PERIÓDICO TCHÊ QUÍMICA
ARTIGO DE REVISÃO

O USO DE CRISÁLIDAS INFECTADAS POR BACULOVÍRUS NA PRODUÇÃO DE VACINAS DE SUBUNIDADE: UMA MINI-REVISÃO

THE USE OF BACULOVIRUS-INFECTED CHRYSALIDES IN THE PRODUCTION OF SUBUNIT VACCINES: A MINI-REVIEW

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Received 14 June 2025; received in revised form 04 November 2025; accepted 06Nnovember 2025

RESUMO

Introdução: A crescente demanda por produção eficiente e escalável de vacinas levou ao desenvolvimento de plataformas inovadoras, como a CrisBio, desenvolvida pela Cocoon. Essa tecnologia utiliza crisálidas da mariposa *Trichoplusia ni* como biorreatores naturais para a expressão de proteínas recombinantes, empregando o sistema de vetor de expressão por baculovírus (BEVS). Após a inoculação com sequências específicas de DNA, essas crisálidas permitem a síntese de proteínas complexas, com modificações pós-traducionais avançadas, em apenas 3 a 6 dias. Notavelmente, a CrisBio demonstrou a capacidade de produzir partículas semelhantes a vírus (VLPs)**,** incluindo o antígeno PCV2 Cap e a proteína VP60 do RHDV, alcançando rendimentos de até 10 mg de proteína por grama de biomassa. **Objetivo:** Esta mini-revisão visa atualizar o estado da arte sobre o uso de crisálidas infectadas com baculovírus recombinantes para a produção de vacinas de subunidade. **Métodos:** Foi realizada uma revisão sistemática da literatura nas bases de dados PubMed e SciELO para identificar estudos que descrevem o uso de crisálidas e baculovírus modificados na produção de vacinas. A estratégia de busca incluiu os termos: *crisálidas*, *baculovírus* e *produção de vacinas*, utilizados em diversas combinações. **Resultados:** A plataforma CrisBio suporta o manuseio e processamento totalmente automatizado de crisálidas, simplificando significativamente as fases de inoculação e expressão proteica. O uso de baculovírus geneticamente modificados possibilita a produção rápida de proteínas recombinantes com estruturas complexas e altos rendimentos. Além disso, esse sistema prova ser altamente escalável e economicamente viável, com custos de produção reduzidos em até 95% em comparação com métodos convencionais baseados em culturas celulares. **Discussão:** As técnicas tradicionais de fabricação de vacinas enfrentam limitações em escalabilidade, custo e velocidade de produção. Em contraste, a CrisBio oferece vantagens substanciais, incluindo tempo de resposta mais rápido, custos mais baixos e a capacidade de produzir proteínas com modificações pós-traducionais complexas, tornando-a uma plataforma promissora para uma ampla gama de aplicações biofarmacêuticas. **Conclusões:** Em comparação com os métodos tradicionais, a plataforma CrisBio melhora a biossegurança, reduz o tempo e os custos de produção e oferece uma solução escalável para o desenvolvimento de vacinas de subunidade. Sua implementação poderia melhorar significativamente o acesso global a vacinas, especialmente em ambientes com poucos recursos.

Palavras-chave*: Biotecnologia, Baculovírus, Crisálidas, Vacinas.*

ABSTRACT

Background: The increasing demand for efficient and scalable vaccine production has led to the development of innovative platforms such as CrisBio, developed by Cocoon. This technology utilizes chrysalides of the moth *Trichoplusia ni* as natural bioreactors for recombinant protein expression, employing a baculovirus expression vector system (BEVS). Upon inoculation with specific DNA sequences, these chrysalides enable the synthesis of complex proteins with advanced post-translational modifications in just 3 to 6 days. Notably, CrisBio has

