Growth, viral production and metabolism of a *Helicoverpa zea* cell line in serum-free culture

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Abstract

Insect cell cultures have been extensively utilised for means of production for heterologous proteins and biopesticides. *Spodoptera frugiperda* (Sf9) and *Trichoplusia ni* (High FiveTM) cell lines have been widely used for the production of recombinant proteins, thus metabolism of these cell lines have been investigated thoroughly over recent years. The *Helicoverpa zea* cell line has potential use for the production of a biopesticide, specifically the *Helicoverpa armigera* single-nucleocapsid nucleopolyhedrovirus (HaSNPV). The growth, virus production, nutrient consumption and waste production of this cell line was investigated under serum-free culture conditions, using SF900II and a low cost medium prototype (LCM). The cell growth (growth rates and population doubling time) was comparable in SF900II and LCM, however, lower biomass and cell specific virus yields were obtained in LCM. *H. zea* cells showed a preference for asparagine over glutamine, similar to the High FiveTM cells. Ammonia was accumulated to significantly high levels (16 mM) in SF900II, which is an asparagine and glutamine rich medium. However, given the absence of asparagine and glutamine in the medium (LCM), *H. zea* cells adapted and grew well in the absence of these substrates and no accumulation of ammonia was observed. The adverse effect of ammonia on *H. zea* cells is unknown since good production of biologically active HaSNPV was achieved in the presence of high ammonia levels. *H. zea* cells showed a preference for maltose even given an abundance supply of free glucose. Accumulation of lactate was observed in *H. zea* cell cultures.

Introduction

Insect cell cultures have numerous applications in baculovirology, providing a basis not only for studying baculovirus genetics but also for large scale production of insect cells that are used to produce valuable and medically important biologicals by recombinant baculoviruses expressing foreign proteins as well as for the production of viral biopesticides. Insect cell culture is increasingly significant as baculoviruses might be useful as gene

delivery vectors for human gene therapy (Ghosh et al. 2002; Miller and Lu 1997).

Helicoverpa spp. are major pests of field crops worldwide. *Helicoverpa zea* single-nucleocapsid nucleopolyhedrovirus (HzSNPV) and *Helicoverpa armigera* (Ha) SNPV are the two baculoviruses that can be used to control these pests. Although they have not been commercially produced in insect culture for use as biopesticides, research all over the world has been conducted to understand the genetics of *Helicoverpa* baculoviruses (Chen

et al. 2001, 2002; Le et al. 1997) and the physiology of these viruses in susceptible cell lines (Chakraborty et al. 1995, 1996, 1999; Chakraborty and Reid 1999; Granados et al. 1981; Ignoffo et al. 1971; Lenz et al. 1991; Lua et al. 2002, 2003; Lua and Reid 2000, 2003; McIntosh et al. 1995, 1997, 2001; McIntosh and Ignoffo 1981; Rice et al. 1989; Rivkin et al. 1998; Yamada et al. 1982), with the objective of optimising the production of these viruses in cell culture. In recent years, genetic engineering was also attempted on *Helicoverpa* baculoviruses (Chen et al. 2000; Popham et al. 1997) in the hope of generating a more virulent virus against *Helicoverpa* pest species, signifying the magnitude of damage caused by these pests and the urgency to control them.

Helicoverpa zea cell lines are the most commonly used to study the *Helicoverpa* baculoviruses. Increasing the cell density of *H. zea* in culture via a fed-batch strategy was explored (Martelijn et al. 2003), but the nutrient requirements of the cell line have not been investigated. It is important to understand the cell metabolism to aid in the development and formulation of a culture medium for optimal cell growth and virus production using this cell line. Commercial production of baculoviruses for use as biopesticides in insect cell culture can only be economically viable if produced in a serum-free low cost medium (LCM).

The desire to achieve high recombinant protein or virus production at high cell densities using insect cell culture has led to intense research on insect cell culture medium formulation, optimisation and supplementation (Bedard et al. 1993, 1997; Donaldson and Shuler 1998a; Gilbert et al. 1996; Mitsunashi 1982; Mitsunashi and Goodwin 1989; Schlaeger 1996a,b; Weiss et al. 1981, 1982, 1994), most aiming at removing the need of serum in the medium. Substitution of serum in cell culture media has many well-known advantages such as cost reduction, elimination of lot to lot variability in serum and avoidance of the potential risk of mycoplasma contamination from serum (Agathos 1991; Goodman and McIntosh 1994; Granados and McKenna 1995).

