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REVIEW AND PATENTS AND LITERATURE

The Use of Insect Cell Cultures for Recombinant Protein Synthesis: Engineering Aspects

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REVIEW

ABSTRACT

The use of the insect cell/baculovirus expression system for producing recombinant proteins of bacterial, plant, insect, and mammalian origin has become widespread. The popularity of this eukaryotic expression system is due to many factors, including (1) potentially high protein expression levels, (2) ease and speed of genetic engineering, (3) ability to accommodate large DNA inserts, (4) protein processing similar to higher eukaryotic cells (e.g., mammalian cells), and (5) ease of insect cell growth (e.g., suspension growth). The following review of the literature discusses two engineering aspects of recombinant protein synthesis by insect cell cultures: bioreactor scale-up and insect cell line selection. Following this review patent abstracts and additional literature pertaining to expression of recombinant proteins in insect cell culture are listed.

INTRODUCTION

Since its development in the early 1980's (*103*) the use of the baculovirus expression system for the synthesis of recombinant proteins has become widespread. A recent review (*68*) includes an extensive list of recombinant proteins, along with their biological properties, which have been expressed by the baculovirus system. This review and references therein should also be consulted for an overview of the biological aspects of baculoviruses and the methodology involved in the construction of a recombinant baculovirus. Having developed a recombinant baculovirus, the resulting foreign protein can be expressed by infecting either susceptible insect cell cultures or insect larvae (*70, 71*). In spite of the fact that *in vivo* protein synthesis in insect larvae generally results in very high concentrations of the desired product, the *in vitro* approach using insect cell culture is used much more frequently since it more amenable to scale-up and is more easily controlled. The strategy for using the baculovirus expression system to produce recombinant proteins in insect cell culture involves three distinct stages: (1) grow the insect cells to mid- to late-exponential growth phase (*10, 48, 115*), (2) infect the cells with the recombinant baculovirus, and (3) harvest and purify the protein product.

In addition to the use of the baculovirus expression vector, foreign genes have also been stably incorporated into host insect cell genomes (*23, 30, 32, 33, 53*). The major advantage of the

baculovirus vector is that its strong polyhedrin promoter can be used to drive foreign protein synthesis; thus, very high levels of the desired product can be obtained. It should be emphasized that a major disadvantage of using the baculovirus expression system is that the cells are killed; therefore, foreign protein will only be synthesized by infected cells from the time when the polyhedrin gene is turned on until the viral infection kills the cells. Another major concern of using the baculovirus expression system is that protein processing may vary as viral replication proceeds. For example, Davidson and Castellino (*25*) have demonstrated that the processing of N-linked oligosaccharides of recombinant human plasminogen expressed in *Spodoptera frugiperda* (IPLB-SF-21AE) insect cells varies considerably during the infection process. In addition, Jarvis and Summers (*54*) hypothesize that the ability of infected insect cells to perform protein processing may be compromised in later stages of viral replication as cell structures begin to break down. Therefore, there are potential advantages of using a system in which the desired foreign gene is stably incorporated into the host insect cell genome, including continuous and potentially more consistent production of the foreign protein. Currently, however, promoters which are considerably less efficient than the polyhedrin promoter are used to express genes incorporated into the host genome. It should be noted that variations in protein processing can also be a concern in mammalian cell culture; for example, glycosylation is known to be dependent on environmental conditions (*40*). Since environmental conditions change over the course of a batch bioreactor run, protein processing may also vary.

In addition to the recombinant DNA technology, other considerations, which can broadly be classified as engineering considerations, involved in optimizing the synthesis of a biologically functional protein by insect cell cultures include (1) bioreactor scale-up, (2) insect cell line selection, (3) medium development, and (4) effect of environmental factors on cell growth and recombinant protein synthesis. Two recent reviews (*41, 125*) have discussed the scale-up of insect cell bioreactors. In addition, recent advances in medium development for insect cell growth have been discussed by many investigators (*20, 60, 63, 72, 77, 78, 116*), as has the effect of environmental factors on recombinant protein expression in insect cell cultures (*16, 64*). This review will focus on new developments in bioreactor scale-up and insect cell line selection.

