CELL CULTURE MEDIUM

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore patent application No. 10201601284Y, filed 22 February 2016, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to compositions for propagation, preservation, or maintenance of cells. In particular, the present invention relates to culture media. The present invention also relates to compositions for supporting protein production by cultured cells.

BACKGROUND OF THE INVENTION

[0003] From the first day of its inception, cell culture medium has progressively been improved and researched depending on the intention of the culture. The first few cell culture solution provides irrigation, supply of water, inorganic anions required for cell metabolism, osmotic balance, a buffering system, and a carbohydrate source for growth.

[0004] As research into how cells function improves, cell culture medium has evolved to include multitude of components, which may be included depending on the purpose of the medium. However, one that remains a constant for cells (such as mammalian cells) are the provision of glucose as a carbohydrate source.

[0005] As such, there is a need to provide cell culture medium that provides alternative carbohydrate source. The potential use of alternative carbohydrate source, as energy source can have practical implications in biopharmaceutical manufacturing. In particular, the potential use of alternative carbohydrate source may lead to the improvement of carbohydrate loading in the batch culture medium, and possibly lead to the decrease lactate accumulation which may become toxic to cells.

[0006] Accordingly, there is a need to provide a cell culture medium that has an alternative carbohydrate source.

SUMMARY

[0006a] In one aspect, the present disclosure refers to a method of increasing protein yield of

a mammalian cell, wherein the method comprises growing and/or culturing the mammalian cell in a serum-free mammalian cell culture medium comprising maltose and at least one additional saccharide as carbohydrate sources, wherein maltose is present at a concentration of between 0.5 g/litre to 40 g/litre.

[0006b] In another aspect, the present disclosure refers to a method of modulating glycosylation profile of a protein, wherein the method comprises the method of growing and/or culturing a cell as disclosed herein, to thereby produce a cell that expresses the protein with the modulated glycosylation profile.

[0007] In first example, there is provided a serum-free cell culture medium. The cell culture medium comprises maltose as sole carbohydrate source.

[0008] In second example, there is provided a serum-free cell culture medium comprising maltose and at least one, at least two, at least three or more saccharides as carbohydrate sources.

[0009] In third example, there is provided a method of growing and/or culturing a cell, wherein the method comprises growing and/or culturing a cell in the serum-free cell culture medium of the first example or the second example.

[0010] In fourth example, there is provided a method of growing and/or culturing a cell, wherein the method comprising growing and/or culturing a cell in the serum-free cell culture medium as described herein.

[0011] In fifth example, there is provided a method of increasing protein yield, wherein the method comprises growing and/or culturing a cell in the serum-free cell culture medium as described herein.

[0012] In sixth example, there is provided a kit comprising the components of the serum-free cell culture medium as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0014] **Figure 1** shows the results of adaptability tests of Chinese Hamster Ovary (CHO) cells in various disaccharide media. In (A) CHO-K1 cells were cultivated in a serum-free protein-free cell culture medium, HyQ PF-CHO, with 3.6 g/l of different sugars (maltose,

sucrose, lactose, trehalose or glucose) as carbohydrate source. The viable cell densities (lined markers) and culture viabilities (marker only) of these cultures at the beginning and end of each passage over a period of 74 days were plotted. Cultures with sucrose, lactose or trehalose were terminated on Day 31 due to the decreased culture viabilities and reduced viable cell densities. The experiment was repeated with a seeding cell density of 1.0×10^6 cells/ml to obtain similar results. In (B) CHO-DG44 cells were cultivated with a seeding cell density of 1.0×10^6 cells/ml in a serum-free protein-free cell culture medium, HyQ PF-CHO, with 10 g/l of different sugars (maltose, sucrose, lactose, trehalose or glucose) as carbohydrate source. The viable cell densities (lined markers) and cell viabilities (marker

Claims

1. A method of increasing protein yield of a mammalian cell, wherein the method comprises growing and/or culturing the mammalian cell in a serum-free mammalian cell culture medium comprising maltose and at least one additional saccharide as carbohydrate sources, wherein maltose is present at a concentration of between 0.5 g/litre to 40 g/litre.

2. The method of claim 1, wherein the saccharide is a monosaccharide.

3. The method of claim 2, wherein the monosaccharide is glucose.

4. The method of any one of claims 1 to 3, wherein the serum-free mammalian cell culture medium comprises maltose and glucose as sole carbohydrate sources.

5. The method of any one of the preceding claims, wherein the serum-free mammalian cell culture medium is protein-free.

6. The method of any one of the preceding claims, wherein the serum-free mammalian cell culture medium is a chemically defined medium.

7. The method of any one of the preceding claims, wherein the serum-free mammalian cell culture medium is a protein-free chemically defined medium (PFCDM).

8. The method according to any one of the preceding claims, wherein the cell culture medium is further combined with an ionic surfactant or a non-ionic surfactant.

9. The method according to any one of the preceding claims, wherein the cell culture medium further comprises an antibiotic agent.

10. The method of any one of the preceding claims, wherein each ingredient is present in an amount which supports the cultivation of a cell *in vitro*.

11. The method of any one of the preceding claims, wherein said at least one saccharide is present at a concentration of between 0.5 g/litre to 40 g/litre.

12. The method according to claim 1, wherein the serum-free mammalian cell culture medium comprises Dulbecco's minimum essential medium (DMEM), F-12 basal medium (DMEM-F12), L-glutamine, a non-ionic surfactant, geneticin, maltose and glucose, or a modified version thereof.

13. The method of any one of the preceding claims, wherein the serum-free mammalian cell culture medium is in powdered or liquid form.

14. The method of claim 1, wherein the cell is able to metabolize maltose without requiring prior adaptation.

15. The method of any one of the preceding claims, wherein the serum-free mammalian cell culture medium is used to grow and/or culture mammalian cells in a cell culture method selected from the group consisting of batch, fed-batch, continuous, and perfusion.

16. The method of any one of the preceding claims, wherein the culture medium is used as a basal medium, a feed medium, and/or a perfusion medium.

17. The method of any one of the preceding claims, wherein the serum-free mammalian cell culture medium comprises a higher concentration of maltose than the at least one additional saccharide.

18. A method of modulating glycosylation profile of a protein, wherein the method comprises the method of growing and/or culturing a cell according to any one of claims 1 to 17, to thereby produce a cell that expresses the protein with the modulated glycosylation profile.