and are capable of expressing foreign genes under the control of mammalian promoters without any viral replication (Ono et al., 2018).

Baculoviruses are considered promising gene transfer vectors for vertebrate cells, primarily due to the low activity of their promoters in mammalian cells and the high level of biosafety conferred by their non-pathogenic, budding viral forms (Liu et al., 2017).

Baculovirus has been investigated as a potential cancer therapy vector due to its ability to suppress tumor growth (Ono et al., 2018). It has been used to deliver diphtheria toxin A for targeting malignant glioma cells (Wang et al., 2006) and to express herpes simplex virus thymidine kinase (HSVtk), inducing cell death in glioblastoma cells in the presence of ganciclovir (Balani et al., 2009). Espíritu-Ramírez et al. (2018) demonstrated that gene therapy using a baculovirus vector carrying the glutamine synthetase (Bac-GS) gene effectively reduced ammonia levels in a rat model of acute hyperammonemia. In a study (Garcia Fallit et al., 2023) investigating the potential of baculoviral vectors for gene therapy targeting brain cancer, the findings indicated that baculoviruses could serve as effective vehicles for delivering therapeutic transgenes to brain cells, highlighting their potential applicability in both degenerative and neoplastic brain disorders.

Baculovirus vectors have been emerged as effective vaccine carriers in mice and non-human primates by stimulating both humoral and cellular immune responses through antigen expression or surface display of peptides fused to Baculovirus envelope proteins. This approach is being evaluated as a potential strategy for preventing and treating human and animal infectious diseases such as malaria, influenza and rabies (Kwang et al., 2016).

Conclusion

Initially used for pesticide purposes, Baculoviruses have become powerful tools in modern biotechnology. Thanks to their biological properties, such as high biosafety profile and large gene splicing capacity, they have been successfully applied in various fields such as recombinant protein production, vaccine development, and gene therapy. BEVS is a strong and versatile platform tool for producing complex eukaryotic proteins with the appropriate post-translational modifications. Despite some limitations, recent improvements continue to increase the usefulness of baculovirus-based technologies. With further development, baculoviruses will become even more important in research and therapeutic contexts, providing a safe, scalable, and efficient solution for future biotechnological applications.

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