SCALE-UP OF INSECT CELL CULTURES

The goal of scale-up is to duplicate as closely as possible the optimized small-scale conditions in the large-scale system. In the case of bioreactor scale-up for insect cell cultures the optimal situation is to provide a uniform environment (with optimal pH, dissolved oxygen concentration, temperature, etc.) without cell damage. Theoretically, if this optimal situation were achieved, then the resulting product yield in the large-scale system would be the same as that obtained under optimal conditions in the small-scale system. This optimal situation, however, is not achievable in

practice since point to point variations in environmental conditions within a large scale bioreactor are inevitable (*85*).

Insect cells have been grown both in immobilized and suspension cultures. Immobilized methods have included the use of roller bottles (*121, 122*), microbead culture (*1, 100*), and microcapsules (*57*). The microcapsule work is unique in that a temperature-sensitive baculovirus was used. A cell/virus mixture was incorporated into the microcapsules and the cells grown at 33°C (temperature at which the virus was not infective); viral infection was initiated by decreasing the temperature to 27°C. Insect cells have been grown in suspension by using both airlift (*72, 79*) and agitated bioreactors. Oxygenation methods which have been used in the agitated bioreactors include: (1) oxygenation through oxygen-permeable silicone tubing (*31, 42, 76*), (2) oxygenation by surface aeration, i.e., actively blowing oxygen over the culture surface (*28, 79*), and (3) direct sparging (*49, 79*). Most of these bioreactors were operated in the batch mode, exceptions include work performed by de Gooijer *et al.* (*28*) and van Lier *et al.* (*114*). Continuous insect cell culture requires at least two stages when the baculovirus system is used due to the eventual cell death caused by the viral infection: at least one stage is required for cell growth to provide cells to replace those in following stage(s) which are killed by the viral infection. It has been demonstrated, however, that a "passage effect" occurs in continuous culture where defective virus particles become dominant and productivity rapidly declines (*28, 114*). Wickman *et al.* (*124*) have demonstrated that infecting insect cells at a low multiplicity of infection (MOI) can counteract the "passage effect." Therefore, if an innovative continuous design could be developed in which cells were always infected at a low MOI, then the productivity of insect cell/baculovirus system would probably not decline.

In general, the most efficient and convenient scale-up methodology involves suspension culture and the most efficient oxygenation method is direct sparging. Therefore, only aspects involved with the scale-up of airlift and agitated, sparged bioreactors will be considered in this review. The major concern with these two types of bioreactors is providing sufficient oxygen without damaging the cells. Insect cells are relatively large (typically, 10 μm diameter) and lack cell walls, therefore they are potentially sensitive to hydrodynamic stress caused by sparging and agitation. The structure of many insect cells is similar to that of attachment-independent animal cells; thus, much of the information pertaining to the scale-up of attachment-independent animal cells is applicable to the scale-up of insect cell cultures.

Many studies of the effect of agitation on insect (*81*) and animal cells (*37, 61, 84*) have been conducted. The results of these studies indicate that the sensitivity of insect and animal cells to hydrodynamic stress is generally comparable. In addition, moderate agitation rates generally have a minimal effect on insect and animal cells grown in suspension in the absence of bubbles. Thus, it appears as though the perception that has lingered in the literature pertaining to the extreme fragility of insect and animal cells has generally been exaggerated. In contrast to the effects of

moderate agitation, however, sparging (*37, 45, 46, 55, 56, 79-81, 84, 108-111, 127*), cavitation (*81*), and bubble incorporation via vortexing (*81, 61, 101*) have been demonstrated to cause severe damage to both insect and animal cells grown in suspension. Therefore, it appears as though damage from these phenomena and not from agitation is the major obstacle to the scale-up of suspension insect cell culture. This section will address the issue of the effect of these these phenomena on insect and animal cell cultures and methods of minimizing the resulting cell damage.

