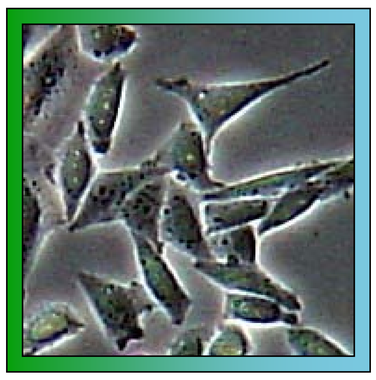


A Comparison of Performance Enhancing Synergy Among Ultrafiltered Yeast Extracts and Recombinant Human Serum Albumin in CHO-K1 Cells

Babcock, J.F.
Benedict, K.A., Perlman, A.L., and Wilcox, C.P.

Sheffield Center for Cell Culture Technology, Sheffield Bio-Science, A Kerry Group Business, Ithaca, NY USA

Introduction

We have previously demonstrated a synergistic reaction between a wheat hydrolysate and recombinant human serum albumin used to supplement a chemically defined growth medium for SP2/0 hybridoma cells. The data presented here illustrates the synergystic performance enhancing effect obtained when ultrafiltered yeast extract and recombinant human serum albumin are co-supplemented in CHO cell media. Each combination has its own distinctive effect on the growth and productivity of transfected cells. Cell viability, cell proliferation and target protein production all may be improved, yet these effects are not necessarily observed concurrently in a given system.

Materials and Methods

Data were collected using a transfected CHO-K1 line, adapted to serum-free suspension culture, and engineered to constitutively express secreted embryonic alkaline phosphatase (SEAP) by means of a modified human cytomegalovirus (hCMV) promoter.

Cultures were grown in 125 ml shake-flasks containing a final medium volume of 35 ml. The basal medium consisted of 100% chemically defined medium (CDM) supplemented with 1 mg/ml G-418. Triplicate flasks were seeded at 4.0 x10^5 cells/ml, and incubated at 37°C in 5% CO_2 at 130 rpm for 12 days. Medium supplement stock solutions were prepared at 100 g/l in the basal medium and sterilized through a 2.0 µm filter**.** Ultrafiltered yeast extracts used in these studies were Sheffield's HyPep™ YE and UltraPep™ YE. The rHSA is Sheffield rAlbumin ACF, an animal free, recombinant version of human serum albumin.

At days 5, 7, 8, 9, and 12, 1 ml of the culture supernatants were removed for assessing cell counts and viability. Cells were counted using a Nova BioProfile Flex automated analyzer. At Day 12, 1 ml of the culture supernatants were removed for SEAP analysis. Levels of SEAP in the supernatants were measured using anion-exchange HPLC.

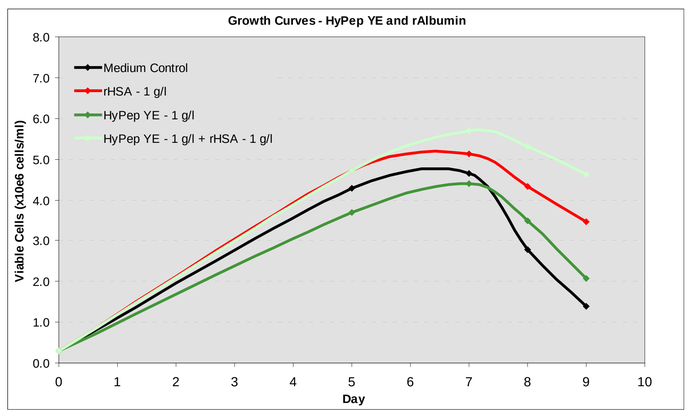


Figure 1: Growth Curves for rHSA and HyPep™ YE Supplemented Batch Cell Cultures

Maximum cell density increased with respect to the Medium Control when cultures were dosed with rHSA at 1 g/l, but not when supplemented with HyPep™ YE at 1 g/l. When both supplements were used together, an even greater increase in cell density was observed.

