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Novel Cell-Ess ® supplement used as a feed or as an initial boost to CHO serum free media results in a significant increase in protein yield and production

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ABSTRACT

Many metrics, including metabolic profiles, have been used to analyze cell health and optimize productivity. In this study, we investigated the ability of a lipid supplement to increase protein yield. At a concentration of 1% (v/v) the lipid supplement caused a significant increase in protein titer (1118 ± 65.4 ng 10^5 cells^{-1} days^{-1}) when compared to cultures grown in the absence of supplementation (819.3 ± 38.1 ng 10^5 cells^{-1} days^{-1}; $p < 0.05$). This equated to a 37% increase in productivity. Furthermore, metabolic profiles of ammonia, glutamate, lactate, and glucose were not significantly altered by the polar lipid supplement. In a separate set of experiments, using the supplement as a feed resulted in 2 notable effects. The first was a 25% increase in protein titer. The second was an extension of peak protein production from 1 day to 2 days. These results suggest that lipid supplementation is a promising avenue for enhancing protein production. In addition, our results also suggest that an increase in protein production may not necessarily require a change in the metabolic state of the cells.

1. Introduction

Using proteins as therapies was ushered in during the early 1980's with the introduction of EPO. Since then, the biologics market has seen a significant increase in the number of products and the total revenue generated each year. The sales of biologics have surpassed $125 billion [1]. As the market has grown, there has been increased competition as numerous companies target therapeutics for the same indication as well as new indications. In addition, biosimilars, already popular in Europe, are growing rapidly in the United States and elsewhere. This creates competitive price pressure and the need for biotech companies to optimize their protein production processes.

Enhancing protein production of cultured cells is a common goal throughout the biomanufacturing industry. This commonly consists of an ongoing process of optimizing cell lines in serum free conditions in order to balance cell proliferation and protein production.

Serum free media have been optimized based on certain principles. The original serum free media was made by Ham in the 1960s [2]. It was based on adding the amino acids, vitamins, and salts needed for cells to grow. The same concept is generally used as a base media today but as technology has improved so have the methods of analysis. Assays testing metabolic byproducts and investigating proteomics are used today to better optimize a media for CHO cell lines [3–7]. The use rate of amino acids or glucose can be determined to identify the constituents rapidly used during a run and may need replenishment, usually in the form of a feed and or feeds. None of these assays screen for lipids or cholesterol. In fact, the typical metabolic analysis looking at the TCA cycle would not be able to predict a benefit or show the harm of not having lipids and cholesterol. It has been reported that essential fats can be beneficial for cell health and protein production [6,8–10].

Here we investigate the utility of a novel approach to optimization using Cell-Ess® as a feed or an initial supplement to show proof of principle of adding lipids to culture. Furthermore Cell-Ess is tested as a feed supplement and in a small bioreactor as a model for use in large scale bio-production and use in a single use bioreactor. In this report we hypothesize that adding lipids will benefit protein production and then set out a set of experiments to test the hypothesis.

2. Materials and methods

2.1. Cell culture

Suspension Chinese Hamster Ovary (CHO) cells expressing several different monoclonal antibodies (mAb) from multiple individual clones

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were grown in 50 mL suspension cultures for 7 days. The lipid supplement was administered using a dosing paradigm as indicated for each experiment. The effect on protein titer and common metabolic profiles were determined and analyzed.

For these experiments two different stably transfected mAb single isolated CHO cell clones were used. The CHO cells were either grown in SAFC (Sigma, St. Louis, MO) serum free media or FortiCHO (Thermo Life). CHO cells were thawed and expanded prior to seeding in shake flasks.

2.2. Supplement with Cell-Ess

Cells were seeded in shake flasks. Cell-Ess was given as a supplement independent of other supplements and in addition to normal feeds at increasing concentrations. Cell-Ess (Essential Pharma, Ewing, NJ) was either added at the beginning of culture for protein expression or added as a feed as indicated.

2.3. Protein analysis

The mAb titers were calculated using two different methods. For the GS0 cells grown in SAFC media the cells were purified on a protein A column and then quantified by in a spectrophotometer at a wavelength of 280. The mAb from the DG44 cells grown in FortiCHO were quantified by an antibody specific ELISA.

2.4. Metabolic analysis

Samples were taken at the indicated time points and frozen. At the end of the run the samples were thawed and run on the BioProfile Flex analyzer (Nova Biomedical, Waltham, MA).

