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OPTIMIZATION OF SERUM-FREE CULTIVATION OF RECOMBINANT SHEEPPOX VIRUS SSPV (TK) OMP16 IN VERO CELLS

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ABSTRACT

Brucellosis remains a major zoonotic disease of global public health and veterinary importance. Recombinant capripox-virus vectors have emerged as promising platforms for the development of next-generation vaccines owing to their favorable safety profile, genomic stability, and ability to induce robust immune responses. The aim of this study was to optimize serum-free cultivation conditions for the recombinant sheeppox virus strain SSPV (TK) OMP16 expressing the *Brucella* outer membrane protein OMP16 in Vero cell culture.

The recombinant virus was propagated on Vero cell monolayers using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2% fetal bovine serum or serum-free OptiPro™ medium containing 1% L-glutamine. Adaptation to serum-free conditions was achieved by five sequential blind passages in Vero cells. Viral biomass accumulation was evaluated at multiplicities of infection (MOI) of 0.01, 0.05, 0.1, and 0.5 TCID₅₀/cell during cultivation periods ranging from 12 to 120 h. Infectious activity was determined by TCID₅₀ assay in Vero cell culture.

Serum-free OptiPro medium supported efficient replication of the recombinant virus and produced infectious titers comparable to those obtained in conventional serum-containing DMEM. The highest infectious activity of recombinant strain SSPV (TK) OMP16 was 6.25 log TCID₅₀/cm³ at an MOI of 0.1 TCID₅₀/cell at 96 h post-infection. Sterility testing confirmed the absence of bacterial and fungal contamination in all virus suspensions.

The optimized serum-free cultivation protocol provides a standardized approach for propagation of recombinant capripox-virus vectors and represents a practical basis for further development and scale-up of recombinant vaccine production against brucellosis.

Keywords: recombinant sheeppox virus; SSPV (TK) OMP16; Vero cells; serum-free cultivation; recombinant viral vector; brucellosis vaccine.

INTRODUCTION

Brucellosis remains one of the most important zoonotic bacterial diseases worldwide and continues to pose a major challenge to both public health and veterinary medicine, particularly in countries with extensive livestock production. Caused by bacteria of the genus *Brucella*, the disease is primarily transmitted to humans through direct contact with infected animals or consumption of contaminated animal products [1,2]. Despite long-standing control programs, brucellosis remains endemic in many regions, including Kazakhstan, resulting in considerable economic losses and persistent epidemiological risks [2,3].

To date, no licensed vaccine has been approved for the prevention of human brucellosis despite decades of intensive research [4,5]. Currently available live attenuated veterinary vaccines have several well-recognized limitations, including residual virulence, safety concerns, and the inability to reliably differentiate infected from vaccinated animals (DIVA) [5,6]. Consequently, the development of safe, genetically stable, and highly immunogenic recombinant vaccine platforms has become one of the major priorities of modern biomedical and veterinary biotechnology [6,7].

Recombinant poxvirus vectors are increasingly recognized as versatile platforms for vaccine development owing to their ability to induce both humoral and cellular immune responses, together with their genomic stability and favorable safety pro-

file [8,9]. Among them, capripoxviruses have attracted considerable attention because of their restricted host range and their capacity to express heterologous antigens efficiently [10]. The recombinant strain SSPV (TK) OMP16 investigated in the present study is derived from a thymidine kinase-negative attenuated sheeppox virus engineered to express the *Brucella* outer membrane protein OMP16, one of the major protective antigens considered promising for recombinant vaccine development against brucellosis [11,12].

Among the outer membrane proteins of *Brucella* spp., OMP16 has been identified as a promising antigen for recombinant vaccine development because of its ability to induce protective cellular and humoral immune responses [11,13]. Experimental studies have demonstrated that recombinant OMP16 elicits antigen-specific immune responses and contributes to protection against *Brucella* infection, supporting its potential use in next-generation vaccine development [11].

Optimization of virus cultivation technology represents a critical step in the development of recombinant viral vaccines, as it directly influences virus yield, process reproducibility, and manufacturing efficiency. In recent years, serum-free cultivation systems have attracted considerable interest owing to their improved batch-to-batch consistency, reduced risk of adventitious contamination, simplified downstream processing, and compliance with modern Good Manufacturing Practice (GMP) requirements [14,15]. Furthermore, elimination of fe-

tal bovine serum reduces biological variability and contributes to better standardization of vaccine production processes. Despite these advantages, information regarding serum-free cultivation and optimization of recombinant capripoxvirus propagation remains limited. Therefore, optimization of cultivation parameters under serum-free conditions is essential for the development of scalable and standardized manufacturing technologies for recombinant viral vectors [16].

