HEME-CONTAINING CELL CULTURE MEDIA AND USES THEREOF

BACKGROUND

Fermentation and cell or tissue culture involve culturing cells or tissue in a growth medium that includes nutrients needed for survival or growth of the cells. Yeast extracts, peptones and animal serum are commonly used as a source of nutrients for fermentation and cell and tissue culture. However, these sources of nutrients can be cost limiting or deficient in some nutrients to promote optimal growth of the cells. Other nutrient sources for fermentation and cell or tissue culture are needed to support efficient commercial processes.

SUMMARY

The present disclosure provides cell or tissue culture or fermentation media and ingredients for the same, including a biomass or derivative thereof of a hemoprotein-producing C_1 metabolizing non-photosynthetic bacterium, methods of using the culture or fermentation medium, and products such as food ingredients that are produced from the methods of using the culture or fermentation media.

In one aspect, the present disclosure provides a method of cell or tissue culture or fermentation comprising culturing animal, fungal, or algal cells or tissue or bacterial cells selected from the group consisting of: *Bacillus licheniformis, Lactobacillus reuteri, Corynebacterium glutamicum, Pseudomonas putida,* and *Xanthomonas sp.*, *Bifidobacterium lactis*, *Bf. bifidum*, *Bf. longum*, *Bf. infantis*, *Bf. animalis*, *Bf. breve*, *Saccharomyces boulardii*, *Streptococcus Thermophilus*, and *Bacillus Coagulans,* and marine-dwelling bacterial cells in a culture or fermentation medium that comprises a biomass or derivative thereof of a hemoprotein-producing C_1 metabolizing non-photosynthetic bacterium, wherein the amount of heme in the biomass or derivative thereof is in the range of from 0.01 to 10 mg/g protein of the biomass or derivative thereof, and/or wherein the amount of the biomass or derivative thereof in the culture or fermentation medium is in the range of 0.1 to 50 g/l.

In another aspect, the present disclosure provides a cell or tissue culture or fermentation medium, comprising biomass or derivative thereof of a hemoprotein-

producing C_1 metabolizing non-photosynthetic bacterium, wherein the amount of heme in the biomass or derivative thereof is in the range of from 0.01 to 10.0 mg/g protein, and/or wherein the amount of the biomass or derivative thereof in the culture medium is in the range of from 0.1 to 50 g/l.

In another aspect, the present disclosure provides a product of cell or tissue culture or fermentation produced by a method of the disclosure.

In another aspect, the present disclosure provides a product of non-bacterial cell or tissue culture or fermentation having a heme iron content that is higher than that of a reference product produced using a culture or fermentation medium that does not comprise biomass or derivative thereof of a C_1 metabolizing non-photosynthetic bacterium.

In another aspect, the present disclosure provides a method of making a food product or ingredient, comprising: producing a product of cell or tissue culture or fermentation by a method of the disclosure, and processing the product to produce the food product or food ingredient.

In another aspect, the present disclosure provides a food product or food ingredient produced by a method of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1 is a graph of molecular weight distributions of 4 autolysate samples (samples B135, B139, B140 and B141) produced from a biomass of a hemoprotein-producing C_1 metabolizing non-photosynthetic bacterium (*Methylococcus capsulatus* Bath) according to Example 1. Sample B145 is a sample of biomass homogenate that has not undergone autolysis.

Figure 2 is a graph of cell growth as measured by spectrophotometry for two marine dwelling bacterial species, *Moritella marina* and *Shewanella pneumatophori*, and a marine algae, *Schizochytrium sp.* ATCC 20888, each cultured in a culture medium without a biomass of a hemoprotein-producing C_1 metabolizing non-photosynthetic

CLAIMS

That which is claimed is:

