SAFC®
3050 Spruce Street
Saint Louis, MO 63103 USA
Phone: 1-800-244-1173 • 1-314-534-4900
Fax: 1-888-289-8449
Global Email: safcglobal@sial.com

Research Report

Commercially Available Serum-Free Insect Media: A Comparison of Sf9 Growth Dynamics and Protein Production

Diane E. Potts[1], Justine A. Malinski[1], Laura T. Kakach[1] and Sara L. Gilliland[2]

[1] ATG Laboratories, Inc., Suite 107, 10300 Valley View Road, Eden Prairie, MN 55344
[2] SAFC, 13804 West 107th Street, Lenexa, KS 66215

Abstract

In this study *Spodoptera frugiperda* (Sf9) cells were adapted to and grown in four different types of serum-free media: EX-CELL® 420 (SAFC), Sf-900 II SFM (Life Technologies, Inc.), HyQ® SFX-Insect™ (HyClone Laboratories, Inc.) and High Five™ (Invitrogen Corporation). After the cells were adapted to each serum-free medium, three independent growth studies were performed. In each study, the performance of each medium was monitored and cell densities and viabilities were determined. Each culture was also infected with recombinant baculovirus expressing ß-galactosidase and protein productivity was determined.

Cells grown in EX-CELL 420 consistently attained the highest cell densities and produced the highest levels of ß-galactosidase ($p<0.05$). Cells grown in High Five and Sf-900 II SFM performed well but had lower cell densities, and produced less ß-galactosidase than the EX-CELL 420 cultures. Cells grown in HyQ SFX-Insect consistently had the lowest cell densities and the lowest levels of recombinant protein production.

Introduction

Baculoviruses are powerful expression systems for the production of biologically active recombinant proteins. These recombinant proteins can be used for a variety of applications including diagnostics, biopesticides and vaccines. The Sf9 cell line, derived from *Spodoptera frugiperda,* has been the cell line of choice for the expression of recombinant proteins and virus production using baculovirus expression vector systems (BEVS). Sf9 cells have proved to be versatile, i.e. they have fast doubling times (approximately 18 - 22 hours), may be grown in either monolayer or suspension culture, and are easily scaled up to large culture volumes. Sf9 cells have also been reported to produce some proteins at levels approaching 20% or more of the total cell protein[1]. Sf9 cells are capable of expressing full-length proteins, and in many cases the recombinant protein produced is indistinguishable in biological activity and structure from the native protein. Given the increased use of BEVS and Spodopteran cell lines for specialized protein production, there has been an increased need for specialized insect cell culture products.

Until the late 1980s the media most widely used for propagation of insect cells required supplementation with serum and various other components including yeast hydrolysate, lactalbumin hydrolysate and tryptose phosphate broth. Due to the costs, regulatory concerns and purification issues associated with serum, many media manufacturers have developed cost-effective, serum-free formulations for the culture of insect cells.

SAFC introduced EX-CELL 420, a complete medium designed and developed specifically for the serum-free, protein-free growth of Spodopteran cell lines. Previous studies indicate that Sf9 cells can be transferred directly from adherent cultures grown in serum-free or serum-containing media into EX-CELL 420 suspension cultures without weaning or adaptation[2]. Sf9 cells cultured in EX-CELL 420 have previously been shown to routinely achieve cell densities > 10^7 cells/mL with viabilities > 95%, and can be maintained for more than 10 days at these densities[2].

The intent of this study was to compare the growth characteristics and recombinant protein expression of Sf9 cells in EX-CELL 420 and other commercially available serum-free media marketed for Spodopteran cell lines. All studies were performed by ATG Laboratories, Inc., Eden Prairie, Minnesota.

Materials

Cells

- Sf9 cells were obtained from American Type Culture Collection, Catalog No. CRL-1711

Serum-Free Media

(all media were supplied by the individual manufacturers)

- EX-CELL 420, Catalog No. 14420C, SAFC
- Sf-900 II SFM, Catalog No. 10902C, Life Technologies, Inc.

