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Usability of size-excluded fractions of soy protein hydrolysates for growth and viability of Chinese hamster ovary cells in protein-free suspension culture

Bok-Hwan Chun, Jong-Hwang Kim, Ho-Joung Lee, Namhyun Chung *

College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Republic of Korea

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Abstract

To investigate the effect of size-excluded fraction of non-animal protein hydrolysate on growth, viability and longevity of Chinese hamster ovary (CHO) cells, several commercially available protein hydrolysates were evaluated as a feed supplement to chemically-defined protein-free suspension culture. Soy protein hydrolysates showed better supporting capability for cell growth and viability than the other types of hydrolysates. Maximal cell growth was not affected greatly by size exclusion of some soy hydrolysates such as bacto soytone and soy hydrolysates. CHO cells supplemented with size-excluded fractions of the two hydrolysates showed viable cell density and viability almost equal to those with their crude hydrolysates, although soy hydrolysates showed a little better performance. This suggested that the size-excluded hydrolysate fractions of some soy hydrolysate might be a potential culture medium additive to achieve better downstream operation in a large-scale production as well as enhanced productivity.

Keywords: Cell growth; Cell viability; CHO cells; Protein hydrolysate; Size exclusion

1. Introduction

The use of a typical animal-derived component, such as fetal bovine serum, for the production of human therapeutic proteins by mammalian cell culture may cause an infective risk by infectious viruses, mycoplasma, and prions (Kallel et al., 2002). Development of protein-free media (PFM) has successfully eliminated the use of animal-derived components, which was strongly discouraged by the regulatory authorities in Europe and the United States (Castle and Robertson, 1999). Currently, the trend of cell culture media formulations has migrated from PFM to chemically-defined medium (CDM). Although much effort has been made to develop CDM for various cell lines, nutritional requirements were generally known cell line-specific so that the use of non-optimized PFM and/or CDM often resulted in a decrease in cell growth, productivity, and product quality (Stoll et al., 1996).

Protein hydrolysates were commercially available from animal tissues, milk products, microorganisms, and plants and were known as a potential source of metabolizable materials including amino acids, oligopeptides, iron salts, some lipids, and trace elements (Franek et al., 2000; Gu et al., 1997; Martone et al., 2005). Several studies reported the hydrolysates as excellent nutrient supplements for the enhancement of cell growth, productivity, and product quality (Franek et al., 2000; Gu et al., 1997; Heidemann et al., 2000; Jan et al., 1994; Martone et al., 2005; Schlaeger, 1996; Sung et al., 2004; Zhang et al., 1994).

Recently, focus on the use of protein hydrolysates in PFM has switched over from crude protein hydrolysates to ultrafiltered non-animal (especially, plant) protein hydrolysates. This is because some fractions of the ultrafiltrates have exerted many positive effects on cultured animal

* Corresponding author. Tel.: +82 2 3290 3026; fax: +82 2 953 0737.
E-mail address: nchung@korea.ac.kr (N. Chung).

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cells (Franek et al., 2000; Sung et al., 2004) and also because limited presence of high molecular weight proteins might provide a better purification processing.

The aim of this study was to assess cell growth-supporting efficiency of various non-animal hydrolysates and their ultrafiltrates, which were added as a nutrient supplement for suspension culture of Chinese hamster ovary (CHO) cells.

2. Methods

2.1. Cell line and cell culture

A CHO cell line (CHO DG44/*dhfr*-/-) was grown in Minimal Essential Medium alpha modification (Sigma, USA) with 5% (v/v) dialyzed fetal bovine serum (Sigma, USA) and was adapted directly to suspension growth in chemically-defined protein-free CHO medium (HyQ®CDM4-CHO, Hyclone, USA). For maintenance, cells were seeded at a concentration of 5×10^5 cells/ml in fresh medium every 3 days in 250 ml Erlenmeyer flasks (Corning Inc., USA) when cell density reached about $2–3 \times 10^6$ cells/ml. The flasks were placed in a shaker with an agitation rate of 120 rpm and maintained in a 37 °C incubator with a 5% CO_2 overlay. The CDM, which contained no animal-derived components, was specifically developed for growth of CHO cells in a suspension culture. The cells showing exponential growth in 500 ml spinner flasks (Corning Inc., USA) were used as seed. The osmolality was controlled at 320 ± 10 m Osm/kg water with NaCl. Samples were taken everyday for cell counting, and cell-free samples were stored at −20 °C for other analyses.

