

Development of a New Animal Component-Free, Protein-Free Medium for Sf21 and Sf9 Insect Cell Lines

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Abstract

The Baculovirus Expression Vector System¹ (BEVS), when used with insect cell lines such as Sf21 or Sf9 (derived from *Spodoptera frugiperda*), is a powerful tool for producing recombinant proteins. Traditionally, insect cells are grown in media that contain serum or other animal-derived products. As more recombinant proteins are being employed as therapeutic agents, the methods implemented in their production are coming under increasing regulatory scrutiny. A major area of concern is the presence of animal-derived components in media used to culture cells for recombinant protein expression.² To address this matter, a new animal component-free (AF) insect cell culture medium has been developed. One of the foremost challenges in creating an animal component-free insect medium is the replacement of the traditional lipid source, methyl ester fatty acids from cod liver oil, with a non-animal derived substitute. Experiments utilizing factorial design were used to solve this problem. Further medium optimization and replacement of animal-derived components led to the development of TiterHigh™ Sf Insect Medium (Product Code I 5408). TiterHigh™ Sf supports high cell densities ($>2.0 \times 10^7$ cells/mL with Sf21 cells in shaker culture), high wild-type and recombinant AcMNPV titers, and high level recombinant protein production using BEVS. Finally, with the utilization of TiterHigh™ Sf Insect Medium, regulatory concerns associated with the use of animal-derived components have been eliminated.

Introduction

The utilization of insect cells for the production of recombinant proteins has recently increased due to the relative ease of use and the ability to make large amounts of protein. Advances in creating cell lines with more human-like glycosylation patterns have also led to renewed interest. Cell lines derived from *Spodoptera frugiperda* pupal ovarian tissue, such as Sf9 and Sf21, are routinely used in conjunction with the Baculovirus Expression Vector System (BEVS), which takes advantage of viruses that will infect these cell types. The most commonly used baculovirus is *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV). In BEVS, a nonessential baculoviral gene is replaced with the gene-of-interest and put under the control of a very late viral promoter, such as polyhedrin or p10. During the very late stages of a recombinant baculoviral infection, large amounts of the desired protein can be produced, sometimes reaching 50% of the total insect cell protein.

In response to increased regulatory scrutiny of cell culture media, the need for media that are free of any animal-derived components has come to the forefront. Many animal component-free formulations have been developed for other cell culture platforms, such as CHO and NS0. However, the options for insect cell culture are limited. By using an animal component-free medium, the possible threat of adventitious agent contamination from animal-sourced material can be eliminated.

One of the challenges for the development of an animal component-free insect cell culture medium is the replacement of the lipid source. Traditionally, methyl ester fatty acids that are prepared by the transmethylation of cod liver oil have been used. An identical animal-free replacement would be difficult to design, however the critical lipids could be determined through experimental analysis. Testing lipids one at a time would be time consuming and costly. Instead, a factorial matrix experimental design was applied. This approach allows the researcher to test many variables at once, while reducing of the number of test conditions and uncovering any interactions between the variables. The data from more complex experiments, such as this, cannot be interpreted without the help of a statistical analysis program, such as Design-Expert®.*

Additional experimental data with TiterHigh™ Sf Insect Medium (Product Code I 5408) is displayed to examine the growth and productivity potential with this new medium.

Materials and Methods

Sigma-Aldrich Corporation (St. Louis, MO) supplied all chemicals, media and solutions unless otherwise stated.

Cell Lines and Baculovirus

Sf9 cells were obtained from the European Collection of Cell Cultures (ECACC), #89070101. Sf21 cells were obtained from Invitrogen, catalog #11497013.

A recombinant baculovirus, AcP1-57GAL,³ was used to study recombinant β -galactosidase production (β -gal) with both the Sf9 and Sf21 cell lines.