SAFC®
3050 Spruce Street
Saint Louis, MO 63103 USA
Phone: 1-800-244-1173 • 1-314-534-4900
Fax: 1-888-289-8449
Global Email: safcglobal@sial.com

- HyQ® SFX-Insect™, Catalog No. SH30278, HyClone Laboratories, Inc.
- High Five™ Serum-Free Medium, Catalog No. B100-01, Invitrogen Corporation

Serum-Containing Media

- Grace's media: Life Technologies, Inc., Catalog No. 11605-094
- Fetal Bovine Serum (FBS): Life Technologies, Inc., Catalog No. 16000-044

Protein Expression Components

- Recombinant baculovirus BacPak6 Virus Stock, Clontech Laboratories, Inc., Catalog No. K1601-C
- ß-Gal Assay Kit, Invitrogen Corporation, Catalog No. K1455-01

Methods

Culture initiation in serum-containing media

An adherent culture of Sf9 cells (passage 18) was initiated in Grace's medium supplemented with 10% FBS. The culture was expanded, then subcultured into suspension culture and maintained for several passages. A master cell stock was generated at passage 23. At passage 25, the cells were used to seed cultures for adaptation into the four serum-free media types.

Adaptation to serum-free media

Suspension cultures (100 mL culture in 250 mL spinner flasks) were initiated at a cell density of 3.0 x 10^5 cells/mL in a 1:1 ratio of Grace's plus 10% FBS to serum-free media. All cultures were incubated at 27 C on the same magnetic stir plate operating at 85 - 90 rpm. During adaptation, cell counts and cell viability measurements were determined regularly using trypan blue exclusion methods. Based on cell density, the cells in each culture were subcultured as needed, for two more adaptation steps. The amount of serum-containing media was reduced sequentially to approximately 25% and then to approximately 10% in these two steps. All subsequent passages were made in serum-free media. After 3 passages in 100% serum-free medium, the cells were frozen at 1.0 x 10^7 cells/mL, according to the specifications provided by the media manufacturer and stored in liquid nitrogen until needed. These serum-free master cell stocks were used to initiate cultures for each growth study.

Growth studies

Three independent growth studies were initiated from the frozen stocks of cells adapted to serum-free medium. One vial of serum-free master cell stock from each test media was thawed into one stationary flask, then expanded to several flasks. Suspension cultures (100 mL culture in 250 mL spinner flask) were initiated and the cells passaged at least once for adaptation into suspension culture. Growth studies were initiated with all cultures at a cell density of 3.0 x 10^5 cells/mL, and were subcultured every 3 - 4 days for a total of 5 passages. Cell densities and viabilities were determined frequently during the growth studies. After 18 days the cells were subcultured at a density that would produce a culture with a density of approximately 1.0 x 10^6 cells/mL in 2 - 3 days. The seeding density ranged from 3.0 x 10^5 - 5.5 x 10^5 cells/mL, and was based on the growth rates previously observed. On day 20 or day 21 each culture was infected at a multiplicity of infection (MOI) of 4 with a recombinant baculovirus that expresses ß-galactosidase. The cells from each culture were harvested 48 hours post-infection, and the cell pellets were washed once with phosphate buffered saline.

Protein expression

ß-galactosidase production in each culture was quantified by spectrophotometric assay of enzyme activity using the chromagenic substrate ortho-nitrophenyl-ß-D-galactopyranoside (ONPG). After each growth study, two independent assays of ß-galactosidase production were performed for each test culture using the ß-galactosidase assay kit according to the manufacturer's instructions.

The soluble lysates from each of the four cultures were processed in parallel and the activity was reported as specific activity, i.e. nanomoles ONPG hydrolyzed/minute/mg total protein in the lysate. Total protein was determined by Bradford assay using Bovine Serum Albumin as the standard[3].

A washed cell pellet (1 mL of culture) from each of the test cultures was resuspended in 50 μL of 250 mM Tris(HCl) pH = 8.0 (lysis buffer) and subjected to three freeze/thaw cycles (-80 C/37 C) to lyse the cells. The insoluble material was removed by centrifugation at 13,000 rpm for five minutes at 4 C. The supernatants were transferred to fresh tubes and the pellets were discarded. An aliquot of each supernatant was diluted 1:2000 (v/v) with lysis buffer and the diluted lysates were assayed (n = 3 for each lysate). Since the soluble lysates from Sf9 cells lack the ability to hydrolyze ONPG, samples prepared with lysates from uninfected cells were used to calibrate the spectrophotometer at 420 nm. The low levels of absorbance due to the spontaneous hydrolysis of ONPG were automatically subtracted from the absorbance of each assay sample.

Results

Cell culture growth

Sf9 cultures grown in EX-CELL® 420 media consistently reached the highest cell densities, frequently achieving densities of 4-5 x 10^6 cells/mL (See Figures 1A, 1B and 1C). The Sf-900 II SFM and High Five cultures typically had cell densities ranging from 2-3.5 x 10^6 cells/mL. The HyQ® SFX-Insect cultures consistently had the lowest cell densities, with cell counts rarely above 1.0 x 10^6 cells/mL.

