OPTIMIZATION OF REPLICATION AND MAINTENANCE CONDITIONS OF VARIOUS BACULOVIRUSES (HELICOVERPA ZEA AND SPODOPTERA FRUGIPERDA) IN INSECT CELL LINES

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Abstract: *This article highlights the replication of various baculoviruses (Helicoverpa zea and Spodoptera frugiperda) in insect cells, as well as the optimization of their maintenance and propagation conditions. The study thoroughly investigated the growth efficiency of these viruses in vitro (under laboratory conditions) using insect cell lines, analyzing the effects of multiplicity of infection (MOI), temperature, pH, and nutrient media on viral proliferation. Virus titers in Sf9 and HzAM1 cell lines were determined using microscopic, molecular, and biological evaluation methods. The obtained results contribute to optimizing the virus replication process for the production of biological insecticides. This research supports the development of more effective biological control tools and promotes ecologically safe agriculture.*

Keywords: *baculovirus, Helicoverpa zea, Spodoptera frugiperda, cell line, Sf9, HzAM1, virus replication.*

INTRODUCTION

In modern agriculture, protecting crop fields from harmful insect pests is considered one of the most important agro-technical tasks. The excessive and improper use of conventional chemical insecticides not only negatively impacts the agroecosystem, but also poses serious threats to human health. Furthermore, the increasing resistance of pests to pesticides and the accumulation of pesticide residues in the environment have intensified the need for alternative biological control methods. In this context, baculovirus-based biocontrol agents are gaining significant importance as ecologically safe and selectively acting alternative insecticides.

Baculoviruses are double-stranded DNA viruses that primarily infect insect pests belonging to the order Lepidoptera. Certain strains of these viruses exhibit high pathogenicity against major crop pests such as Helicoverpa zea and Spodoptera frugiperda, which are particularly harmful to plants. H. zea causes substantial damage to crops like cotton, maize, tomato, and pepper, while S. frugiperda poses a serious threat to maize plantations, especially in Africa, Asia, and Latin America. Due to their rapid reproduction, high mobility, and migratory capacity, controlling these pests has become a pressing global issue [1].

This study investigates the potential of baculoviruses isolated from or effective against H. zea and S. frugiperda as biocontrol agents against plant-damaging organisms. It focuses on the optimal conditions for viral replication and host cell culture growth under laboratory conditions, including nutrient media, pH, temperature, and multiplicity of infection (MOI).

The expected outcomes of the research aim to contribute to the development of more efficient biological insecticides, promote sustainable crop protection systems, and support the widespread application of biological control methods [2].

LITERATURE ANALYSIS

In recent years, biological control methods—particularly those based on baculoviruses—have been widely employed to protect agricultural crops from insect pests. Scientific literature highlights the high selectivity, ecological safety, and prolonged efficacy of baculoviruses compared to chemical pesticides. Research indicates that baculoviruses become active only after ingestion by the host organism, replicating within the host's cell nucleus and ultimately leading to the disintegration of the infected organism [3].

For instance, King and Possee (1992), in their fundamental study, thoroughly analyzed the genetic and molecular structure of baculoviruses and demonstrated the potential to enhance viral pathogenicity through the creation of recombinant viruses. According to their findings, mass production of biological preparations can be achieved by propagating viruses in vitro using cell lines such as Sf9 and HzAM1[4].

Research by van Oers and Vlak (2007) on baculovirus genomics further uncovered their genetic stability and adaptability to different hosts. Their experiments on Spodoptera frugiperda revealed that both temperature and pH of the growth medium significantly affect viral replication. Specifically, a temperature of 27°C and a pH level of 6.4 were identified as optimal conditions for virus proliferation [5].

Among local researchers, A. Abdullayev and Sh. Bahromov (2018) evaluated the effectiveness of baculovirus-based biological products in combating lepidopteran pests affecting cotton and vegetable crops in Uzbekistan. Their findings suggest that such biocontrol agents can gradually replace chemical insecticides and play a key role in maintaining plant health [6].

At the same time, some studies point out challenges in baculovirus production, including the sensitivity of cell lines, low viral titers, and economic inefficiency. These limitations underline the necessity of improving the production technology.

Overall, the reviewed literature demonstrates the promising potential of using baculoviruses against plant pests. However, further optimization of in vitro viral replication, deeper analysis of virus-cell interactions, and continued research into formulation as a biological product are still needed. Scientifically grounded approaches in this direction will contribute to improving the efficacy and applicability of biological control methods.

DISCUSSION AND RESULTS

As a result of the conducted experiments, the replication efficiency of various baculovirus strains was compared under different conditions in Helicoverpa zea (HzAM1) and Spodoptera frugiperda (Sf9) cell lines. During the trials, the influence of factors such as temperature, pH, nutrient medium, and multiplicity of infection (MOI) on viral replication was assessed.

