Non-animal Peptone for Serum Free Cultivation of Recombinant Mammalian and Animal Cells

Asif Durrani (Corresponding author), Abrar Ali, Shabbir Durrani, Jani.Basha Shaikh, A.Upadhyay & Z.H.Khan

Hislop School of Biotechnology, Biochemistry Department Nagpur, India

Tel: 96-653-419-5201 E-mail: durraniasif1@rediffmail.com

Abstract

Gene expression by large-scale transfection of mammalian and animal cells is becoming an established technology for the fast production of therapeutic biotech products. However, efforts are still needed to optimize production parameters in order to maximize volumetric productivity while maintaining product quality.

Generally, in cell culture 5% to 15% serum is used as supplement in the standard basal media in addition to salts and amino acids for healthy cell growth. To overcome the serum related problems, serum derived and animal derived substances are being used. The serum, serum derived or animal derived substances have some advantages and disadvantages which we have summarized in this review. This article mainly focuses on the replacement of serum, serum derived or animal derived substance with plant derived peptones to fulfill the regulatory requirements.

In addition, this article also highlights the methods of peptone selection, peptone screening and some issues related to peptone utilization and their solutions.

On the basis of review, we found that the usage of ultrafiltered plant peptone in combination with polyamine could be the best composition for cells growth and reproducible expression of the therapeutic Bio- products. The source or type of peptone (soy peptone, wheat and rice peptone) and polyamines (cadaverine, putrescine, spermidine, spermine, agmatine and ornithine etc) are cell dependent and can be selected on the basis of their performance with respect to cell growth, expression of protein of interest with consistency in the production process.

Keywords: Mammalian cell culture, Serum alternatives, Animal peptone, Non-animal peptone

Gene expression by large-scale transfection of mammalian and animal cells is becoming an established technology for the fast production of milligram and even gram amounts of recombinant proteins. However, efforts are still needed to optimize the production parameters in order to maximize volumetric productivities while maintaining product quality (P.L.Pham et al.2005, Grillberger et al.2008,)

Generally, in cell culture 5% to 15% serum is used as supplement in the standard basal media in addition to salts and amino acids for healthy cell growth (Price Paul et al. 2004; James Babcock et al.2007; Tian Chen et al.2003; Hubertus Stockinger et al.1991). Serum is a non-physiological fluid for the cells and could be derived from different sources including newborn calf, horse and human. Serum provides carriers or chelators for labile or water-insoluble nutrients. It binds and neutralizes toxic moieties, provide hormones, growth factors and protease inhibitors. It facilitate the attachment and spreading of the cells. Further it also provides unidentified or undefined low molecular weight nutrients which protect the cells from physical stress and mechanical damage (Kjell Bertheussen et al.2001; James Babcock et al.2007; Tian Chen et al.2003, Masami Mochizuki et al.2009).

In addition of the above advantages, use of serum has some drawbacks; it is not a reproducible and fixed material, because it is derived from the different animal which grows under different climatic conditions. Significant batch to batch variations interfere with the reproducibility of cell culture processes, which also interference in scientific experiments and in production process. Since the blood stream constitutes a transport system for nutrients and waste products, all kinds of waste products from the body's food intake and metabolism is present to the cultured cells which interfere in the purification of the biotech products. As an example, epithelial cell lines and of myeloma lines and their hybrids of the hybridoma type, the presence of serum or of unknown or poorly defined components created difficulty to purify the protein of interest (Chessebeuf et al., Merten OW 1999, James Babcock et al.2007). In addition, the non-define composition of the medium leads to prohibition of the human utilization of these biological products by the international and national health

authorities like Food and Drug Administration (FDA) and Ministry of Health etc. (Tian Chen et al.2003; Willaim.G.taylor et al.1974; Masami Mochizuki et al.2009; Maik W. Jornitz 2009).