Many serum-free media are formulated for *Spodoptera frugiperda* cell lines (SF900II, Life Technologies; Excell 401, JRH Biosciences; SFIM-1, Sigma) and in recent years, serum-free media (HyQ-SFX, HyClone) have been formulated

for the High Five™ (*Trichoplusia ni*) cell line. These media are optimised but very costly. Most reports on metabolism of insect cells have been focused on Sf9 and High Five™ cell lines due to their importance for the production of foreign proteins (Bedard and Kamen 1997; Bedard et al. 1992, 1993, 1997; Marheineke et al. 1998; Ohman et al. 1996; Rhiel et al. 1997; Sugiura and Amann 1996).

In brief, most cell lines utilise sugars, proteins, peptides, amino acids, organic acids, vitamins, lipids and organic salts (Agathos 1991). However, the nutrient requirement of each line is unique and different from each other. The nutrient demands of the High Five™ cells are known to be different from that for Sf9 cells (Rhiel et al. 1997; Sugiura and Amann 1996). High Five™ cells preferentially utilise asparagine vs. glutamine. Glutamine is essential to some insect cell lines but not for others. Glutamine, when present, is generally the most rapidly consumed amino acid in Sf9 cultures (Bedard et al. 1992, 1993; Rhiel et al. 1997; Wong et al. 1994) but it has been shown that it is not an essential amino acid for Sf9 cell growth as long as ammonium ions are supplied (Ohman et al. 1996). The production of a recombinant protein is also not negatively affected by glutamine-free conditions. However, High Five™ cells are not capable of growing in a glutamine-free medium (Ohman et al. 1996). Mitsunashi (1989) has reported insect cell lines that require glutamine will consume it to a great extent, whereas cell lines that do not require glutamine produce it. The latter cell lines consume glutamate and free ammonium in the media, and glutamine is presumably synthesised with the help of glutamine synthetase.

Accumulation of inhibitory waste products such as lactate and ammonia in Sf9 cultures are minimal (Bedard et al. 1994; Rhiel et al. 1997; Sugiura and Amann 1996), whereas High Five cells™ produce both lactate and ammonia (Rhiel et al. 1997; Sugiura and Amann 1996).

The release of specific amino acids, in particular alanine, into the culture medium during cell growth is commonly found (Bedard et al. 1993; Donaldson and Shuler 1998b; Rhiel et al. 1997; Wong et al. 1994). Alanine is the major organic carbon product released and is also one of the nitrogenous metabolic by-products besides ammonia. Alanine was found to accumulate in Sf9 cultures as long as glucose was present (Bedard et al. 1993).

The range and efficiency of carbohydrate utilisation varies for different cell lines. Glucose is usually the preferred carbon and energy source of insect cells (Bedard and Kamen 1997; Bedard et al. 1993; Raghunard and Dale 1996; Rhiel et al. 1997) and is the single most rapidly consumed source of organic carbon. Findings concerning sucrose utilisation are contradictory. Some investigators have found no detectable changes in levels in growing cultures (Bedard et al. 1993; Ferrance et al. 1993) while others report consumption of sucrose in both infected (Wang et al. 1993) and uninfected cultures (Sugiura and Amann 1996).

This paper presents the metabolism of *H. zea* cells in two serum-free media, SF900II and an in-house LCM. SF900II is one of the most common commercially available serum-free medium for use in culturing *H. zea* cells. LCM consists mainly of protein hydrolysates (yeast extract, meat hydrolysate, casein hydrolysate and lactalbumin hydrolysate) as source of amino acids, inorganic salts similar to those in IPL-41 medium and a lipid emulsion (Schlaeger 1996a). Studies were also conducted in an LCM to obtain relevant information for the development of an LCM for optimal cell growth of *H. zea* cells and the production of HaSNPV. Comparisons of the *H. zea* cell metabolism with Sf9 and High FiveTM cells are also discussed.