Accordingly, the aim of this study was to establish a standardized serum-free cultivation protocol for the recombinant sheeppox virus strain SSPV (TK) OMP16 in Vero cell culture by adapting the virus to OptiPro medium and determining the optimal conditions for viral biomass accumulation.

MATERIALS AND METHODS

Recombinant virus

The recombinant sheeppox virus strain SSPV (TK) OMP16 expressing the Brucella outer membrane protein OMP16 was used throughout this study [12]. The virus was obtained from the Collection of Microorganisms of the Research Institute for Biological Safety Problems and propagated in Vero cell culture. The infectious activity of the initial virus stock was 4.0 log TCID₅₀/cm³.

Cell culture

Vero cells (African green monkey kidney epithelial cells) were used for virus propagation and titration. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂ and grown to fully confluent monolayers in 150 and 300 cm² tissue culture flasks (TPP, Switzerland).

Media and supplements

Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich, USA) was used as the growth medium for Vero cells. For serum-containing cultivation, DMEM supplemented with 2% fetal bovine serum (FBS, Sigma-Aldrich, USA) and 1% L-glutamine (Gibco, USA) was used as the maintenance medium.

For serum-free cultivation, OptiPro medium (Thermo Fisher Scientific, USA) supplemented with 1% L-glutamine (Gibco, USA) was used. No animal serum components were added to the serum-free medium.

Adaptation to serum-free conditions

The recombinant virus was adapted to serum-free OptiPro™ SFM by five sequential blind passages in Vero cell culture. At each passage, virus-containing material was transferred to fresh confluent Vero cell monolayers maintained in serum-free medium. Successful adaptation was confirmed by the stable development of virus-specific cytopathic effect and reproducible virus replication throughout consecutive passages.

Virus cultivation and biomass accumulation

The recombinant sheeppox virus strain SSPV (TK) OMP16 was propagated on fully confluent Vero cell monolayers. Following virus adsorption for 60 min at 37°C in a humidified atmosphere containing 5% CO₂, the inoculum was removed and replaced with the corresponding maintenance medium. Infected cultures were incubated under the same conditions, and cytopathic effect (CPE) development was monitored daily using an inverted microscope.

To determine the optimal conditions for viral biomass accumulation, confluent Vero cell monolayers were infected at multiplicities of infection (MOI) of 0.01, 0.05, 0.1, and 0.5 TCID₅₀/cell. Virus-containing samples were collected every 6 h beginning at 12 h post-infection and continuing until 120 h post-infection. Four independent culture flasks were sampled at each time point for subsequent determination of infectious activity.

Following sample collection, the culture medium was removed and infected monolayers were gently washed with phosphate-buffered saline (PBS). The cell-associated virus was released by a single freeze–thaw cycle at –20°C and subsequently used for infectious activity determination.

Determination of infectious activity

Infectious activity of the recombinant virus was determined by end-point titration using ten-fold serial dilutions in 96-well microplates containing confluent Vero cell monolayers. All experiments were performed in quadruplicate. Virus titers were calculated according to the Reed and Muench method and expressed as log TCID₅₀/cm³.

Statistical analysis

All experiments were performed in four independent replicates. Quantitative data are presented as mean ± standard deviation (SD). No variation was observed among replicate measurements obtained under identical experimental conditions; therefore, the calculated SD values were zero. Descriptive statistics were used for data analysis.

Sterility testing

Sterility testing of virus-containing suspensions was performed according to the requirements of GOST 28085-89. Samples were inoculated into Sabouraud medium, Meat-Peptide Broth (MPB), Meat-Peptide Liver Broth (MPLB), and Meat-Peptide Agar (MPA), followed by incubation under appropriate conditions for 14 days. The absence of bacterial and fungal growth throughout the incubation period confirmed the sterility of all virus preparations.