One would expect that different cell lines have different sensitivities to hydrodynamic stress. One method which has been used to measure the sensitivity of animal cells to hydrodynamic stress is to expose them to known levels of laminar shear in viscometers. Although it is not clear how a cell line's ability to withstand laminar shear can be translated into its ability to withstand the ill defined hydrodynamic stresses encountered in bioreactor environments, the viscometer results should give some indication of a cell line's relative robustness. Peterson *et al.* (*89*) found that hybridoma cell death in a viscometer exhibited trends similar to cell death caused by excessive agitation in spinner flasks. In a more recent study (*75*), however, it was demonstrated that Pluronic F-68 and polyoxyethylene were able to protect hybridoma cells from agitation damage in a bioreactor, but were incapable of increasing cell resistance to shear damage in a viscometer. Contrary to these results, Goldblum *et al.* (*39*) demonstrated that the addition of either methyl cellulose, dextran, or Pluronic F-68 to the medium helped protect both *Trichoplusia ni* (TN-368) and *Spodoptera frugiperda* (SF-9) insect cell lines from shear damage in a viscometer. Goldblum *et al.* also showed that the shear sensitivity of these two cell lines is comparable, although the TN-368 cell line appears to be somewhat more sensitive. It was found that shear stresses of 0.1 N/m^2 and 0.59 N/m^2 resulted in the significant cell lysis of the TN-368 and SF-9 cell lines, respectively, after only 5 minutes of exposure in the absence of protective agents. Tramper *et al.* (*108*) found that a shear stress of 1.5 N/m^2 resulted in minimal lysis of *S. frugiperda* (IPLB-SF-21) insect cells after 3 hours of exposure. The medium, however, contained 0.1% (w/v) methylcellulose and they did not test lower shear stresses. As expected, Tramper *et al.* (*108*) and Goldblum *et al.* (*39*) both observed increased cell damage as the magnitude of the laminar shear stress was increased. Peterson *et al.* (*89*) used a viscometer to demonstrate that hybridoma cells cultured in tissue culture flasks (static culture) were more sensitive to laminar shear than cells cultured in spinner flasks (suspension culture). They also found that growing the cells under high agitation increased the cells' resistance to shear stress. Stationary phase cells, both from the tissue culture flasks and spinner flasks, were more sensitive than cells in exponential growth. Ten minute exposure to 1.0 N/m^2 resulted in significant cell damage in most experiments with the hybridoma cells.

Results obtained in sparged bioreactors (*79*) suggest that virally-infected insect cells are more sensitive to hydrodynamic stress than uninfected cells. Murhammer and Goochee (*79*) hypothesized that this is due to a significant stretching of the cell membrane caused by the accumulation of baculovirus within the cell.

As mentioned previously, sparging, cavitation, and bubble incorporation via vortexing can be very damaging to cultured insect and animal cells. Supplementing the medium with the surfactant Pluronic F-68 has been demonstrated to protect these cells from sparging (*37, 45, 46, 56, 79-81, 87, 91, 96*), cavitation (*81*), and bubble incorporation via vortexing (*81*). Murhammer and Goochee (*80*) have also demonstrated that a wide variety of other Pluronic and reverse Pluronic polyols provide protection to insect cells in sparged bioreactors. Pluronic polyols are nonionic block co-polymers consisting of a center block of polyoxypropylene with polyoxyethylene blocks on both ends. Reverse Pluronic polyols consist of a center block of polyoxyethylene with polyoxypropylene blocks on both ends. It was demonstrated that the ability of a polyol to serve as a protective agent correlates well with the hydrophilic-lipophilic balance (HLB). HLB is an empirical measure of the emulsifying ability of a surfactant molecule (*43*) and is related to its relative solubility in oil and water. These results raise two fundamental questions: (1) What is the mechanism of cell damage resulting from sparging, cavitation, and bubble incorporation via vortexing? (2) What is the mechanism of the protection provided by Pluronic and reverse Pluronic polyols?

