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The Development of IS Sf Insect – A New Medium Supporting High-Density Insect Cell Growth

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Introduction

Insect cells derived from *Spodoptera frugiperda* have been widely used with the baculovirus expression vector system (BEVS) for the production of biological products such as recombinant proteins, adeno-associated viruses (AAVs), and virus-like particles (VLPs) due to their ease of culture, scalability in high cell density suspension cultures, and high protein expression levels.

Traditionally, insect cells are cultured in an undefined medium containing yeast hydrolysate and cod liver oil; however, there is an increasing push to use chemically-defined, animal component-free (ACF) medium to minimize any potential contaminants and decrease lot-to-lot variability. This poster presents the development and application data of IS Sf Insect medium. It is a ready-to-use, serum-free, and animal component free (ACF) insect cell medium that supports high density cell growth and protein production while minimizing lot-to-lot variability. Utilizing a traditional formulation as a starting point, we first optimized multiple nutrient groups including amino acids, vitamins, and metals in the basal medium with the help of design of experiments software. Then, the animal-derived components in the starting formulation, cod liver oil and cholesterol, were replaced with chemically-defined lipids (CDL) developed by FUJIFILM Irvine Scientific.

Finally, yeast hydrolysate is a well-known, undefined component that is crucial for insect cell growth and productivity. To minimize lot-to-lot variability, the yeast hydrolysate concentration was significantly lowered, and multiple yeast hydrolysate sources were evaluated to determine the highest quality raw material. As a result, IS Sf Insect medium was developed that had improved growth performance and comparable productivity to widely used, commercially available, animal-derived media.

Methods

Cell Cultures

- Sf9 cells
- Seeding density: 0.8–1.0 x 10^6 cells/mL
- Vessel: 125 mL Thomson shake flasks
- Incubator settings: 27°C, 0% CO_2, no humidity
- Multiplicity of Infection: 0.1–1

Culture Analysis

- Multifactor design of experiments software: Design-Expert
- Viable cell density and cell viability: Beckman Coulter Vi-Cell XR
- Transfection efficiency: BD Biosciences FACSVerse
- Protein quantification: ELISA

Media Development Overview

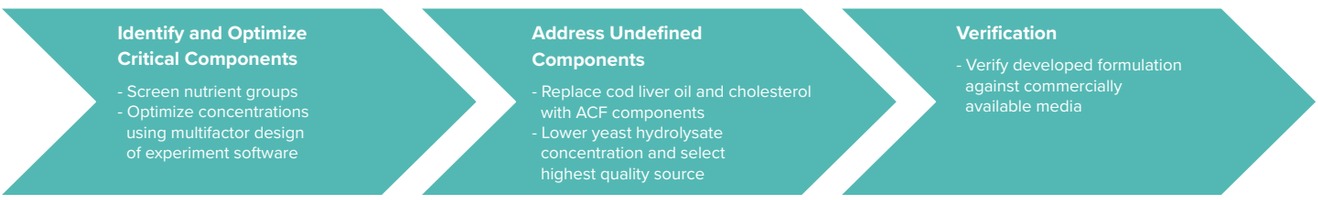

Figure 1. Flow chart of FUJIFILM Irvine Scientific's Media Development Overview for an ACF insect medium.

Results

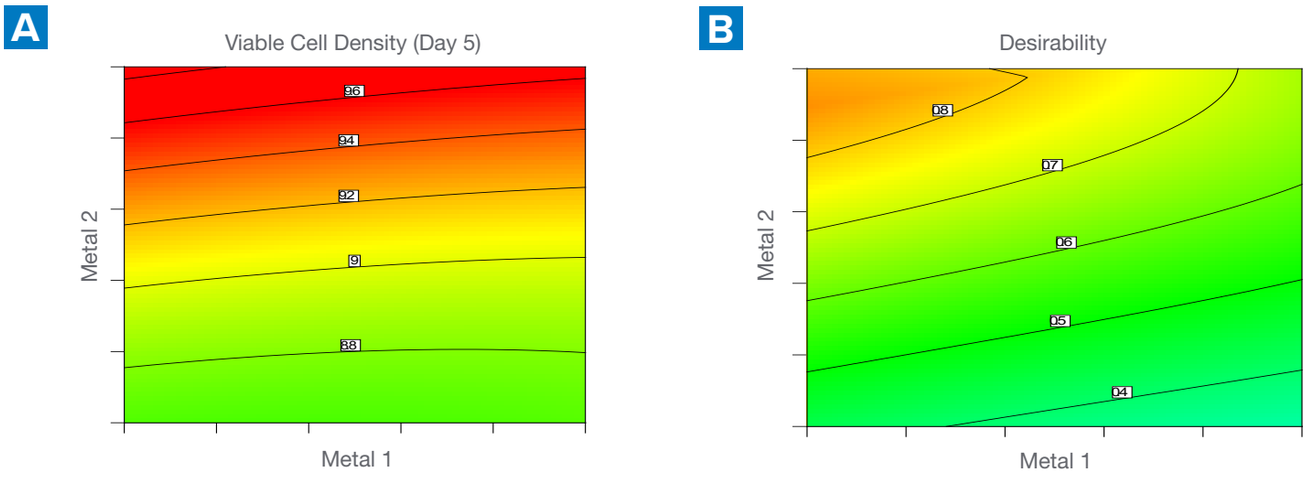

Figure 2. Contour plots of metal response surface design of experiment. Various nutrient groups were screened including amino acids, vitamins, and metals (data not shown) for cell growth. **(A)** Viable Cell Density and **(B)** Overall Desirability of Metal 1 and Metal 2.