1. A method of cell or tissue culture or fermentation comprising culturing animal, fungal, or algal cells or tissue or bacterial cells selected from the group consisting of: *Bacillus licheniformis, Lactobacillus reuteri, Corynebacterium glutamicum, Pseudomonas putida,* and *Xanthomonas sp.*, *Bifidobacterium lactis*, *Bf. bifidum*, *Bf. longum*, *Bf. infantis*, *Bf. animalis*, *Bf. breve*, *Saccharomyces boulardii*, *Streptococcus Thermophilus*, and *Bacillus Coagulans,* and marine-dwelling bacterial cells in a culture or fermentation medium that comprises a biomass or derivative thereof of a hemoprotein-producing C_1 metabolizing non-photosynthetic bacterium,

wherein the amount of heme in the biomass or derivative thereof is in the range of from 0.01 to 10 mg/g protein of the biomass or derivative thereof, and/or

wherein the amount of the biomass or derivative thereof in the culture or fermentation medium is in the range of 0.1 to 50 g/l.

2. The method of claim 1, wherein the cells or tissue comprise animal cells or tissue, and optionally the culture or fermentation medium further comprises serum, a growth hormone, a growth factor, an antibacterial agent and/or an antifungal agent.

3. The method of claim 2, wherein the animal cells or tissue comprise fish or shellfish cells or tissue, and optionally the culture or fermentation medium further comprises fish serum.

4. The method of claim 2, wherein the animal cells or tissue comprise avian cells or tissue.

5. The method of claim 1, wherein the animal cells comprise insect cells or tissue.

6. The method of claim 2, wherein the animal cells or tissue comprise mammalian cells or tissue.

7. The method of claim 6, wherein the animal cells comprise embryonic stem cells, adult stem cells, myosatellite cells, myoblasts, myocytes, and/or muscle cells.

~~2. The method of claim 1, wherein the cells or tissue comprises bacterial cells.~~

~~3. The method of claim 2, wherein the bacterial cells comprise *Bacillus subtilis*, *Bacillus licheniformis*, *Escherichia coli*, *Lactobacillus reuteri, Corynebacterium glutamicum*, *Pseudomonas putida* or *Xanthomonas* sp.~~

~~4. The method of claim 2, wherein the bacterial cells comprise *Bifidobacterium lactis*, *Bf. bifidum*, *Bf. longum*, *Bf. infantis* , *Bf. animalis*, *Bf. breve*, *Saccharomyces boulardii*, *Streptococcus Thermophilus*, or *Bacillus Coagulans.*~~

~~5. The method of claim 1, wherein the bacterial cells comprise marine dwelling bacterial cells.~~

~~6~~8. The method of claim 1, wherein the cells or tissue comprises non-bacterial cells or tissue.

~~7~~9. The method of claim 1, wherein the cells or tissue comprise algal cells, and optionally the culture or fermentation medium further comprises seawater base.

~~8~~10. The method of claim ~~7~~9, wherein the algal cells comprise microalgae.

~~9~~11. The method of claim ~~7~~9, wherein the algal cells comprise macroalgae.

~~10~~12. The method of claim 1, wherein the cells or tissue comprise fungal cells or tissue.

~~11~~13. The method of claim ~~10~~12, wherein the fungal cells or tissue comprise yeast cells.

~~12~~14. The method of claim ~~10~~12, wherein the fungal cells or tissue comprise mushroom cells or tissue, and optionally the culture or fermentation medium further comprises potato extract, grain, and/or fruiting substrate.

~~13. The method of claim 1, wherein the cells or tissue comprise animal cells or tissue, and optionally the culture or fermentation medium further comprises serum, a growth hormone, a growth factor, an antibacterial agent and/or an antifungal agent.~~

~~14. The method of claim 12, wherein the animal cells or tissue comprise fish or shellfish cells or tissue, and optionally the culture or fermentation medium further comprises fish serum.~~

~~15. The method of claim 12, wherein the animal cells or tissue comprise avian cells or tissue.~~

~~16. The method of claim 1, wherein the animal cells comprise insect cells or tissue.~~

~~17. The method of claim 12, wherein the animal cells or tissue comprise mammalian cells or tissue.~~

~~18. The method of claim 17, wherein the animal cells comprise embryonic stem cells, adult stem cells, myosatellite cells, myoblasts, myocytes, and/or muscle cells.~~

~~19~~15. The method of any one of claims 1 to ~~18~~14, wherein the C_1 metabolizing non-photosynthetic bacterium comprises a methylotrophic bacterium.