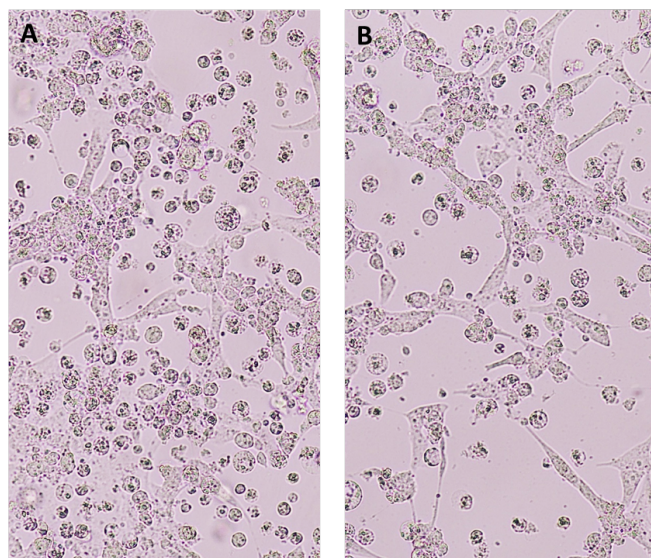
RESULTS

Adaptation of SSPV (TK) OMP16 to serum-free OptiPro medium

The recombinant sheeppox virus strain SSPV (TK) OMP16 was successfully adapted to serum-free OptiPro™ medium after five sequential blind passages in Vero cell culture. Stable virus replication was maintained throughout the adaptation process, and development of virus-specific cytopathic effect remained comparable to that observed in conventional DMEM supplemented with 2% fetal bovine serum. These findings demonstrate that serum-free OptiPro™ medium supports efficient propagation of the recombinant virus without detectable loss of infectivity.

Cytopathic effect of recombinant virus in Vero cells

Cytopathic effect (CPE) induced by the recombinant sheeppox virus strain SSPV (TK) OMP16 was assessed by microscopic examination of infected Vero cell monolayers during cultivation. Typical virus-induced cytopathic changes became evident between 72 and 96 h post-infection (Figure 1). The observed CPE was characterized by cell rounding, cell degeneration, aggregation of infected cells, and subsequent



detachment from the culture surface. By 96 h post-infection, **Figure 1.** Representative cytopathic effect induced by the recombinant sheppox virus strain SSPV (TK) OMP16 in Vero cells: (a) 72 h post-infection; (b) 96 h post-infection.

approximately 80–90% of the cell monolayer exhibited characteristic cytopathic alterations.

Comparison of infectious activity in different maintenance media

The infectious activity of recombinant strain SSPV (TK) OMP16 cultivated in serum-free OptiPro medium was compared with virus propagated in conventional DMEM medium supplemented with fetal bovine serum.

The obtained results demonstrated that serum-free OptiPro medium supported efficient replication of the recombinant virus comparable to serum-containing cultivation conditions (Table 1). The infectious activity of the virus reached 6.25 log TCID₅₀/cm³ in both media systems.

These findings indicate that serum-free OptiPro medium may be effectively used for propagation of recombinant sheppox virus without reduction of viral infectivity.

Optimization of multiplicity of infection MOI for viral biomass accumulation

The influence of multiplicity of infection MOI on viral biomass accumulation was evaluated using infection doses of 0.01, 0.05, 0.1, and 0.5 TCID₅₀/cell during 120 h of cultivation. Viral infectivity was monitored at different time points to determine the optimal infection dose and cultivation period for recombinant virus production.

At an MOI of 0.1 TCID₅₀/cell, infectious activity gradually increased during cultivation and reached the maximum value of 6.25 log TCID₅₀/cm³ at 96 h post infection. After this time point, a gradual decrease in viral titer was observed.

A similar pattern of viral accumulation was observed at

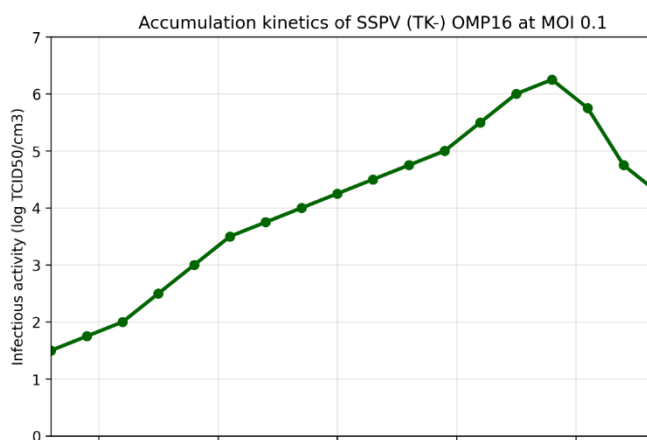


Figure 2. Accumulation kinetics of recombinant virus SSPV (TK) OMP16 in Vero cells at an MOI of 0.1 TCID₅₀/cell. Data are presented as mean ± SD (n = 4)