Culture Media

The media used in this study are TiterHigh™ Sf Insect Medium, Animal Component-Free, Protein-Free (I 5408) and the leading competitors' serum-free, protein-free formulations. A preliminary formulation of TiterHigh™ Sf Insect Medium was used for the animal-free lipids study.

Cell Growth and Recombinant Protein Production Assays

Sf9 and Sf21 cells were routinely cultured in suspension, in TiterHigh™ Sf or the respective competitor's medium, and were used to seed experiments conducted in 125 mL (50 mL working volume) disposable Erlenmeyer shake flasks (Corning). Initial cell density was 3×10^5 viable cells/mL for the growth assays. The productivity assays were infected with a Multiplicity of Infection (MOI) of 5 at a cell density of 1×10^6 viable cells/mL. All conditions were tested in duplicate. The cells were cultured in a ThermoForma incubator at 27 °C and 130 rpm shaker speed.

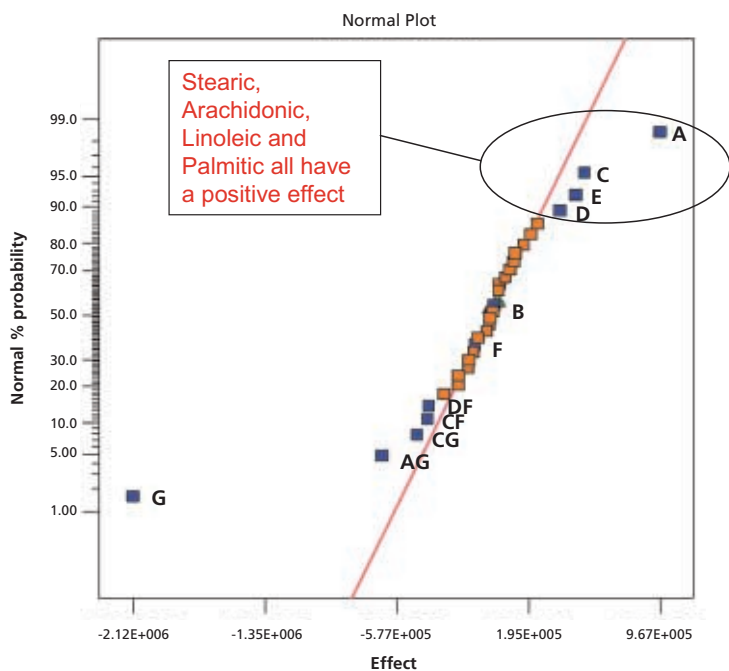
Spent medium samples were collected every day for the analysis of nutrients/metabolites. At the same time, total cell number was determined using a Schärfe System Casy 1® Model TTC and viability was assessed using the Trypan Blue Exclusion Method.

Quantification of Recombinant β -galactosidase

β -gal was quantified using Sigma's β -galactosidase Reporter Gene Activity Detection Kit (Product Code GAL-A). One-milliliter samples were collected every day and the cells were washed with HBSS (Product Code H 6648) after centrifugation. The cell lysates were diluted 1:2000.

Results

Design-Expert® – Normal Plot



DESIGN-EXPERT Plot
Response 1

- A: Stearic
- B: Oleic
- C: Arachidonic
- D: Linoleic
- E: Palmitic
- F: Palmitoleic
- G: Myristic/Lauric/Linolenic

Figure 1: Normal Plot of the relative effects of the lipids tested. This graph is a representation of the relative effects of the lipids tested in a factorial matrix designed experiment (2^{7-2} or $1/4$ matrix). The cell growth data from the experiment was analyzed in Design Expert to elucidate the effects of individual or combinations of fatty acids. The points that fall to the right of the line denote a positive response.