~~20~~16. The method of any one of claims 1 to ~~18~~14, wherein the C_1 metabolizing non-photosynthetic bacterium comprises a methanotrophic bacterium.

~~21~~17. The method of ~~any claim 18~~claim 16, wherein the methanotrophic bacterium is selected from the group consisting of *Methylococcus,* *Methylomonas*, *Methylobacter*, ~~*Methylococcus*,~~ *Methylosinus*, *Methylocystis*, *Methylomicrobium*, *Methanomonas*, and *Methylocella*.

~~22~~18. The method of ~~any of claims 20~~claim 16, wherein the methanotrophic bacterium comprises *Methylococcus capsulatus*.

~~23~~19. The method of claim ~~20~~16, wherein the methanotrophic bacterium comprises *Methylococcus capsulatus* (Bath).

~~24~~20. The method of any one of claims 1 to ~~23~~19, wherein the biomass of the hemoprotein-producing C_1 metabolizing non-photosynthetic bacterium or derivative thereof comprises a hydrolysate, a suspension, an isolate, an autolysate, an homogenate, a digestate, ~~a hydrolysate,~~ an isolate, an extract, or a combination thereof of the biomass.

~~25~~21. The method of any one of claims 1 to ~~24~~20, wherein the biomass or derivative thereof comprises essential amino acids at an amount within the range of 1-100 mg/g each.

~~26~~22. The method of any one of claims 1 to ~~25~~21, wherein the biomass or derivative thereof comprises copper at an amount within the range of 50-500 mg/kg.

~~27~~23. The method of any one of claims 1 to ~~26~~22, wherein the biomass or derivative thereof comprises iron at an amount within the range of 0.05 to 0.6 mg/g.

~~28.~~24. The method of any one of claims 1 to ~~27~~23, further comprising separating the cultured cells from the growth medium to produce isolated culture cells and/or an isolated supernatant.

~~29~~25. The method of any of one of claims 1 to ~~28~~24, further comprising isolating, concentrating, separating or purifying a desired product from the fermented or cultured cells or tissue.

~~30~~26. The method of claim ~~29~~25, wherein the desired product is selected from vitamins, fatty acids, amino acids, nucleosides, peptides, proteins, enzymes, pigments, flavors, fragrances, organic acids, preservatives, small molecule metabolites, ferment, culture, probiotics, carotenoids, and meat alternatives.

~~31~~27. The method of claim ~~29~~25, wherein the desired product is a small molecule metabolite selected from citric acid and lactic acid.

~~32~~28. The method of claim ~~29~~25, wherein the desired product is an enzyme selected from chymosin, protease, lipase, amylase, cellulase, amylase, and carbohydrase.

~~33~~29. The method of claim ~~29~~25, wherein the desired product is an omega-3 fatty acid selected from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

~~34~~30. The method of claim ~~29~~25, wherein the desired product comprises a biomass of cultured cells or tissue.

~~35~~31. The method of claim ~~34~~30, wherein the biomass of the cultured cells or tissue comprises a probiotic, a dairy culture, or a meat-curing culture.

~~36~~32. The method of any of claims 1 to ~~35~~31, wherein the biomass of the cultured cells or tissue comprises an elevated level of iron, heme or hemoprotein as

compared to a biomass of cells or tissue cultured in a reference culture medium that does not comprise a biomass or derivative thereof of a hemoprotein-producing C_1 metabolizing non-photosynthetic bacterium.

~~37~~33. The method of claim ~~36~~32, wherein the biomass or derivative thereof of the hemoprotein-producing C_1 metabolizing non-photosynthetic bacterium comprises an autolysate of the hemoprotein-producing C_1 metabolizing non-photosynthetic bacterium.