To determine the mechanism of cell damage in sparged bioreactors, it is first necessary to determine the region in the bioreactor in which cell damage is occurring. In a sparged bioreactor there are three distinct zones of bubble behavior: (1) the bubble formation region near the gas distributor, (2) the region where bubbles are rising at their terminal velocity, and (3) the bursting bubble region at the medium surface. Handa-Corrigan *et al.* (*46*) and Tramper *et al.* (*109*) demonstrated that damage is not occurring in the region of rising bubbles. Many lines of experimental evidence suggest that cell damage can occur both in the sparger region and in the bursting bubble region at the medium surface. Microscopic visualization (*4*) has shown that cells adsorb to rising bubbles and when these bubbles reach the medium surface they burst. Similar phenomena were observed by Handa-Corrigan *et al.* (*45, 46*). Results obtained by Wudtke and Schugerl (*127*) also support the hypothesis that cell damage occurs in the region of bursting bubbles. They conducted an experiment in which the culture surface was covered with a paraffin layer and compared these results with those obtained in the absence of a paraffin layer. Less cell damage occurred in the presence of the paraffin layer. It was assumed that cells did not penetrate the paraffin layer and therefore were not exposed to the bursting bubbles at the medium surface. It has been hypothesized (*17, 46*) that cell damage at the medium surface can occur by (1) incorporation into foam, (2) oscillations due to the energy released by bursting bubbles at the medium surface (perhaps in the form of turbulent eddies), and/or (3) shearing of cells in draining liquid films (lamellae) in foams. Murhammer and Goochee (*81*) have demonstrated that cell damage can occur in the sparger region and that this damage is a function of sparger design. It appears as though the key to minimizing cell damage in the sparger region is to minimize the pressure drop across the sparger. It has been hypothesized (*81*) that cell damage in the sparger

region can occur by (1) turbulence caused by fluid rushing into the void formed when a bubble detaches from the sparger and/or (2) bubble oscillation following detachment from the sparger.

The effect of cavitation and bubble incorporation via vortexing on insect and animal cells has not been as extensively studied as the effect of sparging. It is known, however, that both of these phenomena can cause severe cell damage. It has been hypothesized (*29*) that cavitation damage to cells results from the formation of turbulent eddies caused by collapsing bubbles. Recent experimental results (*19*) suggest, however, that cavitation damage under many circumstances is more likely to occur during the formation of cavities. It has been hypothesized that cell damage from bubbles incorporated by vortexing is due to the incorporated bubbles (*101*) rather than energy dissipation in the liquid. Kunas and Papoutsakis (*61*) further hypothesize that significant cell damage will only occur when the entrained bubbles interact with a freely moving gas-liquid interface, like that present at the medium surface. Clearly, the mechanisms of cell damage by sparging, cavitation, and bubble incorporation via vortexing are not well understood. A better understanding of these phenomena is a necessary prerequisite for improving the scale-up of insect and animal cell bioreactors.