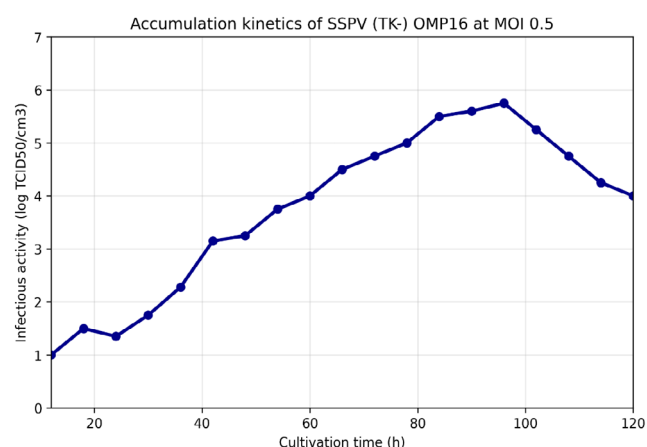


Figure 3. Accumulation kinetics of recombinant virus SSPV (TK) OMP16 in Vero cells at an MOI of 0.5 TCID₅₀/cell. Data are presented as mean ± SD (n = 4)

an MOI of 0.5 TCID₅₀/cell; however, the maximum infectious activity was lower than that observed at an MOI of 0.1 TCID₅₀/cell.

At an MOI of 0.5 TCID₅₀/cell, infectious activity progressively increased during cultivation and reached 5.75 log TCID₅₀/cm³ at 96 h post infection. Further cultivation resulted in a gradual decline in viral infectivity.

Reduction of the infection dose to 0.05 TCID₅₀/cell resulted in slower virus accumulation kinetics and lower infectious activity.

At an MOI of 0.05 TCID₅₀/cell, infectious activity increased gradually throughout cultivation and reached a maximum value of 4.5 log TCID₅₀/cm³ at 102 h post infection. Further incubation resulted in a slight decrease in viral infectivity.

The lowest multiplicity of infection evaluated in this study (0.01 TCID₅₀/cell) resulted in the slowest virus accumulation kinetics among all experimental groups.

Table 1. Comparison of infectious activity of recombinant strain SSPV (TK) OMP16 cultivated in serum-containing and serum-free maintenance media

Maintenance medium	Infectious activity
DMEM + 2% FBS	6.25 ± 0.00 log TCID ₅₀ /cm ³
OptiPro serum-free medium	6.25 ± 0.00 log TCID ₅₀ /cm ³

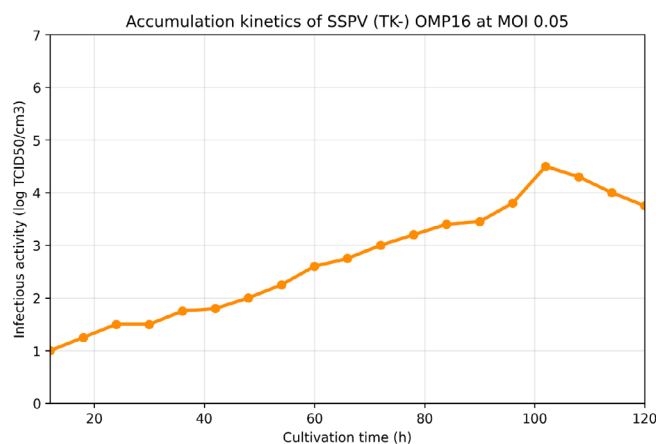


Figure 4. Accumulation kinetics of recombinant virus SSPV (TK) OMP16 in Vero cells at an MOI of 0.05 TCID₅₀/cell. Data are presented as mean $\pm$ SD (n = 4)

