SPRAY DRIED EXTRACT

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application No. 63/396,143, filed August 08, 2022, the disclosure of which is herein incorporated by reference in its entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] The present disclosure provides compositions and methods for producing spray-dried bacterial extracts having enhanced stability after long term storage at different temperatures. The spray-dried extracts can be used in commercial scale cell-free protein synthesis reactions.

BRIEF SUMMARY

[0003] Provided herein are compositions and methods for making spray-dried bacterial extracts for use in cell-free protein synthesis reactions. In one aspect, provided herein is a method for producing a stable, spray-dried bacterial extract for cell-free protein synthesis, the method comprising: i) combining a bacterial extract comprising lysed bacterial components with a composition comprising trehalose, lactose, leucine, and/or raffinose to yield a mixture, wherein the bacterial extract is able to synthesize a target protein from a template nucleic acid encoding the target protein in cell-free protein synthesis reaction; and ii) spray-drying the mixture to produce the stable, spray-dried bacterial extract. In another aspect, provided herein is a spray-dried bacterial extract for cell-free protein synthesis comprising: dried, lysed bacterial components; and a composition comprising trehalose, lactose, leucine, or raffinose, wherein upon rehydration, the extract is able to synthesize a target protein from a template nucleic acid encoding the target protein. In another aspect, provided herein is a spray-dried extract for cell-free protein synthesis, the spray dried extract comprising: dried, lysed bacterial components; and one or more of a

stabilizer, wherein the stabilizer(s) comprise trehalose, lactose, leucine, raffinose, or any combination thereof; and each of the stabilizer(s) has a glass transition temperature (Tg) of at least 90 °C, and wherein the concentration of the stabilizer(s) is between 5 g/L and 200 g/L or between 25 g/kg and 200 g/kg in the liquid extract prior to spray-drying. In another aspect, provided herein is a method for producing a target protein from a spray dried extract, the method comprising: reconstituting the spray dried extract as disclosed herein; providing a template nucleic acid encoding the target protein; and generating the target protein. In one example, the method comprises: i) combining a bacterial extract comprising lysed bacterial components with a composition comprising trehalose, lactose, leucine, or raffinose to yield a mixture, wherein the bacterial extract is able to synthesize a target protein from a template nucleic acid encoding the target protein in cell-free protein synthesis reaction; and ii) spray-drying the mixture to produce the stable, spray-dried bacterial extract.

[0004] In another example, the disclosure provides a method for expressing a target protein in a cell-free protein synthesis reaction, the method comprising: i) combining a bacterial extract comprising lysed bacterial components with a composition comprising trehalose, lactose, leucine, or raffinose to yield a mixture, wherein the lysed bacterial components can synthesize a target protein from a template nucleic acid encoding the target protein in cell-free protein synthesis

reaction; ii) spray-drying the mixture to produce the stable, spray-dried bacterial extract, iii) rehydrating the spray-dried bacterial extract; iv) adding a template nucleic acid encoding the target protein to the rehydrated extract, wherein the template nucleic acid is translated in the rehydrated extract, thereby expressing the target protein.

[0005] In another example, the disclosure provides a method for expressing a target protein in a cell-free protein synthesis reaction, the method comprising: rehydrating a spray-dried bacterial extract comprising lysed bacterial components and a composition comprising trehalose, lactose, leucine, or raffinose; adding a template nucleic acid encoding the target protein to the rehydrated extract, wherein the template nucleic acid is translated in the rehydrated extract, thereby expressing the target protein.

[0006] In some examples, the bacterial extract comprising lysed bacterial components is a liquid or rehydrated bacterial extract.

[0007] In some examples, the composition comprises trehalose or lactose. In some examples, the mixture comprises about 25 to 200 g/kg trehalose. In some examples, the mixture comprises about 50 to 100 g/kg trehalose. In some examples, the trehalose is trehalose dihydrate (TDH).

[0008] In some examples, the mixture comprises about 25 to 200 g/kg lactose. In some examples, the mixture comprises about 50 to 100 g/kg lactose. In some examples, the lactose is lactose monohydrate (LMH).

[0009] In some examples, the mixture comprises about 5 to 10 g/L of leucine. In some examples, the mixture comprises about 25 to 200 g/L raffinose.

[0010] In some examples, the stable, spray-dried bacterial extract comprises about 40 to 70 g/L of the bacterial extract solids. In some examples, the stable, spray-dried bacterial extract comprises about 50 to 60 g/L of the bacterial extract solids.

[0011] In some examples, the bacterial extract is further combined with one or more of high glass transition temperature (Tg), nonpolar, uncharged amino acids. In some examples, the

one or more high Tg nonpolar, uncharged amino acids are selected from Valine, Tryptophan, Isoleucine, Leucine, Alanine, Glycine, Proline, and any combinations thereof. In some examples, the one or more high Tg nonpolar, uncharged amino acids are selected from L-Valine, L-Tryptophan, L-Isoleucine, L-Leucine, L-Alanine, Glycine, or L-Proline, and any combinations thereof.

[0012] In some examples, the bacterial extract is further combined with one or more amino acids selected from leucine, glycine, alanine, valine, isoleucine, proline, tryptophan, serine, threonine, methionine, asparagine, glutamine, cysteine, aspartic acid, glutamic acid, histidine, lysine, arginine, and any combinations thereof.