As indicated above, many studies have been conducted which focussed on the ability of Pluronic F-68 to protect insect and animal cells in sparged bioreactors. In the course of these studies, many hypotheses have been formulated regarding the mechanism of this protective effect. Most of these hypotheses propose that Pluronic F-68's protective effect results from its ability to interact at interfaces. The interfaces in the case of insect and animal cell cultures include the gas-liquid interface and the liquid-solid interface at the cell surface. Handa-Corrigan *et al.* (*45, 46*) hypothesized that Pluronic F-68's protective effect is due to the stabilization of foams. It was further hypothesized that cells do not penetrate the stable foam layer on the medium surface and therefore are not subject to bursting bubbles at the medium surface. Contrary to this hypothesis, Murhammer and Goochee (*79, 80*) have demonstrated that a stable foam layer is neither necessary nor sufficient for cell protection. Murhammer and Goochee (*79-81*) hypothesize that an interaction of Pluronic F-68 with the cell membrane is at least partially responsible for its protective effect. There are many lines of evidence which suggest that Pluronic F-68 interacts with cell membranes. First, it has been observed that addition of Pluronic F-68 to the medium inhibits the ability of insect cells to uptake trypan blue dye (*79*). Second, it has been demonstrated that many Pluronic and reverse Pluronic polyols lyse cells, which is certainly indicative of an interaction with the cell membrane (*80*). Third, it has been shown that the addition of Pluronic F-68 can reduce the rigidity of sickled erythrocytes (*102*). Fourth, it has been demonstrated that addition of Pluronic F-68 to the medium results in reduced membrane fluidity in hybridoma cells (*94*). It is further hypothesized that this interaction with the cell membrane has two possible effects. First, this cell surface interaction, in conjunction with an interaction of Pluronic polyols at gas-liquid interfaces, could inhibit potentially damaging interactions between the cells and air-liquid interfaces. Second,

the interaction of the polyols with the cell membrane could render the cells more resistant to damage from bubbles, e.g., a reduced cell rigidity (*102*) could make the cells more flexible and therefore less likely to be damaged when encountering the air-liquid interface. Michaels *et al.* (*75*) suggest that the protective effect of Pluronic F-68 is not due to changes in the cell itself (i.e., that the cell is not made more shear resistant), but instead is due to changing the level or frequency of the shear forces experienced by the cell. This hypothesis is based on a combination of two observations: (1) Pluronic F-68 provides no protection to hybridoma cells in a viscometer and (2) Pluronic F-68 protects these cells from agitation damage in a bioreactor. It is possible that the actual mechanism of Pluronic F-68 is a combination of effects proposed by the preceding hypotheses. It is also possible that the relative contribution of these various effects is cell line dependent. It is clear that additional study is required to develop a better understanding of this protective effect, which in turn could lead to the design of improved protective agents and thereby aid in bioreactor scale-up.

SELECTION OF INSECT CELL LINES FOR RECOMBINANT PROTEIN SYNTHESIS

When considering an expression system for the production of a recombinant protein it is desirable to have a system capable of producing large quantities of a biologically active protein. The selection of host cells for recombinant protein synthesis is an important consideration since both the level of protein synthesis and protein processing are functions of cell type. It should also be noted that the level of protein synthesis and protein processing are also functions of the type of baculovirus used to infect a particular cell line. This section will consider the currently available information applicable to the selection of a host insect cell line for the production of recombinant proteins with a given baculovirus expression system.

Hink *et al.* (*50*) studied the expression of three different proteins, pseudorabies virus glycoprotein gp50T, human plasminogen (another glycoprotein), and β-galactosidase, in 23 different insect cell lines. They found that no single cell line produced the highest yields for all three recombinant proteins. It was shown that the *S. frugiperda* SF-9 insect cell line, which is the cell line most frequently used with the baculovirus expression system, produced the highest levels of β-galactosidase, but produced levels of the glycoproteins which were considerable lower than that of many other cell lines. The *Mamestra brassicea* IZD-MB0503 insect cell line produced the highest levels of human plasminogen and produced moderately high levels of pseudorabies virus glycoprotein gp50T. Four cell lines, *Heliothis virescens* IPLB-HvT1, *S. frugiperda* IPLB-SF-21AE, *S. frugiperda* IPLB-SF-21AE-15, and *S. frugiperda* IPLB-SF-1254, were the best producers of pseudorabies virus glycoprotein gp50T. It was also demonstrated that many cell lines retain the capacity to produce recombinant proteins after prolonged growth in serum-free medium, the yields in serum-free medium frequently being equal to or greater than those obtained in serum-supplemented medium. Finally, Hink *et al.* found that the expression levels varied considerable

between clones; e.g., *S. frugiperda* IPLB-SF-21AE-15 and *S. frugiperda* SF-9 insect cell lines, which are clones of the *S. frugiperda* IPLB-SF-21AE insect cell line, expressed the proteins in levels considerably different from each other and from the parent cell line. Hink *et al.* suggest that cloning should be considered when maximizing yields of a given recombinant protein.