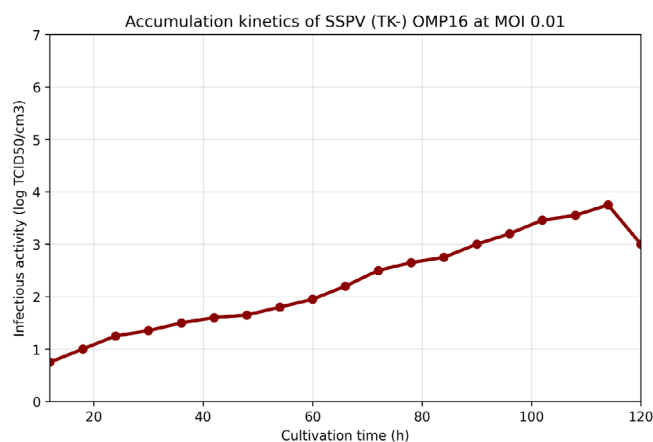


Figure 5. Accumulation kinetics of recombinant virus SSPV (TK) OMP16 in Vero cells at an MOI of 0.01 TCID₅₀/cell. Data are presented as mean $\pm$ SD (n = 4)

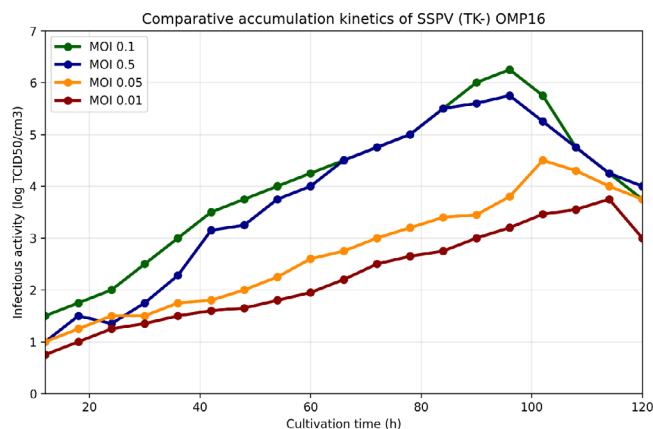


Figure 6. Comparative accumulation kinetics of recombinant virus SSPV (TK) OMP16 in Vero cells at different multiplicities of infection. Data are presented as mean $\pm$ SD (n = 4)

At an MOI of 0.01 TCID₅₀/cell, infectious activity increased slowly throughout the cultivation period and reached a maximum value of 3.75 log TCID₅₀/cm³ at 114 h post infection. Compared with the other experimental groups, virus accumulation occurred more slowly and reached a lower maximal infectious activity.

To facilitate direct comparison of virus accumulation kinetics under different infection conditions, the results obtained for all tested multiplicities of infection were combined into a single comparative graph.

Comparative analysis of viral accumulation kinetics revealed substantial differences depending on the multiplicity of infection. The highest infectious activity (6.25 log TCID₅₀/cm³) was obtained at an MOI of 0.1 TCID₅₀/cell after 96 h post infection.

At an MOI of 0.5 TCID₅₀/cell, infectious activity reached 5.75 log TCID₅₀/cm³ at 96 h post infection, whereas lower multiplicities of infection (0.05 and 0.01 TCID₅₀/cell) resulted in maximum infectious activities of 4.5 and 3.75 log TCID₅₀/cm³ after 102 and 114 h post infection, respectively.

Determination of optimal cultivation period

Based on the comparative analysis of virus accumulation kinetics, the optimal cultivation conditions for recombinant strain SSPV (TK) OMP16 were identified as an MOI of 0.1 TCID₅₀/cell with a cultivation period of 96 h, at which the highest infectious activity of 6.25 log TCID₅₀/cm³ was achieved.

Further cultivation beyond 96 h did not result in additional virus accumulation and was accompanied by a gradual decline in infectious activity. Therefore, 96 h post-infection was selected as the optimal harvesting point for production of recombinant virus under serum-free cultivation conditions.

Sterility assessment of virus suspensions

Sterility testing was performed on virus-containing suspensions obtained following cultivation of recombinant strain SSPV (TK) OMP16 under serum-free conditions. All tested samples complied with the sterility requirements specified by GOST 28085-89. No bacterial or fungal contamination was detected during the 14-day incubation period in any of the inoculated culture media, including Sabouraud medium, meat-peptone broth, meat-peptone liver broth, and meat-peptone agar.