DOI: 10.52571/PTQ.v22.n51.2025_09_ESCARETE_pgs_117_122.pdf

demonstrated the ability to produce virus-like particles (VLPs), including the PCV2 Cap antigen and the VP60 protein of RHDV, reaching yields of up to 10 mg of protein per gram of biomass. **Aim:** This mini-review aims to update the state of the art regarding the use of chrysalides infected with recombinant baculoviruses for subunit vaccine production. **Methods:** A systematic literature review was conducted using PubMed and SciELO databases to identify studies describing the use of chrysalides and modified baculoviruses in vaccine production. The search strategy included the terms: chrysalides, baculoviruses, and vaccine production, used in various combinations. **Results**: The CrisBio platform supports fully automated handling and processing of chrysalides, significantly streamlining the inoculation and protein expression phases. The use of engineered baculoviruses enables rapid production of recombinant proteins with complex structures and high yields. Moreover, this system proves to be highly scalable and cost-effective, with production costs reduced by up to 95% compared to conventional cell culture-based methods. **Discussion:** Traditional vaccine manufacturing techniques face limitations in scalability, cost, and production speed.In contrast, CrisBio offers substantial advantages, including faster turnaround, lower costs, and the ability to produce proteins with complex post-translational modifications, making it a promising platform for a wide range of biopharmaceutical applications. **Conclusions:** Compared to traditional methods, the CrisBio platform enhances biosafety, reduces production time and costs, and offers a scalable solution for subunit vaccine development. Its implementation could significantly improve global access to vaccines, particularly in low-resource settings.

Keywords: *Biotechnology, Baculovirus, Chrysalides, Vaccines.*

1. INTRODUCTION

The pursuit of new strategies to develop more effective vaccine production methods has intensified in recent years. Various biotechnology companies have invested in this scientific field, presenting innovative proposals such as Cocoon's CrisBio technology introduced in 2022.

CrisBio has enabled advances across diverse areas, from cultured meat production to healthcare applications, particularly vaccines. This platform has demonstrated superior efficacy compared to traditional methods by producing simple and complex recombinant proteins. It leverages a unique protein expression system based on the baculovirus expression vector system (BEVS), which initiates the biotechnological process. Baculoviruses play a pivotal role due to their natural ability to infect insect cells, facilitating highly efficient recombination. The desired products are subsequently extracted through purification methods (Hong, 2022).

A distinguishing feature of this technology is the use of natural bioreactors—the chrysalides of *Trichoplusia ni*. These pupae are infected with baculoviruses containing the target DNA sequence, as previously described (Altmann, 1999;, Escribano, 2020).

Although initial tests of this technology have been conducted, further investigation into its advantages and disadvantages is necessary. This would facilitate improvements to future techniques, expanding their application to human vaccine production while maintaining efficacy and increasing vaccine availability in more regions.

2. METHODS

A systematic review of the literature in PubMed and Scielo was performed to search for publications describing the use of chrysalides and Modified Baculoviruses for vaccine production, collecting and analyzing data. To explore this, we used the following words/terms in combination: chrysalides AND Baculoviruses AND vaccine production. The exclusion criteria consisted of limiting papers on the use of any of those drugs from 2001 to 2023. The work was made as a task for the subject Biotechnology, belonging to the Pharmacy and Biochemistry career, and the extension and number of citations were restricted to the indication of the cathedra.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1 The CrisBio Technology

Traditional vaccine production methods often involve *in vitro* cultures of mammalian, insect, bacterial, or yeast cells within bioreactors. These approaches are resource-intensive, costly, and time-consuming, limiting the ability to meet the global demand for recombinant proteins.

The CrisBio platform emerged as a versatile alternative, offering higher yields in vaccine manufacturing. The methodology begins with insect rearing under controlled temperature, humidity, and incubation conditions to optimize the organism's physiology. *Trichoplusia ni* eggs are incubated for eight days at 21-27°C and 50-70% humidity. The resulting pupae are chemically

treated to remove silk and stored in RFID-tagged trays detailing viral characteristics and inoculation dosages (Escribano, 2020). These chrysalides serve as active biocapsules, facilitating production while ensuring easy handling throughout the process.