Materials and methods

Cell line, virus isolate and media

Helicoverpa zea cell line, strain BCIRL-HZ-AM1, was isolated from pupal ovarian tissue (McIntosh and Ignoffo 1981). This cell line has been adapted to serum-free suspension culture in SF900II medium (Life Technologies). Cultures were typically grown in 250 ml Erlenmeyer flasks on an orbital shaker operated at 120 rpm, inside a refrigerated incubator at 28 °C. *Helicoverpa zea* cells were also adapted directly to an LCM prototype. The pH and osmolarity of LCM are adjusted to similar levels as SF900II to enable optimal cell growth, which is pH 6.2–6.3 and osmolarity between 345 and 375 mOsm/kg. Frozen stock cells in SF900II medium were thawed and cultured directly into the LCM prototype. Cells in the LCM prototype were subcultured and maintained regularly as per the

normal working stock maintenance. HaSNPV (UQ-AC53) was first isolated from infectious haemolymph collected from virus infected larvae. Passage one virus (uncloned) was established in cells grown in SF900II supplemented with 10% foetal bovine serum (CSL, Australia) and the details are documented in an earlier paper (Lua and Reid 2000). The virus was titred using a plaque assay (Lua and Reid 2000).

Virus infections

For comparison of *Helicoverpa zea* metabolism in both SF900II and LCM media, both uninfected controls and infected cultures were set-up. For each medium, duplicate flasks were set-up for uninfected cultures, and infected cultures were set-up with cell densities at the time of infection (TOI) of 5×10^5 cells/ml and 1×10^6 cells/ml. All infected cultures were infected with passage 2 virus stock to achieve passage 3 infections. A minimum multiplicity of infection (MOI) of 0.5 plaque forming unit (PFU)/cell were used to infect these cultures. Cell densities and viabilities of all cultures were determined daily in triplicate using a trypan blue exclusion cell count method (Nielsen et al. 1991). To determine polyhedra density, cells were lysed with 0.5% SDS for 1 h at 28 °C before triplicate counts were done in a haemocytometer counting chamber. Samples of spent medium were collected from both uninfected and infected cultures, and stored at –20 °C for analysis of amino acids, carbohydrates, lactate and ammonia. Polyhedra were harvested at the end of the culture (10 dpi) for SDS-Protein Capillary Electrophoresis (CE) analysis.

Dry weight and total cell protein determination

The dry weight of cells grown in SF900II and LCM were determined. To minimise the error of the assay, a large number of cells are required. Each sample was assayed in triplicate and the trays of cells were dried to a constant weight in an oven at 105 °C. The DC Protein Assay (Bio-Rad, USA) was used to determine the total cell protein. All standards and test samples were assayed in triplicate. The protein concentrations of the test samples were obtained using the BSA standard curve.

Amino acid analysis

Amino acid concentrations were determined using the Waters PICO.TAGTM system (Millipore Waters Chromatography Division, USA) and the method is described by Reid et al. (1987). In brief, amino acids were derivatised with phenylisothiocyanate (PITC) to form phenylthiocarbamyl (PTC) derivatives of the amino acids. The derivatised amino acids undergo separation in a PICO.TAGTM column and UV detection at 254 nm. For determination of free amino acids, cell-free supernatant samples were filtered through 10 000 MW cut-off ultrafiltration membranes (Ultra-free[®] MC, Millipore, USA) before derivatisation. For total amino acid analysis, a hydrolysis step with 6 N HCl/1% phenol for 20–24 h at 105 °C is introduced before the derivatisation step.

Carbohydrate and lactate analysis

Carbohydrates and lactate were analysed by HPLC. Cell-free supernatant samples were deproteinated using 10 000 MW cut-off ultrafiltration membranes (Ultra-free[®] MC, Millipore, USA) before analysis. The glucose concentrations were obtained directly from the HPLC system using a Shodex cation exchange sodium column (Bio-Rad, USA). Maltose, sucrose and lactate are determined on one column. Maltose and sucrose tend to co-elute on the column as both have similar molecular weights. Thus, test samples must be hydrolysed before HPLC injection. Twenty- μ l of 6 M HCl was added to 80 μ l of ultrafiltered sample and incubated at 60 °C for 15 min to allow complete hydrolysis of sucrose to glucose and fructose. Fructose produced from hydrolysis of sucrose is used as an indirect measure of the sucrose concentration. The hydrolysed samples were separated on a 30-cm long HPX-87H cation exchange hydrogen column (Bio-Rad, USA). The column operates at 65 °C using 0.008 N H₂SO₄ as eluent buffer.