~~38~~34. The method of claim ~~37~~33, wherein the cultured cells or tissue, biomass thereof, or derivative of the biomass is suitable for preparing a cell-based meat product or a meat alternative product.

~~39~~35. The method of any of claims 1 to ~~38~~34, wherein the culturing results in an improved growth rate, yield or productivity of the cells or tissue or the final target ingredient of the culture, as compared to culturing the cells or tissue in a reference culture medium that does not comprise a biomass or derivative thereof of a hemoprotein-producing C_1 metabolizing non-photosynthetic bacterium.

~~40~~36. A cell or tissue culture or fermentation medium, comprising biomass or derivative thereof of a hemoprotein-producing C_1 metabolizing non-photosynthetic bacterium,

wherein the amount of heme in the biomass or derivative thereof is in the range of from 0.01 to 10.0 mg/g protein, and/or

wherein the amount of the biomass or derivative thereof in the culture medium is in the range of from 0.1 to 50 g/l.

~~41~~37. The culture or fermentation medium of claim ~~40~~36, wherein the amount of essential amino acids in the biomass or derivative thereof is in the range of from 1 to 100 mg/g each.

~~42~~38. The culture or fermentation medium of claim ~~40~~ 36 or ~~41~~37, wherein the amount of bioavailable copper in the biomass or derivative thereof is in the range of from 50 to 500 mg/kg.

~~43~~39. The culture or fermentation medium of any of claims ~~40~~ 36 to ~~42~~38, wherein the amount of heme is in the biomass or derivative thereof is in the range of from 0.1 to 1 mg/g protein of the biomass or derivative thereof.

~~44~~40. The culture or fermentation medium of any of claims ~~40~~ 36 to ~~43~~39, wherein the culture or fermentation medium comprises a derivative of the biomass.

~~45~~41. The culture or fermentation medium of claim ~~44~~40, wherein the derivative is an autolysate, lysate, extract, isolate, suspension, homogenate, or digestate of the biomass of the hemoprotein-producing C_1 metabolizing non-photosynthetic bacterium.

~~46~~42. The culture or fermentation medium of any of claims ~~40~~ 36 to ~~45~~41, wherein the culture or fermentation medium is suitable for bacterial cell culture.

~~47~~43. The culture or fermentation medium of claim ~~46~~42, wherein the culture or fermentation medium is suitable for culture of marine-dwelling bacteria.

~~48~~44. The culture or fermentation medium of any of claims ~~40~~ 36 to ~~45~~41, wherein the culture or fermentation medium is suitable for non-bacterial cell culture.

~~49~~45. The culture or fermentation medium of any one of ~~40~~ 36 to ~~45~~41, wherein the culture or fermentation medium is suitable for algal cell culture and optionally further comprises seawater base.

~~50~~46. The culture or fermentation medium of any one of claims ~~40~~ 36 to ~~45~~41, wherein the culture or fermentation medium is suitable for fungal cell or tissue culture.

~~51~~47. The culture or fermentation medium of claim ~~50~~46, wherein the culture or fermentation medium is suitable for yeast cell culture.

~~52~~48. The culture or fermentation medium of claim ~~50~~46, wherein the culture or fermentation medium is suitable for mushroom cell or tissue culture and optionally further comprises potato extract, grain, and/or fruiting substrate.

~~53~~49. The culture or fermentation medium of any one of claims ~~40~~ 36 to ~~45~~41, wherein the culture or fermentation medium is suitable for animal cell or tissue culture and optionally further comprises serum, a growth hormone, a growth factor, an antibacterial agent and/or an antifungal agent.

~~54~~50. The culture or fermentation medium of claim ~~53~~49, wherein the culture or fermentation medium is suitable for fish cell or tissue culture and optionally comprises fish serum.

~~55~~51. The culture or fermentation medium of claim ~~53~~49, wherein the culture or fermentation medium is suitable for avian cell or tissue culture.

~~56~~52. The culture or fermentation medium of claim ~~53~~49, wherein the culture or fermentation medium is suitable for insect cell or tissue culture.

