FEED SUPPLEMENT FOR MAMMALIAN CELL CULTURE AND METHODS OF USE

FIELD OF THE INVENTION

[0001] The present invention relates generally to media supplements for use in culturing cells for the production of recombinant proteins, such as antibodies.

BACKGROUND OF THE INVENTION

[0002] Chinese hamster ovary (CHO) cell culture is frequently used to produce proteins for use as therapeutic agents, such as therapeutic antibodies. Growth medium for such cell cultures has historically included supplements of animal origin, but such supplements have recently been linked with the appearance of transmissible spongiform encephalopathies (TSEs), such as bovine spongiform encephalopathies (BSE, mad cow disease), which is linked to variant Creutzfeldt-Jakob disease (vCJD) in humans. *See, e.g.*, Cleland *et al.* (2007) *J. Microbiol. Meth.* 69:345. In light of the resulting concern regarding such contamination, it is preferable to use production media that do not include animal-derived components for the manufacture of pharmaceutical agents.

[0003] Protein hydrolysates, such as soy hydrolysate, have also been used as supplements in cell culture medium to enhance productivity. However, the quality of such hydrolysates can vary from lot-to-lot, affecting both the quantity and quality of the product produced.

[0004] Accordingly, the need exists for improved methods for producing therapeutic proteins in CHO cells in culture that do not involve addition of animal-derived components that could introduce troublesome contaminants. Preferably, such methods would also support high-level expression of therapeutic polypeptides from CHO cells in culture.

SUMMARY ~~OF THE INVENTION~~

[0005] The present ~~invention~~disclosure meets these needs and more by providing a feed supplement concentrate based on a modified 20X DMEM/F12, termed "SP feed" herein, devoid of animal components and protein lysates, and methods of using this supplement for the culture of CHO cells producing therapeutic polypeptides. In a first aspect, there is provided the use of a concentrate comprising components 1- 47 of Table 3 and;

a) sodium selenite; and

b) vitamin E; as a cell feed supplement.

In one embodiment the therapeutic polypeptide is an antibody, or antigen binding fragment thereof. In one embodiment the therapeutic polypeptide is an IgG antibody, or antigen binding fragment

thereof. In some embodiments the therapeutic antibody is a chimeric, humanized or fully human antibody that specifically binds to human IL-23p19.

[0006] In one embodiment, the production feed supplement of the present ~~invention~~disclosure comprises vitamin E and/or sodium selenite (Na_2SeO_4). In another embodiment, the vitamin E is present in the supplement concentrate at approximately 30.2 mg/L. In another embodiment, the sodium selenite is present in the supplement concentrate at approximately 0.3 mg/L. In a further embodiment, the production feed supplement concentrate comprises the components listed in Table 3.

[0007] ~~In another aspect, the invention~~The disclosure provides methods for using the supplement the present ~~invention~~disclosure to produce therapeutic proteins. In one embodiment, the method involves a forward-feeding rationale in which the amount of nutrient provided to the cell culture is based on the growth rate of the cells and nutrient consumption. In various embodiments the feed supplement of the present ~~invention~~disclosure is added to a cell culture on more than one occasion during production, e.g. in as series of two or more bolus feedings, for example on days 3, 5 and 10. In another embodiment, the supplement of the present ~~invention~~disclosure is added during early exponential phase, late exponential phase, and stationary phase. In various embodiments the cultures are supplemented one, two, three, four, five or more times. In still other embodiments, the supplement can be added daily, or on a continuous or semi-continuous basis, to ensure a steady concentration of components over time. In various embodiments the ~~invention involves~~disclosure involves recovery of the protein from the culture after a suitable growth period, which recovery can be from the culture medium (supernatant), the cells (*e.g.* by lysis), or both.

[0008] In one embodiment, one or more components of the production feed supplement of the present ~~invention~~disclosure is added to the culture separately, e.g. one, two, three or more components may be added separately from a mixture of the remaining components. In one embodiment, tyrosine and/or cysteine is added separately from the mixture of other feed components.

[0008a] In a second aspect, there is provided a method of producing a protein comprising:

a) growing mammalian cells expressing the protein in culture;

b) supplementing the culture with the supplement as defined herein; and

c) recovery of the protein from the culture.

[0008b] In a third aspect, there provided a method of producing a protein comprising:

a) growing mammalian cells expressing the protein in culture;

b) supplementing the culture with sodium selenite and vitamin E; and

c) obtaining the protein from the cell culture.

[0009] ~~In another aspect, the invention~~The disclosure provides a method of supplementing mammalian cells in culture for the production of a protein comprising adding vitamin E and/or sodium selenite to the culture medium at least once during a production run. In various embodiments, cultures are supplemented with vitamin E to a final concentration of approximately 2 mg/L, 4 mg/L or 6 mg/L. In various other embodiments, cultures are supplemented with sodium selenite to a final concentration of approximately 0.02 mg/L, 0.04 mg/L or 0.06 mg/L.

[0010] In some embodiments, the methods and feed supplement of the present ~~invention~~disclosure support production of therapeutic proteins at levels (titers) at least as high as levels achieved with growth media supplemented with plant hydrolysate, such as soy hydrolysate. In various embodiments, the feed and methods of the present ~~invention~~disclosure support production of a therapeutic monoclonal antibody at approximately 1.2-, 1.4-, 1.6-, 1.8- or 2.0-fold or higher titer than basal feed (glucose and glutamine only).

[0011] In some embodiments, the method and medium of the present ~~invention~~disclosure support production of therapeutic proteins that are at least as pure as is achieved with soy hydrolysate containing feed medium. In some embodiments purity is assessed after purification of an antibody by Protein-A chromatography. Purity may be determined, for example, by reverse-phase HPLC or size-exclusion chromatography (HP-SEC).

[0012] In one embodiment, the temperature of the culture is shifted during production from 37°C to 34°C, *e.g.* on day 3, 4 or 5 of production.

[0013] In one embodiment the supplement of the present ~~invention~~disclosure is prepared as a 20X concentrate and added to 1.33X, 2.66X and/or 4X final concentration in production runs. In other embodiments, the supplement is prepared and used as a 2X, 3X, 4X, 5X, 6X. 7X, 8X, 9X, 10X, 11X, 12X, 12X, 13X, 14X, 15X, 16X, 17X, 18X, 19X concentrate or more. In still other embodiments, the supplement is prepared and used as a 21X, 22X, 23X, 24X, 25X, 26X, 27X, 28X, 29X, 30X concentrate or more.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1A shows the relative titer enhancement for three antibody production cell lines grown using commercial base media as a function of how (and if) the cultures were fed during production. The antibodies produced by the cell lines are referred to herein as antibodies A (open bar), B (hatched bar) and C (filled bar). All cells were cultured in commercial base medium with supplementation as indicated. The base media used were Sigma C5467 EX-CELL® ACF CHO medium, animal-component free, with HEPES, without L-glutamine, liquid, sterile-filtered, cell culture tested, either with aurin tricarboxylic acid (ATA) (for cells producing antibodies A and C) or without ATA (for cells producing antibody B, and antibodies D, E and F, which are discussed below). All cultures in FIGS. 1A and 1B were grown at 37°C. Cultures were supplemented with either soy hydrolysate, a commercially available feed medium concentrate, or SP feed. The commercially available feed medium was Sigma C1615 CHO Feed Bioreactor Supplement (Sigma-Aldrich, St. Louis, Mo., USA). Antibody titers were determined by reverse-phase-HPLC.