These findings confirmed the microbiological purity of all virus-containing suspensions and their compliance with established sterility requirements.

DISCUSSION

The transition from serum containing to serum-free cultivation systems represents an important direction in the development of modern cell culture-based vaccine manufacturing. Fetal bovine serum provides essential growth-promoting components but is associated with batch-to-batch variability, undefined composition, potential introduction of adventitious agents, and additional downstream purification requirements. Accordingly, serum-free and chemically defined media are increasingly considered preferable for the development of standardized and scalable viral production processes [14,15,17]. Recent studies have demonstrated that serum-free media can support both Vero cell proliferation and efficient production of different viral vectors and vaccine candidates, confirming the suitability of Vero-based serum-free platforms for biopharmaceutical manufacturing [17–21].

In the present study, recombinant sheeppox virus strain SSPV (TK) OMP16 was successfully adapted to serum-free

OptiPro™ SFM, while maintaining an infectious activity comparable to that obtained in conventional DMEM supplemented with fetal bovine serum. This observation indicates that removal of serum from the maintenance medium did not adversely affect propagation of the recombinant virus in Vero cells. Similar findings have been reported for other viral production systems in which serum-free Vero cultures maintained virus yields comparable to serum-containing processes. Alfano et al. demonstrated that chemically defined serum-free formulations supported robust virus production in Vero cells, while serum-free production platforms have also been successfully applied to recombinant vesicular stomatitis virus-based vaccine vectors [17–19]. These observations support the broader applicability of serum-free Vero cell systems for recombinant viral vector production.

An important outcome of the present study was the identification of the multiplicity of infection as a critical parameter affecting the kinetics and efficiency of recombinant SSPV (TK) OMP16 accumulation. Among the tested infection doses, an MOI of 0.1 TCID₅₀/cell provided the most favorable virus accumulation profile, with peak infectious activity achieved at 96 h post-infection. From a bioprocess perspective, optimization of MOI is important because the initial proportion of infected cells influences the subsequent spread of infection, duration of productive replication, extent of cytopathic damage, and harvesting time. Studies involving other viruses propagated in Vero cells have similarly shown that infection parameters, including MOI and time of infection, can substantially influence final virus yield and process productivity [18–20].

Increasing the MOI from 0.1 to 0.5 TCID₅₀/cell did not improve the final infectious virus yield. Although a higher inoculum may increase the proportion of cells infected during the initial phase of cultivation, it can also accelerate development of cytopathic changes and reduce the period during which metabolically active cells remain available to support virus production. The lower maximal infectious activity observed at MOI 0.5 in the present study is therefore consistent with the general principle that increasing the infection dose does not necessarily result in proportionally higher virus productivity. Rather, successful optimization requires a balance between rapid initiation of infection and maintenance of a sufficiently viable cell population throughout the productive phase of cultivation. Similar process-dependent effects of infection conditions have been emphasized in studies of cell culture-based viral vaccine production and bioreactor process optimization [19,20].

Conversely, lower multiplicities of infection (0.05 and 0.01 TCID₅₀/cell) resulted in slower virus accumulation and delayed achievement of peak infectious activity. This pattern can be explained by the smaller proportion of initially infected cells, requiring additional replication cycles for infection to spread throughout the monolayer. Consequently, lower MOI values prolonged the cultivation period and resulted in lower maximal infectious activities under the conditions evaluated in this study. Taken together, these findings indicate that both unnecessarily high and excessively low infection doses may reduce overall process efficiency, highlighting the importance of experimentally defining the MOI for each virus–cell culture system rather than directly extrapolating conditions es-

tablished for other viral vectors.

The selected harvesting time of 96 h post-infection also has practical technological significance. Prolongation of cultivation beyond the period of maximum infectious activity did not improve virus yield and was followed by a gradual decline in infectivity. Determination of the appropriate harvesting point is an important component of viral process development because extended cultivation may increase cell debris and released host-cell components while providing no additional gain in infectious virus. Modern viral vaccine manufacturing therefore increasingly relies on coordinated optimization of MOI, infection time, harvesting strategy, and cultivation platform rather than optimization of individual parameters in isolation [18–20].