Simultaneously, baculoviruses are prepared to deliver the target protein's DNA sequence. These virus primarily infect insects, especially Lepidoptera (moths and butterflies), and are non-pathogenic to humans, enhancing biosafety. Additionally, the BEVS system accommodates large DNA insertions, enabling the production of a wide range of proteins [Zhang, 2008; Felberbaum, 2015). It is noteworthy that other vectors are used, as Adenivirus, with mRNA as a genetic material to deliver, instead of DNA (Gebre, 2013).

To evaluate CrisBio's productivity, Cocoon generated two recombinant baculoviruses expressing the Cap protein of PCV2 and VP60 protein of RHDV, forming VLPs in insect cells. These proteins were chosen due to their established market presence in animal vaccines. Genetic sequences were synthesized, cloned into optimized plasmids, and transfected into E. coli to produce recombinant baculoviruses through a two-step selection and cloning process (Hong, 2022).

The pupae and baculoviruses were then used for automated inoculation. Plastic trays containing pupae were processed by a machine equipped with a needle-bearing arm, injecting up to 5 mL of virus with adjusted concentrations to maximize recombinant protein production. The system achieved an inoculation speed of approximately 3,000 pupae per hour.

Following inoculation, the pupae were incubated for 3 to 7 days before harvesting. Homogenized pupae were processed with PBS, reducing agents, protease inhibitors, salts, and detergents at the optimal pH for each protein. Purification steps included clarification, diafiltration, ultrafiltration, and purification to obtain purified VLPs. Protein concentration, yield, and purity were assessed using SDS-PAGE analysis (Escribano, 2020).

CrisBio technology demonstrated fully automated handling and processing of chrysalides, significantly accelerating the inoculation and production stages. Using baculoviruses with specific DNA sequences, recombinant proteins with complex structures and advanced post-translational modifications were produced within 3 to 6 days post-infection (Felberbaum, 2015).

The system excelled in producing proteins for biotechnological applications in healthcare, achieving post-translational modifications comparable to those of eukaryotic cells and maintaining stability for up to two years at -20°C (Hong, 2022). Successful VLP formation was observed for the Cap antigen of PCV2 and the VP60 protein of RHDV, demonstrating CrisBio's versatility in generating complex recombinant vaccines (Altmann, 1999).

Protein yields ranged from 2 to 5 mg per gram of biomass, with some trials exceeding 10 mg/g. This scalability and cost-effectiveness position CrisBio as a viable option for mass vaccine production, with the use of biocapsules optimizing efficiency and reducing costs by up to 95% compared to cell culture-based methods (Felberbaum, 2015).

3.1.2 Comparison with Traditional Methods

Traditional vaccine production methods face several limitations that hinder their efficacy and applicability. Producing vaccines for immunocompromised individuals can be challenging due to insufficient personalization and higher reactogenicity, which can cause adverse effects post-administration (Felberbaum, 2015; Ghattas,2021; Hayman, 2021).

Reactogenicity is a critical consideration in vaccine development, as the ideal vaccine should be effective in preventing diseases while minimizing adverse reactions. The CrisBio technology mitigates this risk by employing non-pathogenic vector-based expression systems.

Another significant challenge is the prolonged development time associated with traditional methods, such as cell culture or embryonated eggs, which delays vaccine availability during emergencies like pandemics. In contrast, CrisBio reduces production time to just 3 to 6 days. In the same sense, plant-made vacccines are another example of molecular farming with several developments, mainly used in veterinary (Rybicki, 2010).

High infrastructure costs also limit traditional methods. Mammalian cell fermentation requires significant investments in cell line maintenance, large bioreactors, and controlled facilities. CrisBio's use of natural bioreactors—chrysalides—eliminates the need for costly machinery, reducing expenses by up to 95%.

3.1.3 An application: Hepatitis B Vaccines

The first hepatitis B vaccine, introduced in 1982, consisted of purified HBsAg derived from the plasma of individuals with chronic HBV infection. This vaccine has since been replaced by recombinant vaccines, which eliminate concerns associated with human blood products. The HBsAg gene has been inserted into yeast and mammalian cells using appropriate expression vectors, with the antigen expressed in several yeast species.

