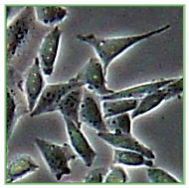

An inverse relationship between cell proliferation and target protein production in CHO-K1 cells cultivated in a protein hydrolysate-supplemented medium.

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Introduction

CHO-K1 cells were cultivated in a basal medium supplemented with protein hydrolysates of plant and animal origin. An inverse relationship became apparent when cell proliferation and target protein production in the hydrolysate-supplemented medium were compared with the control medium. If cell proliferation was enhanced by the hydrolysate supplement, target protein production was inhibited. If cell proliferation was inhibited, target protein production was enhanced. In all cases, cell viability was comparable or improved in the hydrolysate-supplemented media. The manifestation of this relationship was influenced by the raw materials used to manufacture each hydrolysate, ultra-filtration, as well as hydrolysate dosage. However, the inverse relationship was consistent across the range of treatments.

Materials and Methods

Sheffield™ Clone B.1 is a transfected CHO-K1 line engineered to constitutively express secreted embryonic alkaline phosphatase (SEAP) by means of a modified human cytomegalovirus (HCMV) promoter. Monolayers cultures were grown in six-well microplates containing a final medium volume of 3 ml/well. The basal medium consisted of 50% chemically defined medium (CDM) and 50% Ham's F12-K, 1 mg/ml G-418, supplemented with 5% FBS. Cultures were seeded at $1x10^5$ cells/well, and incubated at 37°C in 5% CO_2. After 5 days, 200 µl of the culture supernatants were removed for SEAP analysis. Levels of functional SEAP in the supernatants were measured using an absorbance-based activity assay. Cell monolayers were rinsed, trypsinized and neutralized for counting. 50 µl samples from replicate wells were removed for cell counts and viability assessment by trypan blue dye exclusion analysis.

Results

Figure 1: Comparison of CHO-K1 performance in a basal medium supplemented with two ultra-filtered animal-derived protein hydrolysates manufactured from dissimilar raw materials.

Figure 2: Comparison of CHO-K1 performance in media supplemented with a plant-derived protein hydrolysate manufactured from the same raw material, with and without ultra-filtration.

Figure 3: Comparison of CHO-K1 performance in a basal medium supplemented with an ultra-filtered plant-derived protein hydrolysate at two dosages.

Figure 4: Comparison of CHO-K1 performance in a basal medium supplemented with a third ultra-filtered plant-derived protein hydrolysate at two dosages.

Summary

Supplementation of culture media with protein hydrolysates can provide a number of benefits to cell culture systems. Cell viability, cell proliferation and target protein production all may be improved. However, these effects might not be observed concurrently in a given system. As each cell culture platform is unique, so are the protein hydrolysates used to supplement the culture medium. Each may have its own distinctive effect on the growth and productivity of transfected cells.

The results shown here demonstrate that a number of factors can influence the functional properties of an individual hydrolysate within one specific system. These factors include the raw materials used to manufacture the hydrolysate, the manner in which the hydrolysate is produced, and the dosage at which the hydrolysate is used as a medium supplement.

Further experiments will be required to determine whether the inverse relationship demonstrated here is a function of the cell culture system used in this investigation, or if this phenomenon occurs across a range of cell lines, expression systems, and culture platforms.

It is noteworthy that in this experimental system, the inverse relationship between cell proliferation and target protein production is more pronounced in media supplemented with plant-derived hydrolysates, as opposed to animal-derived hydrolysates. This suggests that when transitioning from animal- to plant-derived hydrolysate supplements, it may be necessary to use a variety of approaches when optimizing the culture medium.

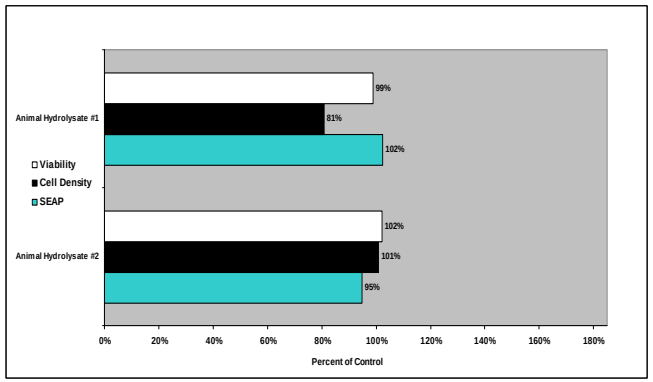

Figure 1. These results demonstrate the inverse relationship between cell proliferation and SEAP production, as well as the effect of raw materials on the relationship. With Animal Hydrolysate #1, cell proliferation is inhibited as compared with the control medium, while SEAP production is slightly enhanced. With Animal Hydrolysate #2, the relationship is less pronounced. Cell proliferation is nearly equivalent to the medium control, while SEAP production is slightly reduced. The two hydrolysates were manufactured from dissimilar raw materials of animal origin.