The present findings are also relevant to subsequent process scale-up. Serum-free Vero systems have been successfully transferred from conventional adherent cultures to microcarrier, fixed-bed, and suspension-based bioreactor platforms for production of recombinant viral vectors [18–20]. More recently, serum-free cultivation of recombinant capripoxvirus in Vero cells has been demonstrated using dynamic microcarrier- and macrocarrier-based culture systems, providing direct evidence that recombinant capripoxvirus production can be transferred toward scalable cultivation formats [22]. Together with the results of the present study, these data support the feasibility of developing a standardized upstream production platform in which serum-free medium, optimized infection parameters, and controlled harvesting time can subsequently be integrated into larger-scale cultivation systems.

The present study was focused primarily on optimization of upstream cultivation parameters under laboratory-scale monolayer conditions. Therefore, further work should evaluate process reproducibility during scale-up and determine whether the selected cultivation parameters remain optimal in dynamic culture systems. Additional assessment of recombinant virus stability during serial serum-free propagation and confirmation of consistent expression of the inserted OMP16 antigen would further strengthen characterization of the production platform. Nevertheless, the comparable virus yield obtained under serum-free and serum-containing conditions, together with the identified optimal MOI and harvesting time, provides a practical basis for further development of a standardized production process for recombinant capripoxvirus-based vaccine candidates.

CONCLUSION

This study established optimized serum-free cultivation conditions for the recombinant sheeppox virus strain SSPV (TK) OMP16 using Vero cells. Adaptation of the recombinant virus to OptiPro™ serum-free medium maintained infectious activity comparable to that achieved under conventional serum-containing cultivation, demonstrating the suitability of serum-free conditions for propagation of the recombinant virus. Among the evaluated cultivation parameters, an MOI of 0.1 TCID₅₀/cell and a harvesting time of 96 h post-infection provided the optimal balance between virus accumulation, infectious activity, and cultivation efficiency. Sterility testing confirmed compliance of the obtained virus suspensions with established microbiological quality requirements.

The optimized cultivation strategy developed in this study

provides a standardized laboratory-scale platform for production of recombinant SSPV (TK) OMP16 and may serve as a basis for further optimization and scale-up of recombinant capripoxvirus manufacturing processes. The obtained results contribute to the development of serum-free cultivation technologies for recombinant viral vectors and support future application of standardized production systems for veterinary vaccine development.

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AUTHOR CONTRIBUTIONS

Conceptualization, Ch.O.V.; methodology, O.Z.D.; investigation, A.N.A.; validation, Ye.T.T.; formal analysis, T.A.B.; data curation, S.S.O.; writing original draft preparation, R.R.A. and B.Ye.D; writing and editing, B.Ye.D. and Zh.K.D.; visualization, A.T.U.; supervision, Ch.O.V.; project administration, Zh.K.D. All authors have read and agreed to the published version of the manuscript.

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VERO ЖАСУША ӨСІНДІСІНДЕ SSPV (ТК) OMP16 РЕКОМБИНАНТТЫ ҚОЙ ШЕШЕГІ ВИРУСЫН ӨСІРУДІҢ ҚАН САРЫСУСЫЗ ЖАҒДАЙЛАРЫН ОҢТАЙЛАНДЫРУ**ТҮЙІН**

Бруцеллез бүкіл әлемде қоғамдық денсаулық сақтау және ветеринария салалары үшін елеулі қауіп төндіретін маңызды зооноздық аурулардың бірі болып табылады. Каприпоксвирустар негізіндегі рекомбинантты вирустық векторлар қауіпсіздігімен, генетикалық тұрақтылығымен және жоғары иммуногенділігімен ерекшеленетін жаңа буын вакциналарын әзірлеуге арналған перспективалы платформа ретінде қарастырылады. Осы зерттеудің мақсаты *Brucella* туысына жататын бактериялардың OMP16 антигенін экспрессиялайтын SSPV (ТК) OMP16 рекомбинантты қой шешегі вирусының штаммын Vero жасуша дақылында қан сарысусыз жағдайда өсірудің оңтайлы параметрлерін анықтау болды.

Рекомбинантты вирус Vero жасушаларының өсіндісінде эмбрионалды ірі қара мал сарысуымен толықтырылған Dulbecco's Modified Eagle Medium (DMEM) коректік ортасында және құрамында 1% L-глутамин бар OptiPro қан сарысусыз ортасында өсірілді. Вирусты қан сарысусыз жағдайларға бейімдеу кезекті пассаждар жүргізу арқылы жүзеге асырылды. Вирустық биомассаның жинақталуы жұқтырудың әртүрлі еселіктерінде (MOI) – 0,01; 0,05; 0,1 және 0,5 ТЦД₅₀/жасуша деңгейінде, 12–120 сағат аралығындағы өсіру кезеңдерінде бағаланды. Инфекциялық белсенділік Vero жасушаларында титрлеу әдісімен анықталды.