SAFC®
3050 Spruce Street
Saint Louis, MO 63103 USA
Phone: 1-800-244-1173 • 1-314-534-4900
Fax: 1-888-289-8449
Global Email: safcglobal@sial.com

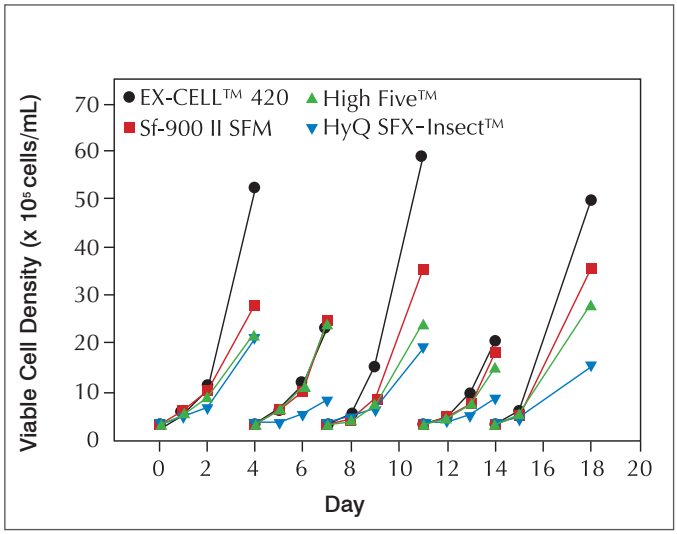
High cell viabilities were obtained in all culture media throughout the study. Typical measurements were 96% or higher and no viability determination was less than 93%.

Protein expression

Active recombinant ß-galactosidase was expressed in every culture (see Figure 2). Due to the instability of ß-galactosidase activity, especially at high dilution, it is not appropriate to directly compare the specific activity of samples that were not processed in the same assay set. The activity data from all three of the growth studies therefore was normalized and is plotted as the combined percent of the maximum activity measured in each assay set.

Under the infection and culture growth conditions used in these studies, the highest average production of ß-galactosidase was observed in cells grown in EX-CELL® 420. Cultures grown in EX-CELL 420 produced significantly higher quantities of ß-galactosidase than all other cultures (ANOVA, Tukey Test, $p < 0.05$, SigmaStat®, SPSS). The levels of protein production in cultures maintained in Sf-900 II SFM and High Five™ were about 10 to 15% less than EX-CELL 420. The cells grown in HyQ® SFX-Insect™ consistently produced the lowest levels of recombinant protein, 43% less than the cells grown in EX-CELL 420.

Figure 1A. Growth Study #1



Growth of Sf9 cells in EX-CELL 420, Sf-900 II SFM, High Five and HyQ SFX-Insect. Cultures were seeded at 3 x 10⁵ cells/mL in 250 mL spinner flasks (100 mL media volume).

Figure 1B. Growth Study #2

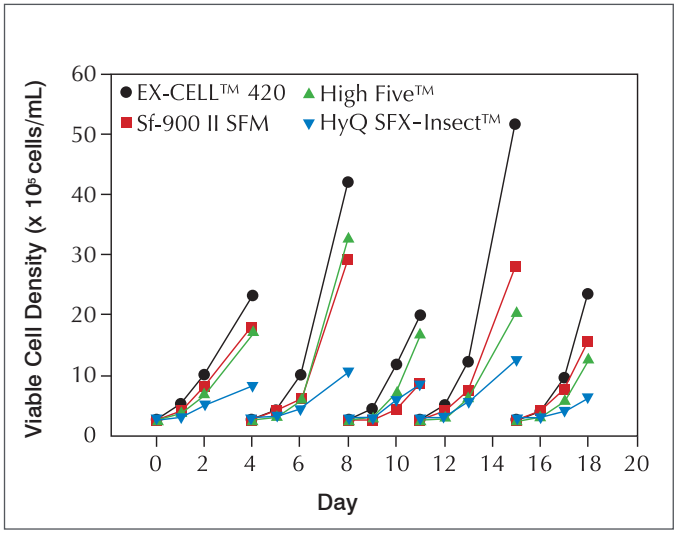
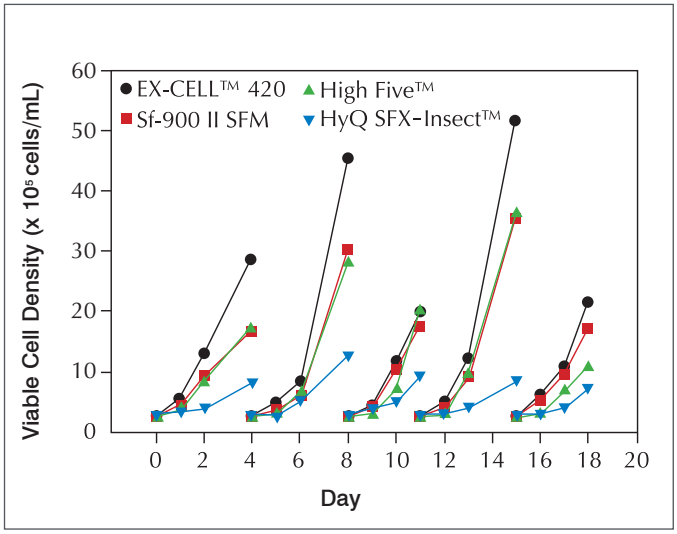


