Journal of Biotechnology 233 (2016) 34–41

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Targeted supplementation design for improved production and quality of enveloped viral particles in insect cell-baculovirus expression system

Francisca Monteiro[a,b], Vicente Bernal[c,1], Maxime Chaillet[d,e], Imre Berger[d,e,f,**], Paula M. Alves[a,b,*]

[a] iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
[b] Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal
[c] Departamento de Bioquímica y Biología Molecular B y Imunología, Facultad de Química, Campus Internacional de Excelencia "Mare Nostrum", Universidad de Murcia, Murcia, Spain
[d] The European Molecular Biology Laboratory, Grenoble, France
[e] Unit of Virus Host Cell Interaction UVHCI, Université Grenoble Alpes – EMBL – CNRS Unité Mixte de Recherche Grenoble, France
[f] The School of Biochemistry, University of Bristol, Bristol BS8 1TD, UK

ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form 20 June 2016
Accepted 30 June 2016
Available online 1 July 2016

Keywords:
Baculovirus-insect cell system
Metabolism
Productivity
Product quality
Bioprocess optimization
VLPs

ABSTRACT

The recent approval of vaccines and gene therapy products for human use produced in the Insect Cell-Baculovirus Expression Vector System (IC-BEVS) underlines the high potential and versatility of this platform. The interest in developing robust production processes emerges to cope with manufacturing pressure, as well as stringent product quality guidelines. Previously, we addressed the impact of the baculovirus infection on the physiology of insect host cell lines, identifying key cellular pathways enrolled in heterologous gene/protein expression. In the present work, this knowledge was applied to design tailored media supplementation schemes to boost IC-BEVS production yields and quality of enveloped viral particles: influenza VLPs (Inf-VLP) and baculovirus vectors (BV).

The addition of reduced glutathione, antioxidants and polyamines increased the cell specific yields of baculovirus particles up to 3 fold. Cholesterol was identified as the most critical system booster, capable of improving 2.5 and 6-fold cell specific yields of BV and Inf-VLPs, respectively. Surprisingly, the combination of polyamines and cholesterol supplementation improved baculovirus stock quality, by preventing the accumulation of non-infectious particles during viral replication while selectively increasing infectious particles production. In addition, the specific yields of both enveloped viral particles, BVs and Inf-VLPs, were also increased.

The correlation between supplement addition and systems productivity was extensively analyzed, providing a critical assessment on final product quantity and quality as drivers of bioprocess optimization efforts.

1. Introduction

The insect cell-baculovirus expression system (IC-BEVS) is now on the frontline of both pharmaceutical and biotechnological fields after the recent approval of several human therapeutics produced in this system, namely Cervarix®, Flublok® and Glybera® (Cox and Hollister, 2009; Lowy and Schiller, 2006; Moran, 2012). As the market of IC-BEVS based biopharmaceuticals progresses (Lu et al., 2012; Ylä-Herttuala, 2012), the interest in developing high-titer and robust production processes is expected to rise.

Molecular biology studies have provided exciting discoveries on baculovirus–host interactions (reviewed in Monteiro et al., 2012). However, the biological constraints that govern baculovirus infection in the biotechnological context are poorly understood. The analysis of cell culture parameters and media components influencing productivity has turned possible the implementation of high

* Corresponding author at: Animal Cell Technology Unit, IBET/ITQB, Apartado 12, 27801-901 Oeiras, Portugal.
** Corresponding author at: EMBL, 6 Rue Jules Horowitz, 38042 Grenoble CEDEX 9, France.
E-mail addresses: iberger@embl.fr (I. Berger), marques@itqb.unl.pt (P.M. Alves).
[1] Current address: Área de Biología, Dirección de Nuevas Energías, Centro de Tecnología de REPSOL, Autovía A-5, Km 18, 28935 Móstoles-Madrid, Spain.

http://dx.doi.org/10.1016/j.jbiotec.2016.06.029
0168-1656/

cell density bioprocesses with increased and sustained production of recombinant proteins *via* BEVS (Bédard et al., 1997; Chan et al., 1998; Chiou et al., 2000). The combination of metabolic flux analysis with the rational design of a feeding strategy improved baculovirus yields (6- to 7-fold) in high cell density cultures (Carinhas et al., 2010). Also, the on-line monitoring of the oxygen uptake rate (OUR) in baculovirus infected insect cells supported the design of feeding strategies able to boost up to 13 times the recombinant protein yields (Palomares et al., 2004). Overall, the successful application of metabolic and bioprocess engineering strategies to the IC-BEVS shows that there is room for improvement. Also, it seems clear that knowledge on key parameters of cellular physiology can help in devising such efforts towards increased productivity.