2.2. Protein hydrolysates

Non-animal-derived protein hydrolysates in this experiment were generously provided by Beckton Dickinson Co., Hyclone, JRH Biosciences, and Sigma Chemical Co. (Table 1). All hydrolysate concentrates (20%, w/v) were prepared in deionized distilled water. The size-excluded fractions of ultrafiltrates of hydrolysates were prepared from the hydrolysate concentrates using Centricon® Plus Filter units (nominal molecular weight limits of 10,000, 5000 and 3000 Da; Millipore Co., USA). The ultrafiltrates were filtered finally through a 0.2 μm Durapore® filter (Millipore Co., USA). To assess the growth-promoting activity of each hydrolysate solution, cell cultures were inoculated at a viable cell concentration of 5×10^5 cells/ml. The cultures were conducted in triplicate. Addition of a hydrolysate concentrate solution to 100 ml of cell culture corresponded to 1–3% dilution of the growth culture.

2.3. Analytical methods

During cultivation, samples were taken periodically from cell culture for measurement of cell growth and metabolites. Viable cell concentration was determined by a dye exclusion method using Trypan blue. Concentrations of glucose, lactate, glutamine, and glutamate were measured off-line using a Biochemical Analyzer (Model 7100, Yellow Springs Instruments Inc., USA). Osmolality was measured using an osmometer (Vapro 5520, Wescor Inc., USA).

Table 1
Types of protein hydrolysates used

Vendors	Hydrolysate type	Abbreviations
Becton Dickinson	Phytone peptone	PPb
	TC Yeastolate	TCYb
	Yeast extract UF	YEUFb
	Select soytone	SSb
	Bacto soytone	BSb
Hyclone	Soy hydrolysate	SHh
	Wheat gluten hydrolysate	WGHh
	Yeast extract	YEh
	HyQ soy hydrolysate UF	SHUFh
JRH biosciences	Soy hydrolysate UF	SHUFj
Sigma	Hy-Soy UF	HSUFs
	Wheat-rice hydrolysate	WRHs
	Wheat hydrolysate	WHs
	Yeast hydrolysate	YHs
	Rice hydrolysate	RHs
	Soy hydrolysate	SHs

3. Results

3.1. Effect of hydrolysate type on cell growth

The effect of hydrolysate type was investigated on suspension culture of CHO DG44. Each hydrolysate was added to culture medium of CHO cells in CDM to compare cell growth (i.e., maximal cell density and growth rate) (Fig. 1). The extent of maximal cell density in batch culture ranged from 106% to 144% of the control without hydrolysate. The extent of specific growth rate ranged from 99% to

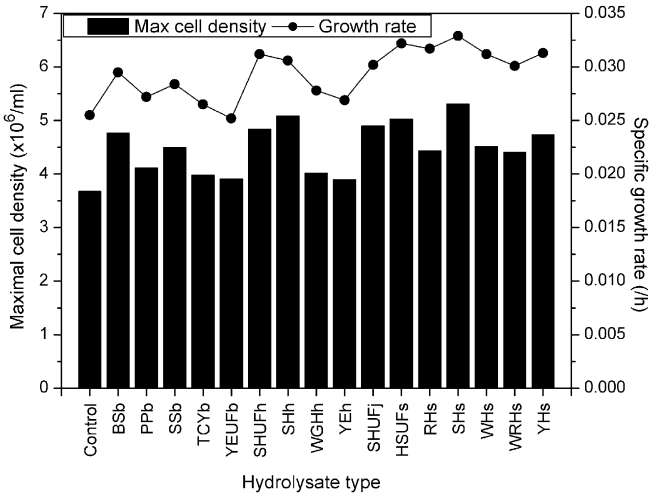

Fig. 1. Effect of protein hydrolysate types on cell growth in protein-free suspension culture. Each hydrolysate was added at a concentration of 4 g/l to the initial cell cultures. All abbreviations are shown in Table 1.