~~57~~53. The culture or fermentation medium of claim ~~53~~49, wherein the culture or fermentation medium is suitable for mammalian cell or tissue culture.

~~58~~54. The culture or fermentation medium of any one of claims ~~40~~ 36 to ~~57~~53, wherein the C_1 metabolizing non-photosynthetic bacterium is a methylotrophic bacterium.

~~59~~55. The culture or fermentation medium of any one of claims ~~40~~ 36 to ~~58~~54, wherein the C_1 metabolizing non-photosynthetic bacterium is a methanotrophic bacterium.

~~60~~56. The culture or fermentation medium of claim ~~59~~55, wherein the methanotrophic bacterium is selected from the group consisting of *Methylomonas, Methylobacter, Methylococcus*, *Methylosinus, Methylocystis, Methylomicrobium, Methanomonas*, and *Methylocella*.

~~61~~57. The culture or fermentation medium of claim ~~59~~55, wherein the methanotrophic bacterium is *Methylococcus capsulatus*.

~~62~~58. The culture or fermentation medium of claim ~~59~~55, wherein the methanotrophic bacterium is *Methylococcus capsulatus* Bath.

~~63~~59. A product of cell or tissue culture or fermentation produced by a method of any one of claims 1 to ~~39~~35.

~~64~~60. The product of claim ~~63~~59, produced by the method of claim ~~28~~24, wherein product comprises the isolated culture cells.

~~65~~61. The product of claim ~~63~~59, produced by the method of claim ~~28~~24, wherein the product comprises isolated, purified or concentrated product of interest derived from the supernatant or the cells themselves.

~~66~~62. The product of any one of claims ~~63~~ 59 to ~~65~~61, comprising an elevated level of one or more desired products, as compared to a reference product produced using a growth medium that does not comprise the biomass or derivative thereof of the C_1 metabolizing non-photosynthetic bacterium.

~~67~~63. The product of claim ~~66~~62, wherein the one or more desired products are selected from vitamins, fatty acids, amino acids, nucleosides, peptides, proteins, enzymes, pigments, flavors, fragrances, organic acids, preservatives, small molecule metabolites, ferment, culture, probiotics, and meat alternatives.

~~68~~64. The product of claim ~~67~~63, wherein the one or more desired products comprise a small molecule metabolite selected from citric acid and lactic acid.

~~69~~65. The product of claim ~~67~~63, wherein the one or more desired products comprise an enzyme.

~~70~~66. The product of claim ~~69~~65, wherein the enzyme comprises chymosin, protease, lipase, amylase, cellulase, and carbohydrase.

~~71~~67. The product of any one of claims ~~63~~ 59 to ~~70~~66, wherein the desired product includes an elevated concentration of omega-3 fatty acids as compared to a reference product produced using a growth medium that does not comprise the biomass or derivative thereof of the C_1 metabolizing non-photosynthetic bacterium.

~~72~~68. The product of claim ~~71~~67, wherein the omega-3 fatty acids comprise eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA).

~~73~~69. The product of any one of claims ~~63~~ 59 to ~~72~~68, wherein the product includes an elevated concentration of one or more carotenoids as compared to a reference product produced using a growth medium that does not comprise the biomass or derivative thereof of the C_1 metabolizing non-photosynthetic bacterium.

~~74~~70. The product of claim ~~73~~69, wherein the one or more carotenoids are selected from beta-carotene, lutein, lycopene, zeaxanthin, and astaxanthin.

~~75~~71. The product of any one of claims ~~63~~ 59 to ~~74~~70, comprising an elevated level of iron, heme iron or hemoprotein as compared to a reference product produced using a growth medium that does not comprise the biomass or derivative thereof of the C_1 metabolizing non-photosynthetic bacterium.

~~76~~72. The product of claim ~~75~~71, comprising a cell-based meat product.

~~77~~73. The product of any one of claims ~~63~~ 59 to ~~76~~72, comprising a probiotic, a dairy culture, or a meat-curing culture.