Ammonia analysis

Ammonia (NH₃) was quantified using an enzymatic ammonia assay kit (Sigma Diagnostics[®], USA). Samples were taken daily from cultures. Cells were spun down and supernatant transferred

Figure 1. *H. zea* cell growth in LCM (circle) and SF900II (triangle). The means of triplicate counts of viable cell densities from duplicate cultures (solid line) and % viabilities (broken line) are shown. Error bars are 95% confidence intervals.

into new tube and kept at –20 °C until the assay was conducted.

SDS–Protein Capillary Electrophoresis

Polyhedrin protein in polyhedra was quantified using the SDS–Protein Capillary Electrophoresis (CE) method using a Biofocus 3000 CE System (Bio-Rad, USA) and the method is described in Biofocus Applications Manual (Bio-Rad). This assay works on the same principle as the conventional SDS–PAGE systems and is designed to detect proteins ranging from 10 to 200 kDa. All standards and samples were injected in triplicate, and separated using the CE SDS–Protein Run Buffer and a 50 μ m $\times$ 24 cm uncoated capillary. The running voltage was 15 kV, a running current of 20 μ A, a run time of 15 min and the capillary temperature was set at 20 °C.

Results and discussion

Cell growth

Comparable maximum viable cell densities and growth rates were obtained in both SF900II and LCM (Figure 1). The maximum viable cell density for LCM was 5.60×10^6 cells/ml and 4.71×10^6 cells/ml for SF900II, with growth rates of 0.0320 h^{-1} and 0.0295 h^{-1} for LCM and SF900II, respectively (Table 1).

The number of cells was not limited in LCM by the very low concentrations of amino acids (especially

Table 1. Summary of typical cell parameters for *H. zea* cells in LCM and SF900II. The means of triplicate determinations and 95% confidence intervals are presented.

Medium	LCM	SF900II
Max. viable cell density ($10^6/\text{ml}$)	5.60 ± 0.21	4.71 ± 0.06
Growth rate (hour^{-1})	0.0320 ± 0.0010	0.0295 ± 0.0004
Population doubling time (hours)	21.8 ± 0.8	23.5 ± 0.4
Dry weight (pg/cell)	324 ± 9	656 ± 57
Total cell protein (pg/cell)	214 ± 7	433 ± 20
<i>Specific uptake or production rates</i>		
Aspartate (10^{-17} mole/cell-s)	0.24	–
Asparagine (10^{-17} mole/cell-s)	–0.02	–1.32
Glutamate (10^{-17} mole/cell-s)	–0.52	–0.14
Glutamine (10^{-17} mole/cell-s)	–0.03	–0.85
Alanine (10^{-17} mole/cell-s)	0.94	0.25
Glucose (10^{-17} mole/cell-s)	–2.79	–3.32
Maltose (10^{-17} mole/cell-s)	–0.07	–0.30
Lactate (10^{-17} mole/cell-s)	0.16	0.59
Ammonia (10^{-17} mole/cell-s)	–0.18	1.57

Figure 2. Comparison of free amino acid composition in LCM and SF900II media.

of asparagine and glutamine) compared to the SF900II medium (Figure 2). On the contrary, the *H. zea* cell line performs better in LCM by the same criteria, achieving a higher growth rate, a shorter cell population doubling time and slightly higher maximum viable cell density. However, a higher biomass yield was obtained in the SF900II medium. Both the dry weight and total cell protein content for cells cultured in the SF900II medium were two times higher than for cells in LCM (Table 1). Uninfected *H. zea* cells also appeared larger in SF900II than in LCM under light microscopy.

The maximum viable cell density of *H. zea* cells in SF900II is relatively low compared to Sf9 cells,

which peak between 7 to 9×10^6 cells/ml in SF900II (Bedard et al. 1997; Rhiel et al. 1997; Wong et al. 1994). A biomass dry weight yield of 3×10^9 pg/ml was obtained for *H. zea* cells in SF900II whereas the biomass dry weight yield of 3.5 to 4.5×10^9 pg/ml (500 pg/cell, (Rosinski 2001)) was achieved for Sf9 cells in SF900II. This suggests that SF900II may be sub-optimal for *H. zea* cell growth.