Figure 1C. Growth Study #3



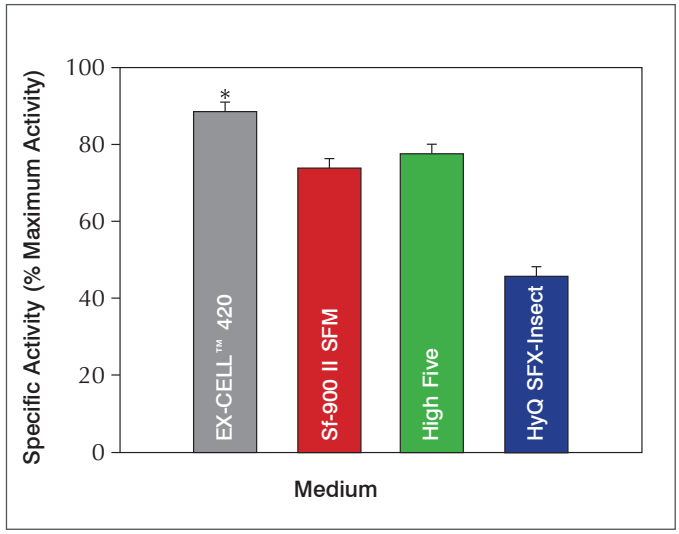
SAFC®
3050 Spruce Street
Saint Louis, MO 63103 USA
Phone: 1-800-244-1173 • 1-314-534-4900
Fax: 1-888-289-8449
Global Email: safcglobal@sial.com

Discussion

The intent of this study was to compare the growth characteristics and recombinant protein expression of Sf9 cells in commercially available serum-free media designed and marketed for Spodopteran cell lines. In this study, Sf9 cells were adapted to and cultured for protein production in four different serum-free media types: EX-CELL® 420, Sf-900 II SFM, HyQ® SFX-Insect™, and High Five™. Sf9 cells could be adapted to and grown in all four media types, i.e. all cultures were viable and produced protein. However, a comparison of the results indicates that there are clear differences in culture growth, and protein production characteristics between the four media.

EX-CELL 420 Serum-Free Medium was developed and optimized to support cell growth, baculovirus production, and recombinant protein expression using BEVS. This study clearly demonstrates that Sf9 cells grown in EX-CELL 420 exhibit superior growth and protein production when compared with other commercially available media. Sf9 cells cultured in this medium achieved the highest cell densities during culture growth, and had the highest levels of ß-galactosidase production.

Figure 2. ß-Galactosidase Activity



***EX-CELL 420 produced significantly more ß Galactosidase than all other media (p <0.05, One-Way ANOVA, Tukey Test). Cultures were infected at 1 x 10^6 cells/mL at a MOI of 4. Data are represented as the mean ± standard error of the mean.**

References

1. O'Reilly, D.R., Miller, L.K., and Luckow, V.A. (1992) Baculovirus Expression Vectors: A Laboratory Manual, CRC Press, Boca Raton, FL

2. Lenk, S.E., Irish, T. W., and Etchberger, K.J. (1998) EX-CELL 420, A New Serum-Free Media for the Growth of Spodopteran (Sf9 and Sf21) Insect Cells. SAFC Literature Reference: A002-99

3. Bradford, M.M. (1976) *Anal. Biochem.,* 72 248-254

For more information, visit safcglobal.com or contact SAFC

USA: + 1-913-469-5580 • info-na@sial.com

Europe: +44 (0)1264-333311 • info-eu@sial.com

Asia/Pacific: +61 (0)2 9841 0555 • auscustserv@sial.com

July 2012 R011
0600 0103 1005 1105 0306

sh5753
1102