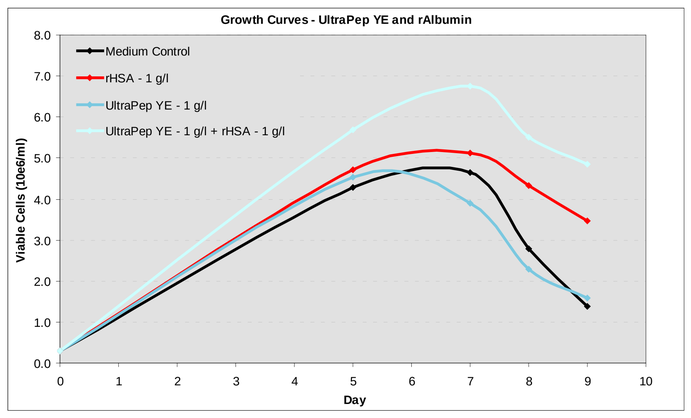


Figure 2: Growth Curves for rHSA and UltraPep™ YE Supplemented Batch Cell Cultures

The synergystic effect was also seen with rHSA and UltraPep™ YE. However, the UltraPep™ YE/rHSA combination out-performed the HyPep™ YE/rHSA with respect to maximum cell density.

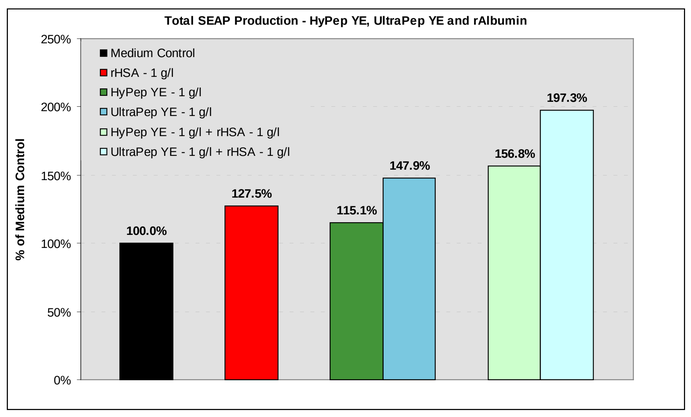


Figure 3: SEAP Titers for rHSA and YE Supplemented Cultures

All cultures were assayed for total SEAP production on Day 12. When dosed at 1g/l, all of the supplements (HyPep™ YE, UltraPep™ YE and rHSA) yielded higher titers than the Medium Control. The greatest increases were seen when HyPep™ YE or UltraPep™ YE were used in conjunction with rHSA.

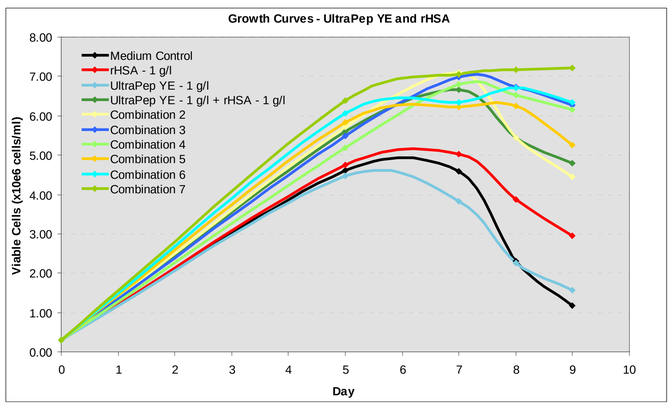


Figure 4: Growth Curves for rHSA and UltraPep™ YE Supplemented Batch Cell Cultures

When the dosage of either or both supplements were varied, the synergistic effect between rHSA and UltraPep™ YE was further enhanced. While all combinations provided a growth benefit, Combination 7 yielded the greatest improvement in maximum cell density.