Infection

Virus infections with HaSNPV were conducted in both LCM (Figure 3a) and SF900II (Figure 3b) media at two different TOIs, at 5×10^5 cells/ml

(a)

(b)

Figure 3. Virus infections in LCM (a) and SF900II (b). Duplicate flasks of uninfected (circle) and infected cultures at two TOIs: 5×10^5 cells/ml (triangle) and 1×10^6 cells/ml (square), were carried out. The means of triplicate counts of viable cell densities (solid line) and volumetric polyhedra yields (broken line) are shown. Error bars are 95% confidence intervals.

and at 1×10^6 cells/ml, using an MOI of 0.5 PFU/cell. At the TOI of 5×10^5 cells/ml, the cell specific yields were 49 polyhedra/cell and 160 polyhedra/cell for LCM and SF900II, respectively (Table 2). Less than one cell doubling was observed with the TOI of 5×10^5 cells/ml and the cell specific yields obtained at a TOI of 5×10^5 cells/ml were higher than that observed at a TOI of 1×10^6 cells/ml for both media. This indicates that either a nutrient limitation or waste product inhibition was occurring at the higher cell density post infection. The biological activities of polyhedra are comparable

for polyhedra produced in the LCM and SF900II media (Table 2), and both are as infectious as the *in vivo* produced HzSNPV standard, Gemstar. Nevertheless, the cell specific yield in LCM was 3.3 times lower than in the SF900II medium, in a nutrient unlimited condition (TOI 5×10^5 cells/ml). In addition, the polyhedra produced in SF900II medium were larger than those produced in LCM, resulting in a higher polyhedrin protein per polyhedron ratio. Lower cell specific yields in LCM indicates that the LCM is not optimal for HaSNPV production as LCM is a leaner medium in terms of amino acid concentrations compared to SF900II (Figure 2). Other media components such as lipids and vitamins which are not investigated here may also have an effect on the virus yield.

Ammonia

A key observation from this study was the major differences in ammonia metabolism by *H. zea* cells in LCM and SF900II. Accumulation of ammonia to high levels in the spent medium by *H. zea* cells was observed in SF900II cultures but not in LCM cultures (Figure 4). The data revealed that ammonia was almost depleted in LCM cultures. By comparison, ammonia accumulated to 16 mM in SF900II cultures. It is important to note that the initial level of ammonia in LCM was very low (<1 mM) as compared to SF900II (>4 mM). Due to low glutamine concentration in LCM, *H. zea* cells may consume glutamate and free ammonium in the media, and glutamine is presumably synthesised with the help of glutamine synthetase (Mitsuhashi 1989). In asparagine and glutamine rich medium such as SF900II, ammonia accumulates in culture media primarily as a result of glutamine and asparagine catabolism (Newland et al. 1990). *H. zea* cells in SF900II have similar ammonia metabolism to High FiveTM cells but significantly different from Sf9 cells. Ammonia accumulates to levels of 25–35 mM in High FiveTM cultures but no significant ammonia accumulation occurred in the Sf9 cultures (Rhiel et al. 1997). The specific ammonia production rate in High FiveTM cultures (4.2×10^{-17} mole/cell s) was 2.4 times higher than in *H. zea* cultures (1.57×10^{-17} mole/cell s).

The toxic effect of ammonia on *H. zea* cells in SF900II cultures is not known but in the literature,

Table 2. Summary of infection of *H. zea* cells in LCM and SF900II. Errors are quoted in 95% confidence intervals.

Medium	TOI (cells/ml)	Peak cell density (10 ⁶ cells/ml)	Specific yield (polyhedra/cell)	Size of polyhedra (μm)	Polyhedrin protein (pg/polyhedron)	Biological activity, LC ₅₀ (polyhedra/mm ²)*
LCM	5 × 10 ⁵	0.94 ± 0.09	49 ± 7	0.78 ± 0.12	0.651 ± 0.046	0.105 (0.018–0.266)
	1 × 10 ⁶	2.34 ± 0.15	28 ± 2	—	—	—
SF900II	5 × 10 ⁵	0.73 ± 0.18	160 ± 6	0.98 ± 0.12	1.093 ± 0.376	0.167 (0.072–0.305)
	1 × 10 ⁶	1.81 ± 0.16	49 ± 6	—	—	—

*Biological activity of control, Gemstar, is 0.113 (0.068–0.174) polyhedra/mm².