Metal group was identified to have the most impact to cell growth

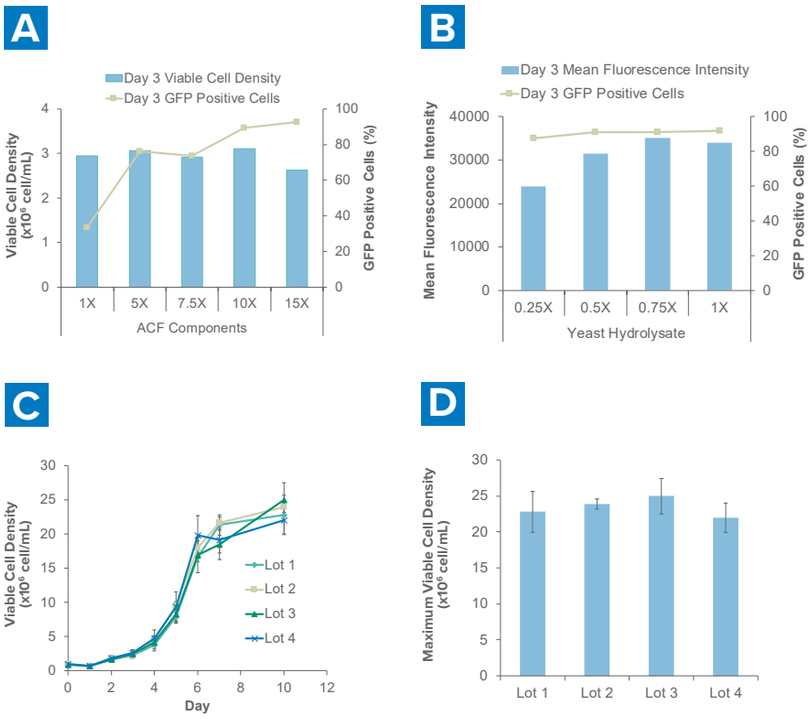

Figure 3. Optimization of ACF components and yeast hydrolysate. (A) Animal-derived components such as cod liver oil and cholesterol were replaced with ACF components and titrated. **(B)** Yeast hydrolysate was titrated to lower concentrations. Eight different yeast sources and multiple lots from each source were tested (data not shown). The **(C)** viable cell density and **(D)** max viable cell density of four different lots of the selected source for the final formulation are shown. Data shown represent mean ± standard deviation, n=4.

AD components were replaced by ACF components

Yeast Hydrolysate content was lowered to one-third of the benchmark medium

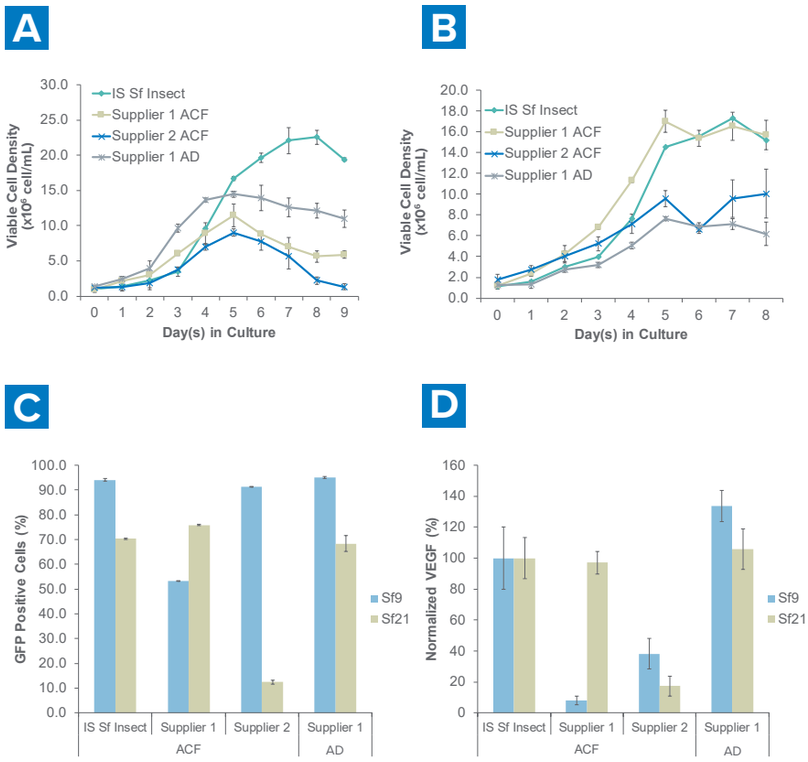

Figure 4. Verification of developed formulation against commercially available media. Sf9 **(A)** and Sf21 **(B)** cells were cultured in IS Sf Insect or commercially available media. Viable cell density was measured. Sf9 and Sf21 cells were infected with BEVS expressing Vascular Endothelial Growth Factor (VEGF) on Day 0 (Sf9:MOI=0.1, Sf21: MOI=0.5). Infection efficiencies were measured by the percentage of cells expressing GFP through flow cytometry on Day 3 post-infection **(C)**. The protein yields were measured by VEGF ELISA on Day 3 post-infection **(D)**; data shown in B were normalized against the average VEGF value expressed in IS Sf Insect. Data shown represent mean ± std deviation, n=4.

IS Sf Insect supports high-density insect cell growth and BEVS for both Sf9 and Sf21

Summary

- An ACF, low-hydrolysate, insect medium formulation was developed that performed better than commercially available ACF media and comparable to commercially available animal-derived medium
- The developed formulation supported high transfection efficiency at low multiplicity of infection
- By reducing the yeast hydrolysate, high cell density was consistently achieved, and lot-to-lot variation was significantly reduced

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