To overcome from the above limitations of the serum, various serum derived substance such as serum albumin, serum transferrin, serum insulin, purified growth factors, lipoproteins, insulin like growth factors (Stephen L. Smith et al.1997) were used for the growth of the mammalian cells. The use of serum derived subtances also carries the risk of presence of unwanted agents {mycoplasma, viruses, virus like particles, Transmissible spongiform encephalopathy agent (TSE), Bovine spongiform encephalopathy agent(BSE)} which can contaminate the cell culture and it may transmitted to the products (Merten OW 1999; Merten OW 2002; Hubertus Stockinger et al 1991; Masami Mochizuki et al 2009). Once serum and serum derives used in cell culture then, there is need to test both cell banks and its product for all the possible viruses, including Hepatitis and Human immunodeficiency virus (HIV). Moreover use of serum and serum derives bear the risk of BSE (Bovine spongiform encephalopathy agent), TSE (Transmissible spongiform encephalopathy agent) and Prion contamination (Stephen L.Smith et al 1997; Grillberger et al 2008; James Babcock et al 2007; Masami Mochizuki et al 2009).

Peptone is one of the good supplements and replacement of serum for the growth of the mammalian cells and production of the biological products. The benefits of peptone supplementation in cell culture applications have been well documented for many years as it contains low molecular weight nutrients, peptides and free amino acids which are good for cell culture. (BD Bionutrients technical manual third addition; James Babcock et al 2007; A. Bernard et al 1999; Merten OW 1999; P.L.Pham et al 2005; Julio Giron-Calle et al 2008; Masaru Shiratori 2007; Masami Mochizuki et al 2009; D. C-H. Jan et al 1994).

Since the nutritional requirements for each cell line are different, so it is very important to identify a peptone that meets the unique requirements of a particular cell line (BD Bionutrients technical manual 3rd addition; Julio Giron-Calle et al 2008).

1. Peptone Selection

There are number of commercially available peptones (animal and plant and yeast derived), it is critical to evaluate the best suited peptone for the healthy growth of the cells of interest and expression of product of interest. The composition of each peptone may vary from one manufacturer to another. Even the multiple lots of peptones produced using the same starting material may also have the different composition which can behave differently in cell culture. That is why selection of the most suitable peptone (to particular cell line) is very important factor (BD Bionutrients technical manual, 3rd addition; James Babcock et al 2007; Price Paul et al 2004; Grillberger et al 2006). While screening for the best suited peptone there is need to consider the current regulation.

In general, the animal-derived peptone shows good performance (Hubertus Stockinger et al 1991; James Wilkins et al 2009; Acumedia 2006). But the use of animal derived peptone will have the risk of the introduction of viruses and virus like particles, so it is necessary to exclude them from the list. This will eliminate the risk and also fulfill the regulatory requirements. If the Endotoxin level is a concern in the process, the screening should be limited to ultra-filtered (UF) peptones only. If the cell line utilizes the Glutamate Synthetase (GS) expression system, wheat peptone to be excluded from the list because it is rich in glutamine and glutamine is toxic to the cells (BD Bionutrients technical manual 3rd addition; Grillberger et.al 2006; PricePaul et.al 2004).

As the use of animal derived peptone posses the risk of contamination of viruses and virus like particles, in order to satisfy the regulatory agencies there is need to replace animal derived peptone with non-animal derived peptone. (Price Paul et al 2000; Kenerson et al 2005 ; Masami Mochizuki et al 2009 ; Price Paul et al 2006).

2. Peptone screening

Paul Price et al (United States Patent 20040171152) used Hydrolysates of wheat gluten (HYPEP 4301 1, "Wheat Hydrolysate 1", and HYPEP 8382, "Wheat Hydrolysate 2"), soy (HY-SOY) and rice (HYPEP 5115), as well as an extract of baker's yeast (HY-YEST 444), Quest International (Norwich, N.Y.) to find out the most suited peptone for growing *Vero cells.* The results obtained in the above study indicate that the use of wheat peptides or hydrolysates is not suitable for the cultivation of *Vero cells* and it should be eliminated from the list of non-animal derived peptone. At the same time the rice peptone gave the good cell number, which indicates that this peptone is suitable for this cell line (Table.1). Both Soya and rice peptone gave approximate same cell number with slight different in relative growth efficiency the further selection of peptone should be based on the expression of product of interest.