Figure 4. Ammonia consumption/production in *H. zea* cell-only cultures in LCM (circle) and SF900II (triangle). Each point is the mean of triplicate assays and error bars represent standard deviations.

the toxic effects of ammonia in mammalian cultures have been frequently discussed (Doyle and Butler 1990; Hassell et al. 1991; Martinelle et al. 1996; Newland et al. 1990; Ryll et al. 1994; Schneider et al. 1996). Ammonia is probably not inhibitory to *H. zea* cell growth or to the production of HaSNPV, since high yields of biologically active polyhedra were obtained in the SF900II medium even though ammonia production was high in this medium (Figures 3b and 4). Nevertheless, it should not be disregarded that the yields of both cells and virus may be a lot higher without ammonia accumulation. The tolerance levels towards ammonia seem to be cell specific and vary widely. Insect cells (Sf9, High FiveTM and *H. zea*) might be less sensitive to ammonia than most mammalian cell lines examined. Sf9 cells were reported to tolerate ammonia levels as high as 10 mM (Bedard et al. 1993) and both High FiveTM (Rhiel et al. 1997) and *H. zea* cells produced ammonia to levels between 16 and 35 mM, depending on the medium used. The difference in ammonia accumulation between cell lines could be due to the presence of enzymes

that might metabolise ammonia, incorporating the nitrogen into other metabolites.

Amino acids

The consumption or production of five amino acids, namely aspartate, asparagine, glutamate, glutamine and alanine, by *H. zea* cells in LCM and SF900II were measured (Figure 5). Aspartate production was observed for the first 120 h during cell growth in LCM but was later consumed when asparagine was exhausted in cultures (Figure 5a). Aspartate can be derived from asparagine (Metzler 1977). Aspartate can also be produced during cell growth due to catalysis of glutamate and oxaloacetate to form α -ketoglutarate and aspartate. No significant net consumption of aspartate was observed in SF900II as a high level of asparagine is present. Asparagine appeared to be the most rapidly consumed amino acid by *H. zea* cells (Figure 5b). Asparagine is present in low concentration in LCM (<0.4 mM) but in higher concentration in SF900II (12 mM). In both cases, asparagine was completely depleted in the cultures. High asparagine consumption was also observed for High FiveTM cells but not for Sf9 cells (Rhiel et al. 1997). Glutamate was consumed by *H. zea* cells in LCM (Figure 5c), probably due to the lack of glutamine in this medium, whereas only a small amount of net glutamate is consumed in SF900II. Glutamine is generally the most rapidly consumed amino acid in Sf9 cultures (Bedard et al. 1992, 1993; Rhiel et al. 1997; Wong et al. 1994), however glutamine was barely consumed for *H. zea* cell growth in LCM (Figure 5d), instead glutamate was consumed. In contrast, glutamine was rapidly consumed by the cells in SF900II, giving a similar consumption rate as High FiveTM cells in S900II (Rhiel et al. 1997). This suggests that *H. zea* cells have similar glutamine metabolism as Sf9 cells as

Figure 5. Aspartate, asparagine, glutamate, glutamine and alanine metabolism in *H. zea* cell-only cultures in LCM (circle) and SF900II (triangle). Each point is the mean of triplicate assays and error bars represent standard deviations.

they will consume glutamine when it is present in higher concentration, but both cell lines can also grow in the absence of glutamine. This trait is not observed for High FiveTM cells as they are not capable of growing in glutamine-free medium (Ohman et al. 1996). However, *H. zea* cells show

a similar preference for asparagine and glutamine, an observation also reported for High FiveTM cells (Rhiel et al. 1997; Sugiura and Amann 1996).

Alanine production peaked around the same time as glucose was depleted in *H. zea* LCM cultures (Figure 5e), which is similar to other

observations reported in the literature. Alanine was found to accumulate in Sf9 cultures as long as glucose was present (Bedard et al. 1993). A higher alanine production rate was detected in LCM compared to SF900II, even though a higher glucose consumption rate was detected in SF900II cultures (Table 1). Consumption of glutamate by the cells can also lead to alanine production. Alanine is produced during transamination of glutamate by alanine aminotransferase.