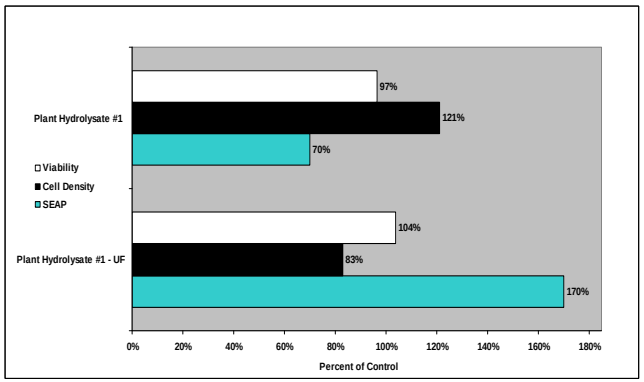

Figure 2. These data demonstrate the effect of ultra-filtration on the inverse relationship. With addition of the non-ultrafiltered version of Plant Hydrolysate #1, cell proliferation is enhanced, while SEAP production is inhibited when compared with the medium control. With the ultra-filtered version, cell proliferation is inhibited, while SEAP production is significantly enhanced. It is evident that ultra-filtration of this hydrolysate significantly alters its functional properties as a cell culture medium supplement.

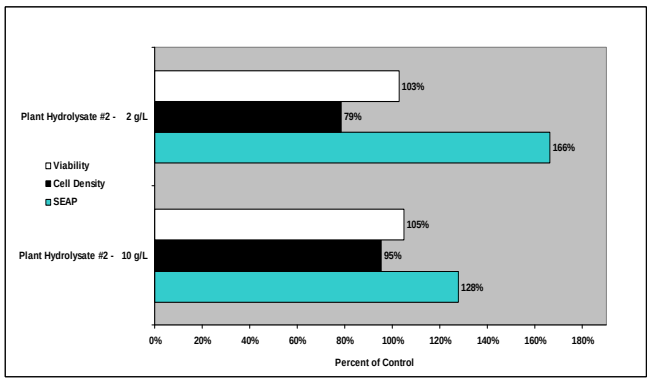

Figure 3. These data demonstrate the influence of hydrolysate dosage on the inverse relationship. This hydrolysate was manufactured from a different raw material than Plant Hydrolysate #1. At a dosage rate of 2g/L, cell proliferation is inhibited, while SEAP production is significantly enhanced. At 10g/L, the manifestation of the inverse relationship is less pronounced. Cell proliferation is only slightly inhibited, while SEAP production is considerably enhanced, though not to the degree as in the 2g/L dosage treatment.

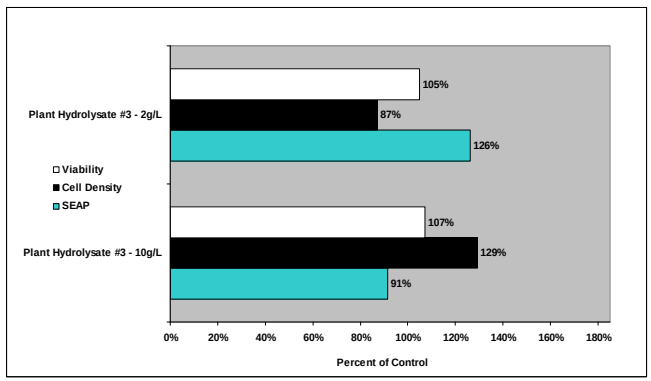

Figure 4. Dose-response of a third plant-derived protein hydrolysate. In this instance, increasing the hydrolysate dosage from 2g/L to 10g/L liter inverts the manifestation of the inverse relationship between cell proliferation and SEAP production. At 2g/L, cell proliferation is inhibited, while SEAP production is enhanced. At 10g/L, cell proliferation is enhanced, while SEAP production is inhibited. This result is a striking contrast to the dose-response effect demonstrated in Figure 3.