There are some reports, where it has been reported that there is a performance variation in the peptone lots which is due to the season affect (Fig1). This variation may affect the consistency of the mammalian cell culture performance and production of the protein of interest. The variation affects can be overcome by subjecting to ultrafiltration or addition of polyamines to the peptone containing media Fig.2. (Paul Price 2004; Masaru Shiratori 2007; Masami Mochizuki et al 2009; James. A. Roszell et al 1977; J. J Harada et al 1981; Weiss T.S et al 2002; Hiroshi Yoda et al 2006' Toshiya Takeda et al 2002; N. Seiler et al 1984; Shewan M.aziz et al 1995; K. M. Milam et al 2008, M. Louise Higgins et al 2005,William Galston, et al 2003).

Polyamines are composed of nitrogen, carbon, hydrogen and contain two or more amino groups. It can control the DNA and RNA synthesis, cell proliferation, cell differentiation, membrane protection and DNA damage.Cadaverine, putrescine, spermidine, spermine, agmatine and ornithine are common polyamines used in the cell culture.

The Ultra filtration (UF) is a membrane filtration process used to separate or concentrate constituents of protein solutions based on molecular weight by using a 10k Dalton Molecular Weight Cut Off (MWCO) membrane. The retentate of the membrane contains molecules over 10kDa MW (BD Bionutrients technical manual 3rd addition; Grillberger et al 2006) which may include fats, larger MW polypeptides and proteins, and permeate contains salts, sugars, peptides, smaller polypeptides and other compounds of less than 10 kDa MW.

The peptone purified by Ultra filtration are pure than not ultrafiltered peptone and different lots of the same kind of peptone shows consistency with respect to performance (Fig.3) further the performance of Ultrafiltered peptone is higher than not ultrafiltered peptone (Fig.4). (James Babcock, et al.2007, BD Bionutrients technical manual third addition; Grillberger et al 2006; Price et al 2000 and Pual Price et al 2004).

3. Conclusion

Historically, serum was used for the mammalian cell culture as one of the main component. As the time passed awareness of major side affect of animal source material forced to replace the serum, its derivatives and switched to the serum free media, in serum free media peptone was the one component which was basically derived from enzymatic digestion of animal tissues.

Due to the tight regulation from the regulatory agency it was impossible to continue the use of animal derived peptone which forced the manufacturing of animal free peptone from various plant sources.

Non-animal peptones, more specifically ultrafiltered non-animal peptone in combination with polyamines are the most preferred, because it is free from all above problems. Some work has been done to select the best source of non-animal peptone for some mammalian cells, one kind of peptone is not good for all kinds of mammalian cells so there is great scope to find out which is good peptone for a specific cell line before entering in to commercial manufacturing of biopharmaceuticals.

References

Anderson, D.V., Tucker, E.M., Powell, J.R. and Porter, P. (1987). Bovine monoclonal antibodies to the F5 (K99) pilus antigen of *E.coli* produced by murine/bovine hybridomas. *Veterinary Immunology and Immunopathology,* 15, *223-237.*

A. Bernard et al. (1999). The use of peptones as media additives for high density perfusion cultures of animal Cells. *Animal Cell Technology: Products from Cells, Cells as Products*, 195–197.

Advanced Bioprocessing. *BD Bionutrients technical manual* (3rd revised edition), 8-20. [Online] Avalable: http://www.bd.com/ds/technicalCenter/misc/br_3_2547.pdf

Andre Siemensma et al. (2002). Non–Animal-Derived Protein Hydrolysates May Bridge Gap. *Genetic engineering news*, Vol 22, No. 12 June15. [Online]Available w w w.g e n e n g n e w s.c o m

BD Product Reference. Bitek-Peptone (Bactopeptone), 433-434. [Online] Available: www.bd.com/ds/technicalCenter/inserts/Bacto_Proteose_Peptone.pdf.

Bok-Hwan Chun et al. (2007). Usability of size-excluded fractions of soy protein hydrolysates for growth and viability of Chinese hamster ovary cells in protein-free suspension culture. *Bioresource Technology, 98(5)*, 1000-1005.

