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Research review paper

Insect cells as factories for biomanufacturing

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ABSTRACT

Insect cells (IC) and particularly lepidopteran cells are an attractive alternative to mammalian cells for biomanufacturing. Insect cell culture, coupled with the lytic expression capacity of baculovirus expression vector systems (BEVS), constitutes a powerful platform, IC-BEVS, for the abundant and versatile formation of heterologous gene products, including proteins, vaccines and vectors for gene therapy. Such products can be manufactured on a large scale thanks to the development of efficient and scaleable production processes involving the integration of a cell growth stage and a stage of cell infection with the recombinant baculovirus vector. Insect cells can produce multimeric proteins functionally equivalent to the natural ones and engineered vectors can be used for efficient expression. Insect cells can be cultivated easily in serum- and protein-free media. A growing number of companies are currently developing an interest in producing therapeutics using IC-BEVS, and many products are today in clinical trials and on the market for veterinary and human applications. This review summarizes current knowledge on insect cell metabolism, culture conditions and applications.

Contents

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1. Introduction

The systematic *in vitro* cultivation of insect cells has been possible since the middle of the past century. At that time, the main motivation for establishing continuous insect cell lines was the study of insect physiology and the *in vitro* production of baculoviruses for the biological control of insect pests. In the early 1980s however, insect cell culture moved into the mainstream of biotechnology when it became possible to genetically modify baculoviruses. Their use as vectors enabled the heterologous expression of proteins in a lytic system involving the infection of lepidopteran cell lines (insect cell-baculovirus expression vector system, IC-BEVS). Nowadays, the IC-BEVS has evolved into a major technology platform for the manufacture of viral particles and recombinant proteins with applications ranging from biopesticides to animal and human vaccines and therapeutics and to vectors for gene therapy. This prominent position reflects the advantages of both the cell culture component and the vectors used for gene transfer. The practice of insect cell culture *in vitro* is very well established and the application of recombinant baculovirus vectors for abundant production of proteins or for manufacture of viral particles is robust, safe and scaleable (Agathos, 2010; Ikonomou et al., 2003; Palomares et al., 2006). The IC-BEVS is a highly versatile system because it can express gene products from almost any organism (from bacteria to human tissue) and from any cellular location (intracellular, extracellular, periplasmic). Unlike many industrial mammalian cell culture systems, it is based primarily on engineering the vector and not the host cell line. This shortens drastically the time from gene cloning to protein overproduction (weeks instead of months). The development of a wide variety of transfer vectors makes the isolation of recombinant virus a simple process, while the non-infectiousness of the baculoviruses to vertebrates guarantees the safety of this expression system. Compared to other biomanufacturing platforms, the IC-BEVS offers consistently high product titers, posttranslational modifications only slightly narrower than mammalian cells, and the capacity to express multimeric proteins or even several distinct proteins using the same vector, as the baculoviral genome can accommodate large fragments of heterologous DNA. Insect cells are readily amenable to suspension culture and the continuous improvement of cell culture media and additives (Agathos, 2007; 2010) is contributing to reliable and robust scale-up practices for commercial applications.

Over the last several decades, hundreds of insect cell lines have been isolated from more than 100 insect species encompassing more than 6 orders (Lynn, 2001). Lepidopteran cell lines are mainly used with the BEVS for the expression of r-proteins and for the production of baculovirus bioinsecticides, particularly lines from *Bombyx mori*, *Mamestra brassicae*, *Spodoptera frugiperda* and *Trichoplusia ni* (Murhammer, 2007; O'Reilly et al., 1992). Among them, Sf-9, Sf-21, Tn-368 and High-Five are the cell lines most widely used in industrial applications. These and related cell lines are highly susceptible to infection by *Autographa californica* multiple nucleopolyhedrosis virus (AcMNPV) and other baculoviruses that provide the basis for the construction of vectors in the IC-BEVS.

The first line to be intensively used in research and technological applications was Sf-21, an ovarian cell line from the fall armyworm, *S. frugiperda* established by Vaughn and co-workers in 1977. The Sf-9 cell line was derived from Sf-21 (Smith et al., 1983). Tn-368, was derived from ovarian tissues of the cabbage looper, *T. ni* (Hink, 1970). BTI-TN-5B1-4 (with sometimes different spelling) cells are a clone of the embryonic Tn-5 cell line isolated from *T. ni* eggs (Hink, 1970). Granados patented this cell line in 1994 (Granados, 1994) and it was subsequently commercialized under the name "High-Five™ cells", thanks to its capacity of reaching higher cell densities with higher growth rate and higher production rate than classical Tn-5 cells. The High-Five cell line showed from the beginning a superior capacity for secreted glycoprotein production compared to Sf cell lines (Davis et al., 1993; Saarinen et al., 1999). Some key characteristics of the Sf-9 and High-Five cell lines are summarized in Table 1.

The Sf lines are adapted to suspension cultivation and are easily detached from cultivation surfaces by gentle agitation without trypsinization (O'Reilly et al., 1992). Tn lines were originally anchorage-dependent, but today they are well adapted to suspension cultivation. Sf-21 are more fragile than Sf-9, less tolerant to osmotic, pH and shear stress than Sf-9, and have lower growth rate (O'Reilly et al., 1992). Today, the use of Sf-21 has diminished to the benefit of Sf-9 cells, thanks to its growth and infection characteristics. The latter are able to better amplify the baculovirus (Wang et al., 1992), while High-Five is the insect cell line that typically produces more r-protein, up to 20 fold more compared to Sf-9 (as a function of the r-protein produced) (Palomares and Ramirez, 1998; Saarinen et al., 1999). High-Five cells are more robust to shear stress and osmotic shocks than Sf-9 (Kioukia et al., 1995), although Sf-9 is more resistant to thermal shock (Gerbal et al., 2000). High-Five cells are larger and with higher protein content than Sf-9 cells, and their cell size distribution is wider than Sf-9 cells (Drugmand, 2007). However, the cell size depends on medium osmolarity, shear stress, cell state (viable, apoptotic, etc.) (Palomares et al., 2001). In recent years, the superior characteristics of *expres*SF+(SF+), a proprietary cell line derived from Sf-9 cells, have prompted its use in the manufacture of several biologicals, including the influenza vaccine FluBlok (Cox and Hollister, 2009).

Insect cell lines have polyploid chromosomes, and changes in this polyploidy could occur during cultivation (Doverskog et al., 2000; Léry et al., 1999). These cell lines are able to grow over long-term passaging, during which, however, morphological and physiological changes may occur: decrease of productivity and increase of growth rate and cell diameter (Donaldson and Shuler, 1998a), and lower susceptibility to growth enhancement by conditioned medium (Calles et al., 2006a; 2006b).

Finally, the tendency of baculovirus-infected cultured insect cells toward production of defective interfering particles ("passage effect") increases with increased cell passage number (Kool et al., 1991; Pijlman et al., 2001; Wickham et al., 1991).

2. Insect cell metabolism

Knowledge of the metabolic requirements of insect cell lines is primordial for the elaboration of new media and for designing effective feeding strategies in order to ensure superior productivities of r-proteins or baculovirus-based products. Despite the numerous individual studies reviewed below, there is a dearth of comprehensive, systems-oriented investigations of insect cell metabolism and only