~~78~~74. The product of any one of claims ~~63~~ 59 to ~~76~~72, comprising a vegetarian meat alternative product.

~~79~~75. The product of any one of claims ~~63~~ 59 to ~~76~~72, comprising a phytoprotective bacterial cell product.

~~80~~76. A product of non-bacterial cell or tissue culture or fermentation having a heme iron content that is higher than that of a reference product produced using a culture or fermentation medium that does not comprise biomass or derivative thereof of a C_1 metabolizing non-photosynthetic bacterium.

~~81~~77. The product of claim ~~80~~76, further comprising an isotopic $\delta\ ^{13}C$ value lower than that of the reference product.

~~82~~78. The product of claim ~~80~~ 76 further having an isotopic $\delta\ ^{15}N$ value lower than that of the reference product.

~~83~~79. The product of claim ~~81~~ 77 or ~~82~~ 78 further having an isotopic $\delta\ ^{34}S$ value lower than that of the reference product.

~~84~~80. The product of any one of claims ~~80~~ 76 to ~~83~~79, wherein the product is selected from non-microorganism cell or tissue culture or fermentation products.

~~85~~81. The product of any one of claims ~~80~~ 76 to ~~83~~79, wherein the product is selected from products of mammalian, algal, yeast, mushroom, fish, avian, and insect~~, and mammalian~~ cell or tissue culture or fermentation.

~~86~~82. The product of claim ~~85~~81, wherein the cell or tissue comprises a yeast or mushroom cell or tissue, and the product comprises a vegetarian meat alternative product.

~~87~~83. The product of any one of claims ~~80~~ 76 to ~~84~~80, wherein the cell or tissue comprises an animal cell or tissue, and the product comprises a cell-based meat product.

~~88~~84. A method of making a food product or ingredient, comprising: producing a product of cell or tissue culture or fermentation by a method of any one of claims 1 to ~~39~~35, and processing the product to produce the food product or food ingredient.

~~89~~85. The method of claim ~~88~~84, wherein the processing the product comprises one or more of separation, filtration, clarification, precipitation, flocculation, evaporation, and drying.

~~90~~86. A food product or food ingredient produced by a method of claim ~~88~~ 84 or ~~89~~85.

~~91~~87. The food product or food ingredient of claim ~~90~~86, wherein the cultured cell comprises yeast, and wherein the food product or food ingredient comprises a starter culture for fermented beverage production.

~~92~~88. The food product or food ingredient of claim ~~91~~87, wherein the food product or food ingredient comprises a starter culture for alcoholic beverage production.

~~93~~89. The food product or food ingredient of claim ~~91~~87, wherein the food product or food ingredient comprises a starter culture of beer, wine, kefir or kombucha production.

~~94~~90. The food product or food ingredient of claim ~~91~~87, wherein the food product or food ingredient comprises a vegetarian meat alternative product.

~~95~~91. The food product or food ingredient of claim ~~94~~90, wherein the cultured cell or tissue comprises fungal cells.

~~96~~92. The food product or food ingredient of claim ~~90~~86, wherein the cultured cells comprise bacterial cells, and wherein the food product or food ingredient comprises a dairy making culture or a meat-curing culture.

~~97~~93. The food product or food ingredient of claim ~~96~~92, wherein the food product or food ingredient comprises a yogurt making culture or a cheese making culture.

~~98~~94. The food product of food ingredient of claim ~~90~~86, wherein the cultured cells comprise bacterial cells, and wherein the food product or food ingredient comprises a probiotic.

~~99~~95. The food product of food ingredient of claim ~~90~~86, comprising a cell-based meat product.

~~100~~96. The food product or food ingredient of any one of claims ~~90~~ 86 to ~~99~~95, comprising a cell-based meat, meat alternative product, amino acid, peptide, protein, fatty acid, organic acid, enzyme, pigment, flavor, fragrance, ferment, culture, probiotic,

food ingredient, nucleoside, vitamin, small molecule, metabolite, flavorant, or preservative.