Growth Test – Sf21

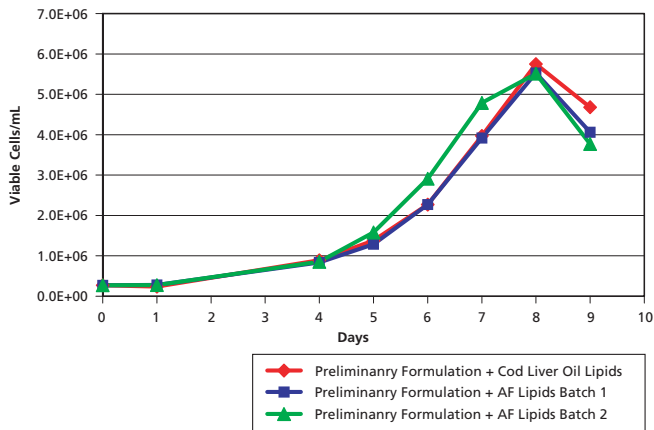


Figure 2: Comparison between the different lipid sources with Sf21 cells. The methyl ester fatty acids from cod liver oil were tested against two batches of the animal-free lipids in shaker cultures using a preliminary formulation of TiterHigh™ Sf Insect Medium. The conditions all preformed similarly.

Growth Comparison – Sf21

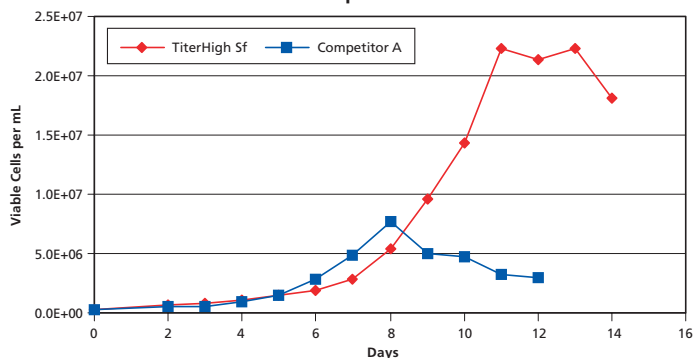


Figure 3: Comparison between TiterHigh™ Sf Insect Medium and the leading competitor for growth with the Sf21 cell line. In this experiment Sf21 cells, grown in their respective media, were seeded in Corning shake flasks (125 mL) at 3×10^5 viable cells/mL. The results show that TiterHigh™ Sf supports excellent cell growth.

Productivity Comparison – Sf21

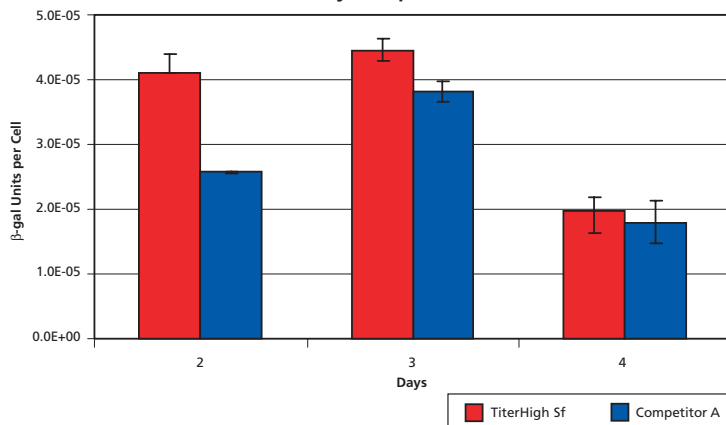


Figure 4: Comparison between TiterHigh™ Sf Insect Medium and the leading competitor for productivity with the Sf21 cell line. In this experiment Sf21 cells, grown in their respective media, were infected with a recombinant (β -gal) baculovirus at an MOI of 5 in Corning shake flasks (125 mL) at 1×10^6 viable cells/mL. The results show that TiterHigh™ Sf supports high level protein production.