A recent application was published by Hussain et al (Hussain, 2005). This study conclusively demonstrates that the recombinant DNA hepatitis B vaccine (Enivac-HB), produced using genetically modified Pichia pastoris yeast cells, appears to be highly immunogenic and safe. It provides seroprotection in 96.5% of participants, with 88.0% exhibiting a hyperresponse. The findings suggest that the vaccine is well-tolerated and support the recommendation of a rapid vaccination schedule (0, 1, and 2 months) for cases where rapid protection is desired.

3.2. Discussions

Traditional vaccine production methods face several limitations that hinder their efficacy and applicability. Producing vaccines for immunocompromised individuals can be challenging due to insufficient personalization and higher reactogenicity, which can cause adverse effects post-administration [Kost, 2016; Hayman, 2021, Hussain, 2005).

Reactogenicity is a critical consideration in vaccine development, as the ideal vaccine should be effective in preventing diseases while minimizing adverse reactions. The CrisBio technology mitigates this risk by employing non-pathogenic vector-based expression systems.

Another significant challenge is the prolonged development time associated with traditional methods, such as cell culture or embryonated eggs, which delays vaccine availability during emergencies like pandemics. In contrast, CrisBio reduces production time to just 3 to 6 days.

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In summary, CrisBio offers significant advantages for recombinant protein production, enabling complex post-translational modifications and providing a cost-effective, scalable solution for various applications (Zhang, 2008)

4. CONCLUSIONS

This study draws attention to the transformative potential of biotechnological advancements in healthcare. Through the innovative CrisBio technology, the complexity of synthesizing recombinant proteins or similar compounds has been addressed. By utilizing natural bioreactors, such as Trichoplusia ni chrysalides, large-scale production of target compounds has become feasible, followed by traditional purification methods.

This comparison of existing vaccine manufacturing possibilities highlights the advantages and limitations of various techniques. Ultimately, CrisBio presents a promising alternative, offering solutions to challenges associated with infrastructure, materials, and the quality and quantity of vaccine production. This technology paves the way for a new era in vaccine development, contributing to enhanced accessibility and global health outcomes.

5. DECLARATIONS

5.1. Study Limitations

This comparative review has several limitations that should be acknowledged. The literature search was restricted to two databases (PubMed and Scielo) and publications from 2001-2023, potentially excluding relevant studies from other sources or time periods. As an academic coursework project, the scope and depth of analysis were constrained by institutional requirements regarding length and citation limits.

Additionally, the rapidly evolving nature of CrisBio technology means newer data may have emerged since the completion of this analysis. Cost-effectiveness comparisons were limited due to variable pricing across healthcare systems and the lack of comprehensive economic analyses in the available literature.

5.2. Acknowledgements

We would like to extend our sincerest

gratitude to our parents, whose unwavering support and encouragement have been instrumental in our academic journey. We also express our deepest appreciation to our professors, who have provided us with invaluable guidance and expertise throughout our studies. Finally, we acknowledge the university's provision of resources and facilities, which have enabled us to grow as professionals and pursue our academic goals. This would not have been possible without the support and opportunities we have received, and for that, we are deeply grateful.

5.3. Funding source

This work was supported by the Universidad Juan Agustín Maza as part of the Biotechnology coursework requirements. No external funding was received for this research. In accordance with the ethical guidelines of the Periódico Tchê Química, which do not allow donations from authors with manuscripts under evaluation (even when research funds are available), or in cases of authors' financial constraints, publication costs were fully absorbed by the journal under our Platinum Open Access policy, through the support of the Araucária Scientific Association (https://acaria.org/). This policy aims to ensure complete independence between the editorial process and any financial aspects, reinforcing our commitment to scientific integrity and equity in knowledge dissemination.

5.4. Competing Interests

The authors declare no financial, professional, or personal conflicts of interest that could have influenced the content or conclusions of this review. All authors are affiliated solely with academic institutions and have no commercial relationships with pharmaceutical companies manufacturing the treatments discussed in this analysis.

5.5. Open Access

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Figure 1. Protein production in the CrisBio system. The figure shows the step-by-step process for protein production using CrisBio technology