- Temperature: The results showed that the most efficient viral replication for both cell lines was observed at 27°C. At 25°C, the replication rate was relatively low, while at

30°C, the increased metabolic activity of the host cells created an unfavorable environment for effective viral replication.

- pH level: Based on observations, the optimal pH range for maximum viral activity was between 6.4 and 6.6. This range appeared to promote viral capsid stability and enhance the efficiency of viral entry into host cells.
- Multiplicity of Infection (MOI): Among the tested infection doses, an MOI of 5 provided the most optimal results. At lower MOIs (0.1–1), the replication rate was limited due to insufficient numbers of infected cells, whereas a higher MOI (10) induced cellular stress and accelerated cell lysis, thus reducing overall productivity.
- Nutrient media: The effects of different growth media (Grace's and SF900 II) were also compared. In Sf9 cells, the SF900 II medium supported 1.4 times higher virus titers. Conversely, in HzAM1 cells, Grace's medium yielded relatively better replication efficiency.
- Confirmation of infection: Baculovirus infection in the cell lines was verified through microscopic morphological analysis, fluorescence markers (if GFP-tagged viruses were used), and quantitative PCR (qPCR) to determine the viral DNA load.

Parametr	Optimal value
Temperature	27°C
pH	6.4-6.6
MOI	5
Sf9 environment	SF900 II
HzAM1 environment	Grace's medium

Furthermore, it was observed that baculovirus replication initiated more rapidly in Spodoptera frugiperda cells, whereas virus titers remained more stable in Helicoverpa zea cells. This indicates that cell line selection plays a critical role depending on the intended purpose—whether rapid infection or stable replication is desired.

The experimental results provided significant scientific and practical insights for improving the development of biological insecticides based on baculoviruses. The variation in replication dynamics under different conditions in H. zea and S. frugiperda cell lines reflects differences in their biological characteristics, metabolic activity, and the complexity of virus-host interactions.

Notably, temperature and nutrient medium composition were found to have a direct impact on the intensity of viral replication, emphasizing the need to individually optimize these parameters for each virus-cell line combination.

In Sf9 cells, higher virus titers were achieved using SF900 II medium, whereas in HzAM1 cells, Grace's medium supported more stable and qualitatively superior virus replication. This highlights the specific advantages of both cell lines: Sf9 for rapid virus propagation, and HzAM1 for stable and high-quality virus production. Consequently, the selection of the cell line can be tailored depending on whether quantity or quality is prioritized.

The identified optimal ranges for pH (6.4–6.6) and temperature (27°C) align with previously reported studies, such as those conducted by van Oers and Vlak (2007), who also reported maximum replication rates for baculoviruses under similar conditions [7].

Additionally, our findings that excessive MOI levels induce cellular stress and reduce virus quality are consistent with the conclusions drawn by King and Possee (1992) [8].

Importantly, this study not only contributes to laboratory-level optimization, but also lays the groundwork for the industrial-scale production of baculovirus-based bioinsecticides. Enhancing the economic efficiency of biological control methods, ensuring ecological sustainability, and developing new, effective solutions against pesticide-resistant pests remain pressing challenges in the agricultural sector.

It is also important to emphasize that Helicoverpa zea and Spodoptera frugiperda are major lepidopteran pests that pose a global threat to food security. Their ability to produce multiple generations during the crop growing season and consume large quantities of biomass makes them extremely destructive. Strengthening biological control strategies plays a critical role in the effective management of these pests.

Overall, the results of this research will support the optimization of production conditions for biological insecticides, reduce technological costs, and facilitate the development of ecologically safe and selective pest control agents.

Conclusion

During this study, important factors affecting baculovirus replication in Helicoverpa zea (HzAM1) and Spodoptera frugiperda (Sf9) cells were systematically studied.uring this study, important factors affecting baculovirus replication in Helicoverpa zea (HzAM1) and Spodoptera frugiperda (Sf9) cells were systematically studied. According to the results obtained, the optimum temperature for effective viral replication was found to be around 27°C, while the ambient pH was around 6.4-6.6, confirming the link between viral titer and cell viability.While Sf9 cells in experiments were characterized by high viral titers, HzAM1 cells were notable for providing a qualitative and stable viral proliferation.hile Sf9 cells in experiments were characterized by high viral titers, HzAM1 cells were notable for providing a qualitative and stable viral proliferation. In particular, the maximum virus concentration was observed when the infection coefficient (MOI) was 5, which makes it possible to increase economic efficiency during the production process. Also, the composition of food environments and their correspondence to cell lines has had a significant impact on viral replication.lso, the composition of food environments and their correspondence to cell lines has had a significant impact on viral replication. For Sf9 cells, SF900 II was identified, and for HzAM1 cells, the grace's food environment was identified as the optimal environment. The results of this study are of significant practical importance in optimizing the production of biological agents based on baculovirus, in particular in the preparation of bioinsecticides based on entomopathogenic viruses.he results of this study are of significant practical importance in optimizing the production of biological agents based on baculovirus, in particular in the preparation of bioins

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