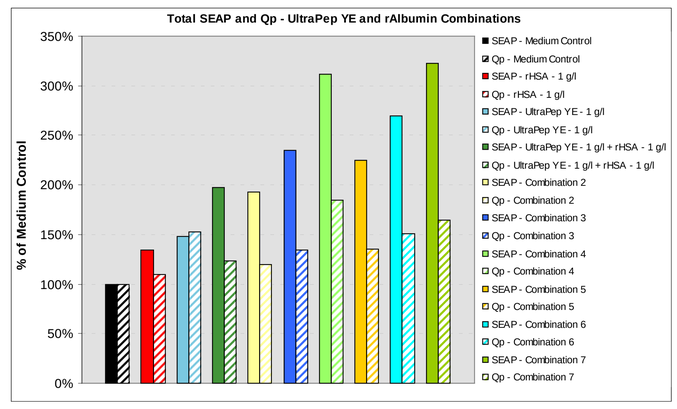
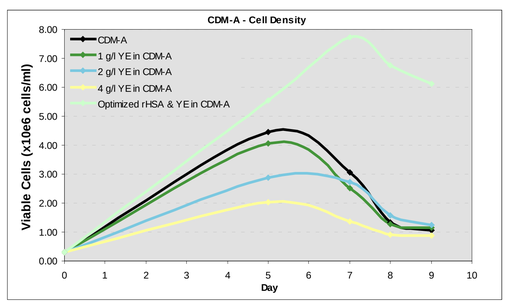


Figure 5: SEAP Titers and Specific Productivity (Qp) for rHSA and YE Supplemented Batch Cultures

Combination 7 not only provided the best growth benefit, it also yielded the greatest amount of SEAP. Combination 4 was second in both maximum cell density and SEAP production, but displayed the greatest specific productivity of all the samples.



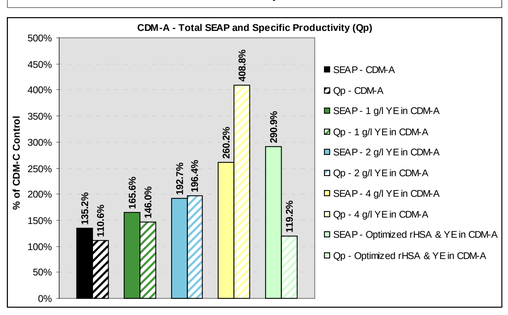
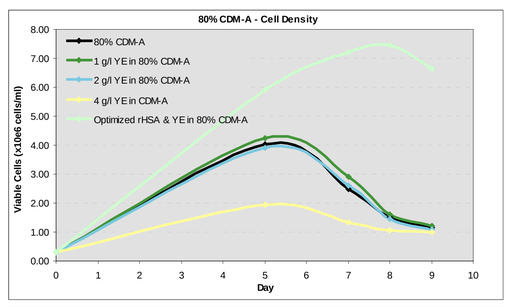


Figure 6: Dose-Response Growth Curves, SEAP Titers and Specific Productivity (Qp) for CDM-A

The best performing, or optimized, combination of dosages of rHSA and YE for overall performance was determined using CDM-A. Growth performance of cultures supplemented only with YE decreased with increasing dosage, while SEAP production and Qp increased in parallel with dosage. The optimized combination of rHSA and YE provided the best overall culture performance. In this medium, the optimized combination would be suitable for batch culture or as a supplement in the basal medium of a fed-batch system, while the 4 g/l YE dosage may be an excellent candidate as a feed supplement.



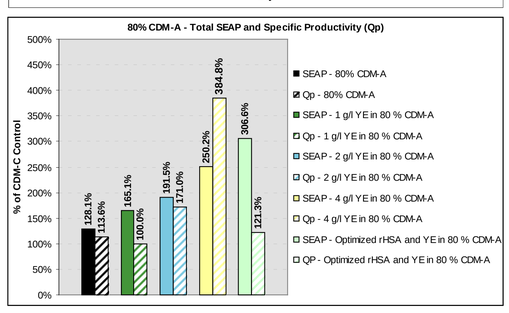
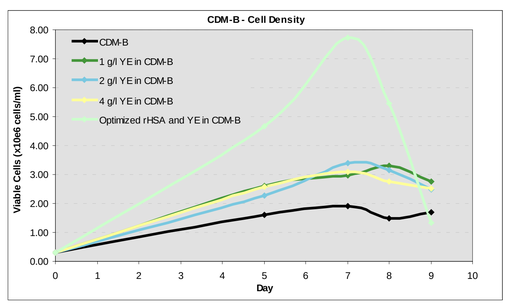


Figure 7: Dose-Response Growth Curves, SEAP Titers and Specific Productivity (Qp) for Diluted (80%) CDM-A

In the CDM-A diluted to 80% strength with PBS, performance of the optimized rHSA/YE combination was nearly identical to its performance in the full strength medium. In contrast with the undiluted medium (Fig. 6), the 1- and 2-g/l dosages of YE provided growth promotion comparable to the Medium Control. Again, at 4 g/l, growth was significantly reduced, but SEAP titer and Qp were vastly improved.