Product guide. (2006). Media Ingredients, Peptones and Hydrolysates. *ACUMEDIA*. [Online] Available: *http://www.neogen.com/acumedia/pdf/mediaingredients.pdf*

Chessebeuf, Martina L. (Dijon, FR) Padieu, Prudent H. (Chevigny St Sauveur, FR) et al. (1988). Serum-free animal cell culture medium and methods for the primary culture and production of cell lines using this medium. *United States Patent, 4786599*. [Online] Available http://www.freepatentsonline.com/4786599.htm.

Coleman WH, Roberts WK. (1982). Inhibitors of animal cell free protein synthesis from grain. *Biochim.Biophys*, Acta, 696, 239-244.

D. C-H. Jan et al. (1994). Peptone, a low-cost growth-promoting nutrient for intensive animal cell culture. *Cytotechnology*, Volume 16, Number 1.

Grillberger et al. (2008). Animal protein free media for the cultivation of cells. *freePatent online United States Patent* 20080064105.

Grillberger, Leopold (Vienna, AU), Reiter, Manfred (Vienna, AU) et al. (2006). Animal protein-free media for cultivation of cells. *United States Patent 20060094104.*

Hiroshi Yoda et al (2006). Polyamine Oxidase Is One of the Key Elements for Oxidative Burst to Induce Programmed Cell Death in Tobacco Cultured Cells. *Plant Physiology,* 142:193-206.

Hubertus stockinger et al. (1991). Serum free culture medium for mammalian cells. *Freepatentonline, United state Patent 5063157.*

J J Harada, et al. (1981). Cell cycle parameters of Chinese hamster ovary cells during exponential, polyamine-limited growth. *Mol Cell Biol*, 1(7):594–599(Abstract).

JameAs.Rosz et al. (1977). Polyamine-stimulated growth of cultured rat urinary bladder epithelial cells. *CANCER RESEARCH,* 37, 239-243.

James Babcock et al. (2007). Enhancing performance in cell culture. *Genetic engineering & Biotechnology news*, 27(20) Nov15. [Online] Available: www.genengnews.com.

James Wilkins(san Francisco, Shiratori masaruken (Burlingame) et al. (2009). Biologically active C-terminal arginine-containing peptides. *FreePatentsonline, Patent application no. 20090143248.* [Online] Avalable: www.freepatentsonline.com.

Julio Giron-Calle et al. (2008). Chickpea protein hydrolysate as a substitute for serum in cell culture. *Cytotechnology*, Vol. 57(3).

K. M. Milam Milam KM, Deen DF, Marton LJ. (2008). Cell proliferation and polyamine metabolism in 9L cells treated with (2R,5R)-6-heptyne-2,5-diamine or α-difluoromethylornithine. *Cell Proliferation*, 22(3), 269 – 277 (Abstract).

Kjell Bertheussen et al. (2001). Serum-free cell culture media. Application No. 878176 filed on 2001-06-12 *Patent storm*, *Patent No 6833271*.

Leda dos Reis Castilho. (2008). Animal cell technology: from biopharmaceuticals to gene therapy. *Google Hand book,* Francis e-library.

Manfred Reiter, Mundt, Wolfgang (Vienna, AT), Dorner, Friedrich (Vienna, AT) et al. (2000). Recombinant cell clone having increased stability in serum and protein free medium and a method of recovering the stable cell clone and the production of recombinant proteins by using a stable cell clone. *freePatents online United States Patent* No.6100061.

Masami Mochizuki et al. (2009). Feline Cell Capable of Being Cultured Without Animal-Derived Protein, and Method for producing virus and method for producing vaccine using thereof. *Paentdocs*, *IPC8 Class: AA61K3912FI.*

Masaru Shiratori Ph.D. (2007). Fractionation and Characterization of a Peptone Used in CHO Cell Culture Processes. *Cell culture development work shop CHI* (Cambridge healthtech institute), 17-18.

Meat peptone (Bactopeptone) "*BD Bionutrients technical manual*", Page 34 [Online] Available www.bd.com/ds/technicalCenter.

Merten OW. (1999). Safety issues of animal products used in serum-free media. *PMID:10404888[PUBMed*-indexed for MEDLINE].

Merten OW. (2002). Development of serum-free media for cell growth and production of viruses/viral vaccines: Safety issues of animal products used in serum-free media. *INIST-CNRS, Cote INIST*: 13557, 35400010413764.0300.