Carbohydrates

Carbohydrates are the main energy source of insect cells. Glucose was consumed rapidly during cell growth in both LCM and SF900II cultures, with consumption rates of 2.72 and 3.32×10^{-17} mole/cell s (Table 1), respectively, and was completely depleted by 168 h post seeding (Figure 6a). Maximum viable cell densities were achieved at 96 h when significant glucose concentration was still available, thus glucose is not a limiting factor in relation to the maximum viable cell density that is achievable in the media. *H. zea* cells continue to consume glucose after maximum viable cell density until exhaustion in both media. The consumption rate of glucose by *H. zea* cells was similar to Sf9 cultures (2.4×10^{-17} mole/cell s) but significantly lower when compared to High FiveTM cells (7×10^{-17} mole/cell s) (Rhiel et al. 1997). Maltose was also depleted completely in both LCM and SF900II cultures (Figure 6b). Although the initial maltose concentration was a lot lower in LCM compared to SF900II, it was still completely consumed by the cells. Maltose, a dimer of glucose, was consumed at high rates even in the presence of significant levels of glucose, indicating that *H. zea* cells might have a preference for consumption of maltose even in the presence of abundant free glucose. Sucrose consumption did not change significantly over the culture period (data not shown), which is similar to what was reported by Rhiel et al. (1997) for Sf9 and High FiveTM cells.

Lactate

Lactate concentrations only increased significantly (up to 6 mM) after 96 h in LCM cultures when the maximum viable cell density was achieved, for a short 24-h period (Figure 7). Lactate was later

Figure 6. Carbohydrate metabolism in *H. zea* cell-only cultures in LCM (circle) and SF900II (triangle). Each point is the mean of triplicate measurements and error bars represent standard deviations.

Figure 7. Lactate production in *H. zea* cell-only cultures in LCM (circle) and SF900II (triangle). Each point is the mean of triplicate measurements and error bars represent standard deviations.

consumed after 168 h which coincided with glucose exhaustion. In SF900II cultures, there was a steady increase in lactate production (up to 9 mM) throughout the culture, with a production rate of 0.59×10^{-17} mole/cell s as compared to 0.16×10^{-17} mole/cell s in LCM (Table 1). Unlike Sf9, both *H. zea* and High Five cells produced lactate during growth. However, *H. zea* cells do not produce as much lactate as High FiveTM cells (accumulated level of 43 mM) (Rhiel et al. 1997). Lactate excretion is due to incomplete oxidation of glucose by the glycolytic pathway. Pyruvate, the end product of this pathway is transformed to lactate in order to maintain the oxidation state of the cell, i.e., to recover the oxidised form of the redox cofactor nicotinamide adenine dinucleotide (NAD). Cells can also produce pyruvate in the absence of glucose by consuming lactate. Pyruvate is derived from lactate via the action of lactate dehydrogenase (Bedard et al. 1993). The toxic effect of lactate is probably due to its effect on pH and osmolarity of the culture medium. LCM cultures were not adversely affected by lactate production as no major fluctuation of pH or osmolarity was observed (Figure 8). In contrast, the pH in SF900II cultures dropped below 6 by the end of the cultures, with osmolarity around 290 mOsm/kg.

Conclusion

Significantly lower virus yields were obtained in LCM compared to SF900II. For economical commercialisation of baculoviruses for use as biopesticides, efforts should be put into developing other LCM prototypes as less expensive options that allow the production yields to match that of SF900II. The metabolism of insect cells is complex and the understanding of its control and regulation is still limited. The depletion of nutrients and accumulation of inhibitory by-products are the two major factors that cause deterioration and the consequent cessation of cell growth. This paper presents the first step in understanding the metabolism of a *H. zea* cell line which will aid in the future development of low cost media. The experimental data shows that *H. zea* cell metabolism is closer to that seen for High FiveTM cells than to Sf9 cells. Both the *H. zea* cell line and High FiveTM cells preferentially utilise asparagine over glutamine and accumulate by-products such as alanine, lactate and

Figure 8. pH (diamond) and osmolarity (triangle) during *H. zea* cell growth (circle) in LCM (a) and SF900II (b). The data of duplicate cultures are presented.

ammonia (in asparagine and glutamine rich medium) to very high concentrations. It appeared that *H. zea* cell growth and the production of biologically active HaSNPV are not adversely affected by the accumulation of ammonia in culture but further research is required. However, unlike High FiveTM cells, *H. zea* cells are capable of growing in asparagine and glutamine free medium. Asparagine and glutamine are expensive free amino acids that should be left out of serum-free medium if they show no benefit to the growth of *H. zea* cells or to the production of HaSNPV. Although the effect of ammonia to *H. zea* cells is unknown, ammonia accumulation can be avoided in asparagine and glutamine free medium.

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