Other studies have also demonstrated significant differences among clonal populations obtained from a heterogeneous insect cell population. Bilimoria and Carpenter (*7*) were able to isolate a cell population from the parent *Trichoplusia ni* TN-368 insect cell line which had a cell attachment level of 95% as compared to the parent cells' level of 5%. Volkman and Summers (*118, 119*) investigated clones from the parent TN-368 cell line and found slight morphological variations and a significant difference in the clones' ability to replicate *Autographa californica* nuclear polyhedrosis virus. Corsaro and Fraser (*21*) investigated 13 clones isolated from the parent *Heliothis zea* IPLB-HZ 1075 insect cell line, finding much variation among the clones in terms of morphology, growth rate, and their ability to support the replication of the singly encapsulated *H. zea* nuclear polyhedrosis virus.

In addition to producing high levels of the desired recombinant protein, it is also imperative that the resulting protein is biologically active. In most cases where the appropriate tests have been performed, recombinant proteins produced with the baculovirus expression system have been found to be antigentically, immunogenically, and functionally similar to the authentic proteins (*68*). Consistent with these findings, insect cells appear to be capable of properly performing most mammalian post-translational modifications. Until recently, however, it was not clear if insect cells had the ability to perform glycosylation in a manner comparable to mammalian cells, i.e., if they were capable of complex-type glycosylation. This is an important consideration in the selection of a host cell line for recombinant protein synthesis since it is known that glycosylation patterns can have a great influence on the biological properties of some proteins (*123*). Complex glycosylation in insect cells was first documented in 1990 when recombinant human plasminogen was produced by the *S. frugiperda* IPLB-SF21-AE insect cell line (*27*). In this study 40% of the N-linked oligosaccharide attached to human plasminogen was of the complex type. The remaining 60% consisted of various oligosaccharide forms, ranging from $Man_9GlcNAc_2$ to $Man_3GlcNAc_2$. Additional studies (*25*) with recombinant human plasminogen synthesis in the *S. frugiperda* IPLB-SF21-AE insect cell line demonstrated that the form of N-linked glycosylation varied with the time post-infection, the oligosaccharides were mainly of the high mannose type early in the infection (e.g., $Man_3GlcNAc_2$) and mainly of the complex type late in infection. These results suggest that the viral infection process alters the glycosylation characteristics of the insect cells. Complex glycosylation was also observed when recombinant human plasminogen was produced by other virally-infected lepidopteran insect cell lines, including *Mamestra brassicae* IZD-MBO503, *Manduca sexta* CM-1, and *S. frugiperda* SF-9 (*26*). Approximately 63% of the total N-linked oligosaccharides present on the recombinant human plasminogen produced in virally-infected

MBO503 cells were of the complex type (*26*). In addition, MBO503 cells were able to add fucose to the oligosaccharides. Svoboda *et al.* (*104*) investigated glycosylation of mouse interleukin-3 expressed in virally-infected *Bombyx mori* larvae and identified both high mannose and complex type N-linked oligosaccharides.