Зерттеу нәтижесінде OptiPro қан сарысусыз ортасының рекомбинантты вирустың тиімді репликациясын қамтамасыз ететінін және оның көрсеткіштері дәстүрлі сарысу мен DMEM ортасымен салыстырмалы екенін көрсетті. SSPV (ТК) OMP16 рекомбинантты штаммының ең жоғары инфекциялық белсенділігі 96 сағаттан кейін MOI 0,1 ТЦД₅₀/жасуша кезінде 6,25 lg ТЦД₅₀/см³ құрады. Стерильділікке жүргізілген сынақтар барлық вирусқұрамды суспензияларда бактериялық және саңырауқұлақтық ластануы болмағанын растады.

Ұсынылған қан сарысусыз өсіру жүйесі рекомбинантты каприпоксвирустық векторларды көбейтудің стандартталған тәсілі болып табылады және бруцеллезге қарсы вакциналық кандидаттарды әзірлеу мен оларды өндірістік деңгейге дейін ұлғайту технологияларын жетілдіру үшін пайдаланылуы мүмкін.

Негізгі сөздер: рекомбинантты қой шешегі вирусы, SSPV (ТК) OMP16, қан сарысусыз өсіру, Vero жасушалары, рекомбинантты вакцина, бруцеллезге қарсы вакцина.

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ОПТИМИЗАЦИЯ БЕССЫВОРОТОЧНОГО КУЛЬТИВИРОВАНИЯ РЕКОМБИНАНТНОГО ШТАММА ВИРУСА ОСПЫ ОВЕЦ SSPV (ТК) OMP16 В КУЛЬТУРЕ КЛЕТОК VERO**АБСТРАКТ**

Бруцеллез остается одним из наиболее значимых зоонозных заболеваний, представляющих серьезную проблему для общественного здравоохранения и ветеринарии во всем мире. Рекомбинантные вирусные векторы на основе каприпоксвирусов рассматриваются как перспективные платформы для создания вакцин нового поколения благодаря их безопасности, генетической стабильности и высокой иммуногенности. Целью настоящего исследования являлась оптимизация условий бессывороточного культивирования рекомбинантного штамма вируса оспы овец SSPV (ТК) OMP16, экспрессирующего антиген OMP16 бактерий рода *Brucella*, с использованием культуры клеток Vero.

В ходе оптимизации рекомбинантный вирус культивировали на монослое клеток Vero с использованием среды Dulbecco's Modified Eagle Medium (DMEM), содержащей эмбриональную сыворотку крови крупного рогатого скота, а также бессывороточной среды OptiPro, дополненной 1% L-глутамин. Адаптацию вируса к бессывороточным условиям проводили методом последовательных слепых пассажей. Накопление вирусной биомассы оценивали при множественности заражения (MOI) 0,01; 0,05; 0,1 и 0,5 ТЦД₅₀/клетку в течение 12–120 ч культивирования. Инфекционную активность определяли методом титрования в культуре клеток Vero.

Установлено, что бессывороточная среда OptiPro обеспечивает эффективную репликацию рекомбинантного вируса, сопоставимую с традиционной сывороточной средой DMEM. Максимальная инфекционная активность рекомбинантного штамма SSPV (ТК) OMP16 составила 6,25 lg ТЦД₅₀/см³ при MOI 0,1 ТЦД₅₀/клетку через 96 ч после заражения. Испытания на стерильность подтвердили отсутствие бактериальной и грибковой контаминации во всех исследованных вирусосодержащих суспензиях.

Разработанная бессывороточная система культивирования представляет собой стандартизированный подход к размножению рекомбинантных каприпоксвирусных векторов и может быть использована для дальнейшего совершенствования и масштабирования технологий получения вакцинных кандидатов против бруцеллеза.

Ключевые слова: рекомбинантный вирус оспы овец, SSPV (ТК) OMP16, бессывороточное культивирование, клетки Vero, рекомбинантная вакцина, вакцина против бруцеллеза.