M. Louise Higgins. (2005). The effect of polyamines on cell culture cells. *Journal of Cellular Physiology,* Volume 74(2), 149 – 154.

Maik W. Jornitz. (2009). Mycoplasma Contamination in Culture Media. *engineering & Biotechnology news,* Vol 29, No.6 Mar15.

N. Seiler et al. (1984). Polyamines and the development of isolated neurons in cell culture. *Neurochemical Research,* 9, 871-886 (Abstract).

P.L. Pham, S. Perret, B. Cass, E.Carpentier, G. St-Laurent, L. Bisson, A. Kamen, Y. Durocher et al. (2005). Transient gene expression in HEK293 cells: Peptone addition posttransfection improves recombinant protein synthesis. *Wiley interscience,Biotechnology and Bioengineering* , 90(3), 332 – 344.

Price Paul et al. (2000). Animal cell culture media comprising peptides derived from rice. *FreePatentsonline, United States Patent No.6103529*. [Online] Available: www.freepatentsonline.com.

Price Paul (Grand Island, NY, US), Gorfien, Steve (Williamsville, NY, US) et al. (2004). Animal cell culture media comprising non-animal or plant-derived nutrients. *FreePatentsonline, United States Patent 20040171152*. [Online] Available: www.freepatentsonline.com.

Reiter, Manfred et al. (2006). Recombinant cell clones having increased stability and methods of making and using the same. *PatentStorm US Patent 7094574*.

Subramanian Ganapathy. (2007). Bioseparation and bioprocessing. *Google Hand book*, Page 74-76 Published by Taylor & Francis e-library.

Stephen L. Smith, Xiaoying Qiao, Susan M. Maciukas et al. (1997). Preparation of serum-free suspensions of human hematopoietic cells or precursor cells. *Patent storm, Application No. 972986* filed on 1997-11-19.

Sadettin S. Ozturk,Wei-Shou Hu. (2006). Cell culture technology for pharmaceutical and cell-based Therapies. *Google Hand book* Page 67-72 Published by Taylor & Francis e-library.

Stacy holdread Stacy Holdread, Cindy Hunt, Perry Haaland, Bryce Chaney, Warren Porter, Mohammad Heidaran and Jon Wannlund. (2005). Advances in media optimization: Two automated approaches that increase expression while reducing development time. *Animal Cell Technology meets Genomics*, 561-563. [Online] Available http://www.springerlink.com/content/q0375685700k2316/

Shewan M.Aziz et al. (1995). Polyamine-polyamine and polyamine-protein transport inhibition conjugates and their use as pharmaceuticals and in research relating to polyamine transport. *United State Patent 5456908*.

Strobe W, Falchuk ZM, Rogentine GN, Nelson DC. (1975). The pathogenisis of gluten sensitive enteropathy. *Ann.Intern.Med*., 83:242-256.

Takuya Sugahara et al. (2008). Effects of polyamines on proliferation and IgM productivity of human–human hybridoma, HB4C5 cells, *Cytotechnology,* 57(2): 115–122.

Tian Chen & Keping Chen et al. (2009). Investigation and Application progress of Vero Cell serum free culture. *International Journal of Biology,* Vol.1, No.2 July 09, E-mail: chenlpwx@163.com.

Toshiya Takeda et al. (2002). Effects of exogenous polyamines on embryogenic carrot cells" *Biochemical Engineering Journal,* 12, 21-28 (Abstract).

Weiss T.S et al. (2002). Collagen sandwich culture affects intracellular polyamine levels of human Hepatocytes. *IngentaConnect Cell Proliferation*, 35(5), 257-267 (Abstract).

William Galston. (2003). Polyamines in plant. *Google hand Book,* Page 365-372.

Willim.G.Taylor et al. (1974). Studies for serum substitute for mammalian cells in culture. *In Vitro,* Vol.9, No.4.