Other studies of glycosylation in insect cells have failed to observe complex glycosylation. It has been demonstrated that the N-linked oligosaccharides present on mosquito cell membrane glycoproteins do not contain the terminal N-acetylglucosamine, galactose, and sialic acid groups characteristic of complex glycosylation (*12, 13*). Hsieh and Robbins (*52*) demonstrated that the most highly processed oligosaccharide obtained in mosquito cells infected with Sindbus virus was of the form $Man_3GlcNAc_2$, as compared to complex oligosaccharides produced by vertebrate cells infected with the Sindbus virus. Bergh *et al.* (*5*) used the baculovirus system to synthesize recombinant human glucocerebrosidase, which has potential therapeutic applications in the treatment of Gaucher's disease. Their results suggested that the N-linked oligosaccharide structure attached to this protein was $Man_3[Fuc]GlcNAc_2$ when synthesized in the *S. frugiperda* SF-9 insect cell line. There is evidence suggesting that human glucocerebrosidase is targeted to macrophages (the desired target of the therapy) more efficiently when its oligosaccharide is $Man_3[Fuc]GlcNAc_2$ or $Man_3GlcNAc_2$ instead of the complex form. Thus, cell lines which produce these oligosaccharide structures would appear to provide a significant advantage over expression systems which process the oligosaccharide to the complex form. Kuroda *et al.* (*62*) found that recombinant influenza virus hemagglutinin produced in *Spodoptera frugiperda* cells processed N-linked oligosaccharides to $Man_3GlcNAc_2$ and $Man_3[Fuc]GlcNAc_2$. Unfortunately, Kuroda *et al.* (*62*) did not indicate the specific *S. frugiperda* insect cell line used in the experiment. Wathen *et al.* (*120*) also observed $Man_3[Fuc]GlcNAc_2$ N-linked oligosaccharides attached to a chimeric protein (combination of two surface proteins of the human respiratory syncytial virus) expressed in SF-9 cells. Reevaluation of these results might be in order in light of the results which demonstrate that the glycosylation capabilities of the insect cells can change during the infection process.

In a study of O-linked glycosylation by insect cells, Thomsen *et al.* (*107*) found that none of the oligosaccharides attached to the pseudorabies virus glycoprotein gp50 produced in the *S. frugiperda* SF-9 insect cell line contained sialic acid. Sialic acid was present on the oligosaccharides attached to gp50 synthesized in CHO and Vero cells. In another study with SF-9 cells, Wathen *et al.* (*120*) failed to find sialic acid in O-linked oligosaccharides of a chimeric protein (combination of two surface proteins of the human respiratory syncytial virus). In addition, sialic acid was not found associated with O-linked oligosaccharides attached to mouse interleukin-3 produced in virally-infected *Bombyx mori* larvae (*104*). Additional studies (e.g., with other cell lines) are needed in order to determine if the inability to produce sialic acid containing O-linked oligosaccharides is a general characteristic of insect cells. It is known that many insect cell lines

are capable of synthesizing sialic acid, as evidenced by its presence in some N-linked oligosaccharides.

In addition to selecting a cell line for its ability to properly process proteins, the ability of the cell line to withstand large-scale bioreactor environments should also be considered. It is known that some cell lines are more resistant to laminar shear than others (*39*) and that some insect cell lines are more conducive to large-scale bioreactor environments. It is also possible that clones isolated from a parent population might have differing susceptibilities to hydrodynamic stress. Therefore, when selecting an insect cell line for producing a given recombinant protein, one should consider (1) the ability to properly process the protein, (2) the level of protein synthesis, and (3) the ability to grow and produce recombinant protein in large-scale bioreactor environments.

CONCLUSION

Two important engineering aspects of using insect cell cultures for recombinant protein synthesis, scale-up and insect cell line selection, have been discussed. It is known that the major obstacle to growing cells in large-scale bioreactors is supplying the cells with sufficient dissolved oxygen without damaging the cells, i.e., developing methods which allow cell growth in sparged bioreactors. Fortunately, supplementing the medium with a protective agent, e.g., Pluronic F-68, can protect insect cells in sparged bioreactors. The selection of host insect cell lines for recombinant protein synthesis should be based on high yields, proper protein modifications, e.g., glycosylation, and the ability to survive in bioreactor environments.

REFERENCES CITED

The references cited in this review are included in the literature list in the following Patents and Literature Section.