NOTE: *All SEAP and Specific Productivity data in Figures 6-9 are presented as a percent of the poorest performing, un-supplemented basal medium (CDM-C). This helps illustrate the relative performance of each separate medium and the unique effect of supplementation on each one.*



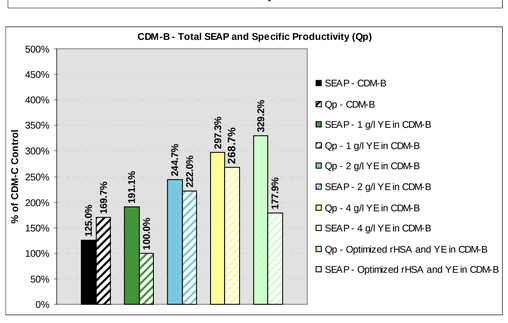
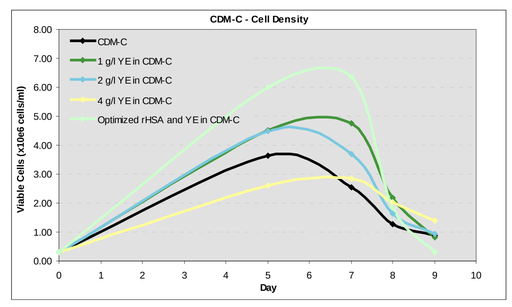


Figure 8: Dose-Response Growth Curves, SEAP Titers and Specific Productivity (Qp) for CDM-B

In CDM-B, growth with and without YE was relatively poor as compared with the other basal media. At all dosages, YE supplemented CDM-B displayed improved growth with respect to the Medium Control. Growth for the optimized rHSA/YE supplemented sample was comparable to results obtained with similarly supplemented CDM-A and 80% CDM-A. The optimized rHSA/YE combination once again yielded the best overall performance. Notably, in CDM-B the total SEAP and Qp obtained using the optimized rHSA/YE combination was highest for all media tested.



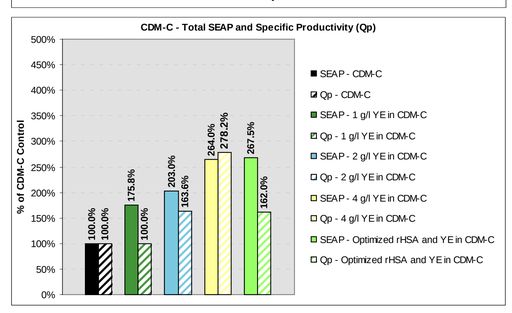


Figure 9: Dose-Response Growth Curves, SEAP Titers and Specific Productivity (Qp) for CDM-C

Cell growth was noticeably improved with respect to the CDM-C control when the medium was supplemented with 1- or 2-g/l of YE. At all dosages, CDM-C yielded the best growth response to YE supplementation as compared with the other basal media. The CDM-C showed the poorest response to the rHSA/YE combined supplement. Total SEAP production in the un-supplemented CDM-C medium was the poorest of all the basal media. However, supplementation of CDM-C improved SEAP titers to levels comparable to the other supplemented media.

Summary

The data presented here illustrate the performance-enhancing synergy that may be realized by supplementing various cell culture media with a combination of yeast extract and recombinant human serum albumin. When the two supplements are used together, cell culture performance results exceed those achieved when using each supplement individually. Overall performance was further improved by varying the individual dosages of YE and rHSA. In four separate basal media, cell response to co-supplementation for each of the yeast extract/recombinant albumin combinations tested was shown to be both medium and dosage dependent. The optimized combination provided significant overall performance improvement in all media tested.