Although progress has been made in tuning the baculovirus to accommodate the expression of challenging targets (Bieniossek et al., 2012; Palmberger et al., 2013), the accumulation of defective interfering particles (DIF) with increasing viral passages constraints baculovirus-based bioprocesses (Pijlman et al., 2001). The loss of expression of heterologous gene(s) and low infectivity of the baculovirus expression vector occurs as a consequence of recombination events during viral replication (Pijlman et al., 2003), and the impact of the heterologous gene(s) on this event is not fully understood. In previous works, metabolic pathways correlated with baculovirus infection and IC-BEVS productivity were identified based on fluxome analysis (Bernal et al., 2009, Monteiro et al. *in preparation*) and metabolomic fingerprinting (Monteiro et al., 2014). Although several metabolic differences were assessed between the two insect cell lines more used for protein and virus production (*Trichoplusia ni* High Five and *Spodoptera frugiperda* Sf9 cells, respectively) responses to infection were very similar (Monteiro et al., 2014).

In the present work, we designed culture supplementation schemes aiming to boost IC-BEVS productivity and quality of complex enveloped viral particles: influenza VLPs (Inf-VLPs) and baculovirus (BV). Supplements selection was based on our previous identification of the metabolic pathways correlated with IC-BEVS productivity (Monteiro et al., 2014), with the goal of enhancing pathway activity and systems performance. An orthogonal screening of culture conditions was performed to pin-point the culture setting leading to maximal productivity in Sf9 cells, addressing target specific key requirements. Finally, an optimized bioprocess for the production of high quality enveloped viral particles *via* BEVS was implemented.

2. Materials and methods

2.1. Cell lines and culture maintenance

Spodoptera frugiperda derived Sf9 cell line was obtained from the European Collection of Cell Cultures (No. 89070101, ECCAC). Cells were maintained in serum- and protein-free Sf900II insect cell culture medium (Gibco, Glasgow, UK) in 500 mL Erlenmeyer flasks (Corning, USA) with 50 mL working volume. Cultures were kept in a humidified incubator operated at 90 rpm and 27 °C. Routinely, Sf9 cells were re-inoculated every 3–4 days at 0.45×10^6 cells mL^{-1}. Cell concentration was determined by hemocytometer cell counts and viability evaluated by the trypan blue exclusion method.

2.2. Baculoviruses and viral stock preparation

The recombinant *Autographa californica* nucleopolyhedrovirus BvHA5M1 was used throughout the work as the expression vector of the enveloped influenza VLPs (Inf-VLPs). This vector is a dual baculovirus, encoding two influenza genes: Hemagglutinin type 5 (H5), under the control of the polyhedrin promoter, and matrix protein 1 (M1), under the control of the p10 promoter.

BvHA5M1 was amplified by infecting Sf9 cells at 1×10^6 cells mL^{-1} with a multiplicity of infection (MOI) of 0.01 IP $cell^{-1}$ in a 10 L bioreactor (ED10, Sartorius AG, Goettingen, Germany). To generate a highly concentrated viral stock, a polyethylene glycol (PEG)-based concentration process was applied. Briefly, virus-containing culture supernatant was mixed with 8.5% (v/v) of a sterile PEG solution prepared in phosphate buffer saline (PBS), and incubated overnight at 4 °C. The mixture was centrifuged at 3200*g* for 30 min at 4 °C, and the collected pellet containing the baculovirus was suspended in 0.5 M sucrose. The concentrated viral stocks were titrated, aliquoted and kept at −80 °C until further use.

2.3. Baculovirus titration and total particles quantification

Baculovirus infectious particles quantification was performed following the MTT assay, as previously described in Roldão et al. (2009). Baculovirus total particles concentration was assessed by counting the baculovirus particles in a Nanosight NS500 (Nanosight Ltd., Salisbury, UK), using the Nanoparticle Tracking Analysis (NTA) software. The average size of purified Inf-VLPs samples and baculovirus was identified *a priori* for the determination of the gates for both particles quantification (data not shown). To exclude minor errors due to counting of cellular debris, exosomes, and other particles that can interfere with the accuracy of the measurements, appropriate controls were performed (supernatant of non-infected cultures at the correspondent cell densities). The measurements were performed at least in triplicates with a typical standard deviation (SD) below 20%.

2.4. Supplements preparation

The culture supplements tested, listed in Table 1, were selected as boosters of the metabolic pathways correlated with IC-BEVS productivity. Supplements concentration was set taking into account the manufacturer instructions and preliminary assays of their impact in the specific productivity and viability of Sf9 cells (data not shown). Supplements were prepared in Sf900II insect culture

Table 1
List of culture medium supplements.

Supplement	Abbreviation	Stock concentration	Concentration added[a]	Supplier	Reference
Antioxidants	AOx	1000 x	1 x	Sigma (Steinheim, Germany)	A1345
Biotin	Bio	1 mM	10 μM	Sigma	B4639
Cholesterol	Chol	250 x	1 x	Gibco (Glasgow, UK)	12531-018
Lipids	Lip	100 x	1 x	Gibco	11905-031
Nucleosides	Nucl	100 x	1 x	Merck Millipore	ES-008-D
Polyamines	Poly	1000 x	1 x	Sigma	P8483
Reduced glutathione	GSH	100 mM	1 mM	Sigma	G1404
Taurine	Tau	100 mM	10 mM	Sigma	T8691

The supplements were added in a two times addition mode (at inoculation and upon infection).
[a] Concentration of supplements added in each addition timing (inoculation and infection).