3. Results

3.1. Addition of Cell-Ess® as a supplement significantly increases titer

Large-scale protein production has predominantly moved to serum free systems developed for CHO cell lines. Serum free media systems are made by multiple companies in the life science industry, like Thermo Fisher Scientific, one of the industry leaders. Thermo provides several brands of CHO cell media including but not limited to FortiCHO and OptiCHO. In theory the complete media systems are ready for use. Interestingly, even though there are readily available products, many biomanufacturers will make internal modifications to optimize protein production for a specific cell line suggesting genetic differences were introduced to each CHO cell clone. In this study we investigated if the addition of a lipid supplement, Cell-Ess®, would lead to an increase in protein production across different clones in two different genetic backgrounds. A multi-step approach was utilized where proof of principle was researched to test the hypothesis of the benefit of adding lipids in a simplified controlled model. The second step was to test a complex model for fed batch in shake flasks where there are multiple variables.

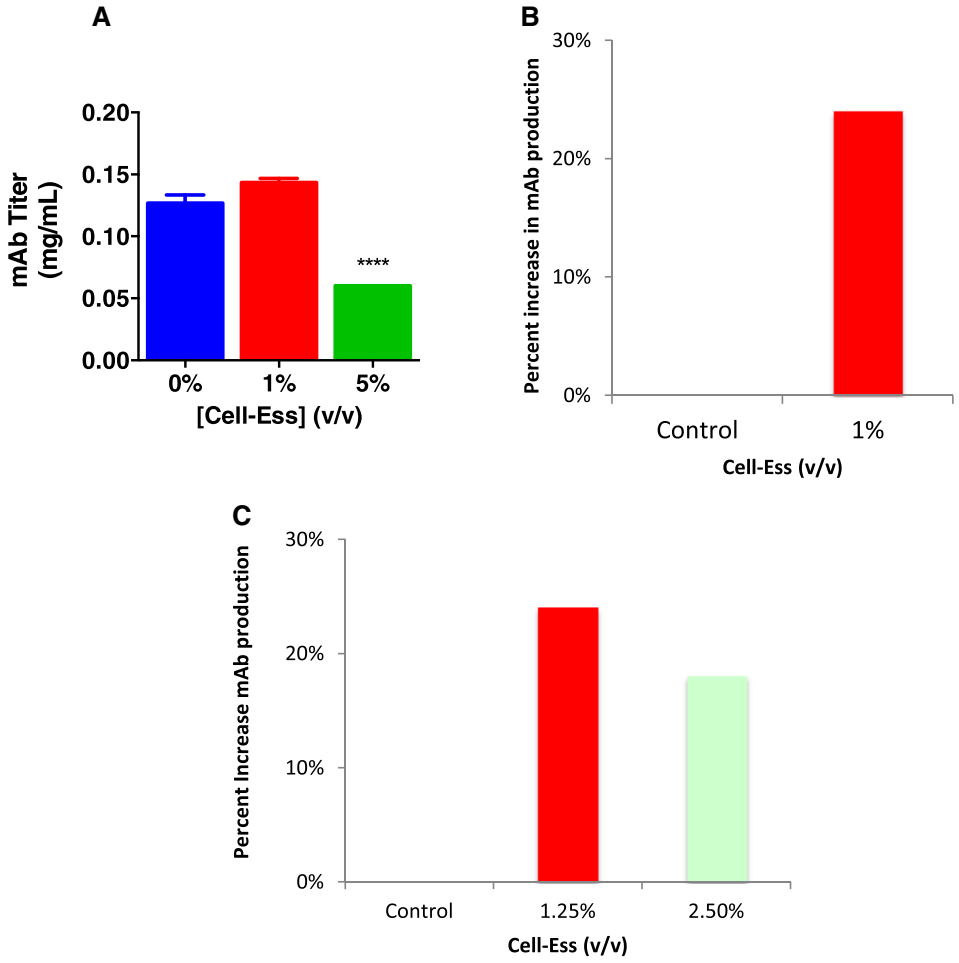

Fig. 1. Increased mAb titer with initial lipid supplement. The addition of Cell-Ess as an initial supplement in a short 7 day culture model was done in multiple media systems investigating the ability of the lipids supplement to increase mAb titers over control. In this figure the increase in titer due to the addition of Cell-Ess to SAFC media is shown in 1A and in 1B the increase is shown as a percent over control. In a similar experiment with addition of lipid at a different site with a different CHO clone the cells were grown in the Thermo media, Forti-CHO and shown as a percent increase over control.