Table 1. Non –Animal peptone screening

Peptone Supplement	Mean cell Count x 10^5	(RGE) Relative growth efficiency
Wheat Hydrolysate 1	12.4	28
Wheat Hydrolysate 2	12.4	28
Soy Hydrolysate	31.3	70
Rice Hydrolysate	35.9	80

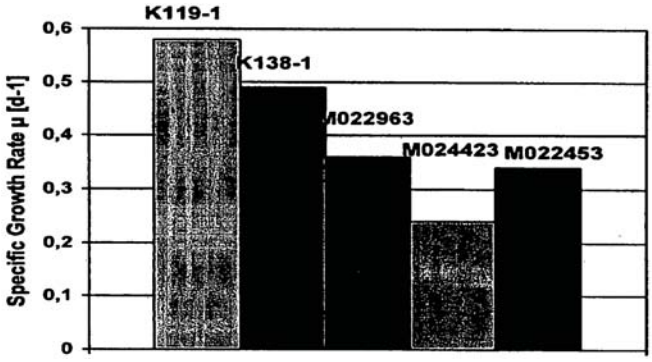

Figure 1. (Inspired by Paul Price et al United States Patent 20040171152) shows a graphical presentation of the performance of the different lots of soy Hydrolysate with respect of specific growth rates of GD8/6 cells

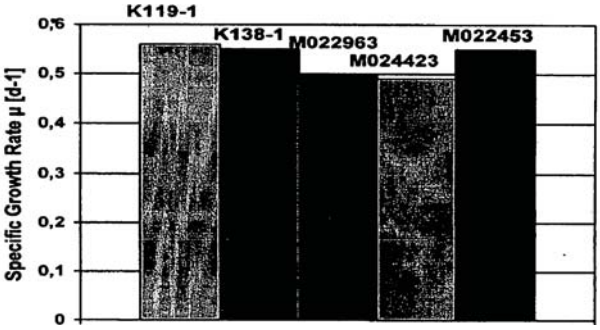

Figure 2. (Inspired by Paul Price et al United States Patent 20040171152) shows a graphical presentation of the performance of the different lots of soy Hydrolysate + polyamine putrescine with respect of specific growth rates of GD8/6 cells

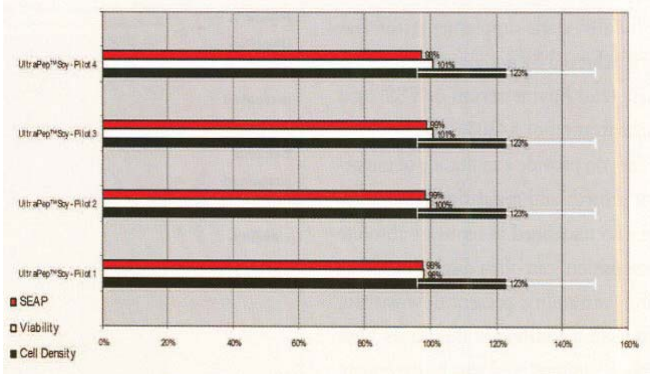

Figure 3. (Inspired by James Babcock,et al. Genetic engineering & biotechnology news, vol.27, No.20, Nov15, 2007) shows performance of four pilot lots supplemented with UltraPep Soycompared as a percentageof a control soy hydrolysate. UltraPep Soy and the lot-to-lot consistency of the four pilot lots

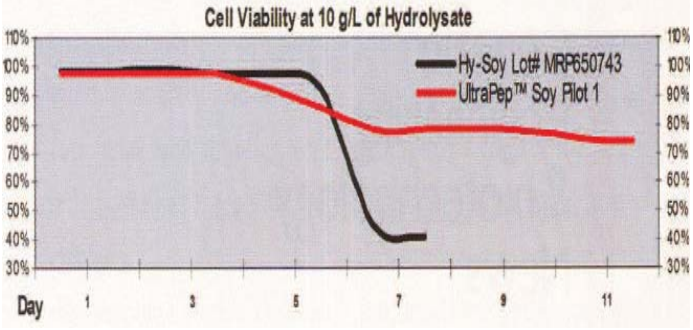

Figure 4. (Inspired by James Babcock,et al. Genetic engineering & biotechnology news, vol.27, No.20, Nov15, 2007) shows performance difference between ultrafiletred peptone (UltraPep Soy) and non-ultrafiletred peptone(Hy-Soy) with respect of %cell viability during 12 days observation