Productivity Comparison – Sf9

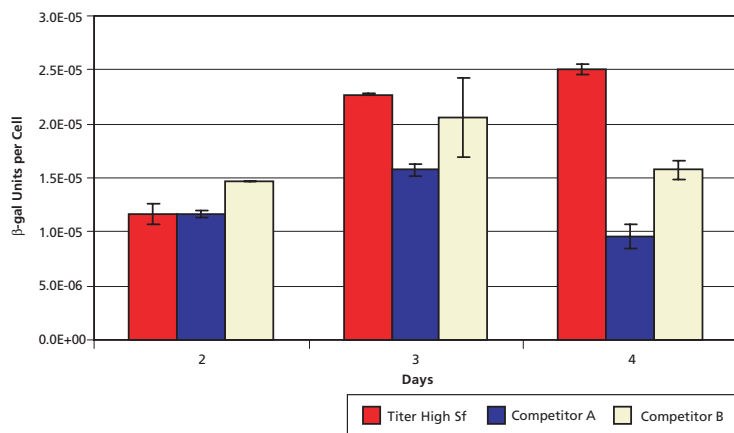


Figure 5: Comparison between TiterHigh™ Sf Insect Medium and the leading competitors for productivity with the Sf9 cell line. In this experiment Sf9 cells, grown in their respective media, were infected with a recombinant (β -gal) baculovirus at an MOI of 5 in Corning shake flasks (125 mL) at 1×10^6 viable cells/mL. The results show that TiterHigh™ Sf supports high level protein production.

Discussion

To generate an animal component-free insect cell culture medium, multiple modifications must be made to current formulations. Primarily, the methyl ester fatty acids from cod liver oil need to be exchanged with a defined lipid mixture. Since cod liver oil is an undefined material, experiments had to be conducted to establish which fatty acids

were critical for the Sf21 and Sf9 cell lines. In this study, a factorial matrix experiment was used to test multiple fatty acids at once. A full matrix experiment testing all of the chosen lipids in all of the various combinations would be 2^7 (128) conditions, but to simplify the experiment a 2^{7-2} (32 conditions) matrix was performed. This allows the researcher to run only $\frac{1}{4}$ of the possible conditions and at the same time keeps the loss of significant data to a minimum. The results (cell growth represented as cell-days) of this experiment were analyzed using Design-Expert® and are shown in Figure 1 as a normal probability plot. In this type of plot the “normal” or insignificant data points lie on a relatively straight line. Any points that do not fall on this line are deemed significant. The points can represent either a specific lipid or a combination of lipids that when used in conjunction have some type of interaction. Those that fall to the left of the line have negative effects, while those that fall to the right have positive effects. The normal plot indicates that stearic, arachidonic, linoleic, and palmitic acids all yielded positive effects on cell growth.

In a subsequent experiment, as depicted in Figure 2, a lipid mixture based on the results from Figure 1 was tested against the cod liver oil fatty acids in a preliminary formulation of TiterHigh™ Sf Insect Medium (Product Code I 5408). All conditions had very similar growth, indicating that the new animal-free lipid mixture is sufficient to sustain Sf21 and Sf9 cells. At this point the preliminary formulation of TiterHigh™ Sf was animal component-free and also supported levels of cell growth that were close to the level of the competition. Further optimization led to much improved cell growth.

Figure 3 demonstrates the excellent growth that is attainable in TiterHigh™ Sf Insect Medium with the Sf21 cell line in shaker culture. Figures 4 and 5 show that this new medium will also support high level recombinant protein production after baculoviral infection. Additionally, TiterHigh™ Sf will support baculovirus titers of greater than 10^8 PFU/mL (data not shown).

Conclusions

- Using factorial matrix experimental design, an animal-free substitute for the traditional insect medium fatty acid source was successfully developed.
- With the utilization of TiterHigh™ Sf Insect Medium (I 5408), regulatory concerns associated with the use of animal-derived components have been eliminated.
- TiterHigh™ Sf Insect Medium is one of the best commercially available media for growth with the Sf21 and Sf9 cell lines.
- TiterHigh™ Sf Insect Medium also supports excellent recombinant protein production using the Baculovirus Expression Vector System (BEVS).

Acknowledgements

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References

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