[0013] In some examples, the mixture comprises 5 to 15 g/kg of amino acids.

[0014] In some examples, in step (i), the mixture further comprises maltodextrin, sucrose, mannitol, sorbitol, polyethylene glycol 200, polysorbate 80 (Tween® 80), polyvinylpyrrolidone (PVP or Kollidon 12 PF), or 2-Hydroxypropyl-β-Cyclodextrin.

[0015] In some examples, the bacterial spray-dried extract comprises less than or equal to about 15% (w/w) residual water. In some examples, the bacterial spray-dried extract comprises less than or equal to about 10% (w/w) residual water. In some examples, the bacterial spray-dried extract comprises less than or equal to about 5% (w/w) residual water.

[0016] In some examples, the bacterial spray-dried extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at 2°C to 8°C for at least 6 months. In some examples, the bacterial spray-dried extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at 2°C to 8°C for at least 12 months. In some examples, the bacterial spray-dried extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at 2°C to 8°C for at least 18 months.

[0017] In some examples, the bacterial spray-dried extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at about -20°C for at

least 6 months. In some examples, the bacterial spray-dried extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at about -20°C for at least 12 months. In some examples, the bacterial spray-dried extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at about -20°C for at least 18 months.

[0018] In some examples, the bacterial spray-dried extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at about room temperature (20°C) for at least 6 months. In some examples, the bacterial spray-dried extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at about room temperature (20°C) for at least 12 months. In some examples, the bacterial spray-dried extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at about room temperature (20°C) for at least 18 months.

[0019] In some examples, the titer is determined by the Phytip® method.

[0020] In some examples, the spray-dried extract and the control extract comprise trehalose, and the spray-dried extract is stored at 2°C to 8°C for at least 12 months and following reconstitution is able to synthesize the target protein with a titer of at least 80% relative to the control extract reconstituted at time zero. In some examples, the protein synthesis activity of the spray-dried extract decreases less than about 5% per month compared to a control extract when the spray-dried extract is stored at 2°C to 8°C and is then rehydrated.

[0021] In some examples, the control extract does not comprise trehalose, lactose, leucine, or raffinose. In some examples, the control extract comprises trehalose, lactose, leucine, or raffinose.

[0022] In some examples, the spray-dried bacterial extract comprises an active oxidative phosphorylation system in cell-free protein synthesis. In some examples, the bacterial extract is from an Escherichia species.

[0023] In some examples, prior to step (i), the bacterial extract is heated at about 20ºC to 45ºC for about 30 minutes to about 10 hours.

[0024] In some examples, the spray-drying in step (ii) comprises atomizing the mixture to produce droplets; contacting the droplets with a gas to evaporate liquid from the droplets; separating the dried extract from the gas and smaller particles; and collecting the spray-dried extract.

[0025] In some examples, greater than or equal to 90% (w/w) of the liquid is removed from the mixture. In some examples, greater than or equal to 95% (w/w) of the liquid is removed from the mixture.

[0026] In some examples, the method further comprises (iii) rehydrating the spray-dried bacterial extract; and (iv) synthesizing the target protein under conditions that support a cell-free protein synthesis reaction. In some examples, the rehydrated bacterial extract comprises about 20% to 60% (by volume) of the cell-free protein synthesis reaction. In some examples, the rehydrated bacterial extract comprises about 30% to 40% (by volume) of the cell-free protein synthesis reaction.

[0027] In another example, the disclosure provides a spray-dried bacterial extract composition for cell-free protein synthesis, the extract comprising dried, lysed bacterial components; and a composition comprising trehalose, lactose, leucine, or raffinose, wherein the extract is able to synthesize upon rehydration a target protein from a template nucleic acid encoding the target protein.

[0028] In some examples, the composition comprises trehalose or lactose. In some examples, the composition comprises about 25 to 200 g/kg of trehalose. In some examples, the composition comprises about 50 to 100 g/kg of trehalose. In some examples, the trehalose is trehalose dihydrate (TDH). In some examples, the composition comprises about 25 to 200 g/kg of lactose. In some examples, the composition comprises about 50 to 100 g/kg of lactose. In some examples, the lactose is lactose monohydrate (LMH). In some

examples, the composition comprises about 5 to 10 g/L of leucine. In some examples, the composition comprises about 25 to 200 g/L raffinose.

[0029] In some examples, the extract further comprises one or more high glass transition temperate (Tg) nonpolar uncharged amino acids. In some examples, the high Tg nonpolar, uncharged amino acids are selected from Valine, Tryptophan, Isoleucine, Leucine, Alanine, Glycine, Proline, and any combinations thereof. In some examples, the high Tg nonpolar, uncharged amino acids are selected from the group consisting of L-Valine, L-Tryptophan, L-Isoleucine, L-Leucine, L-Alanine, Glycine, L-Proline, and any combinations thereof. In some examples, the extract comprises 5 to 15 g/kg of amino acids.

[0030] In some examples, the extract further comprises one or more, a combination of all, or a subset of amino acids selected from leucine, glycine, alanine, valine, isoleucine, proline, tryptophan, serine, threonine, methionine, asparagine, glutamine, cysteine, aspartic acid, glutamic acid, histidine, lysine, and arginine. In some examples, the extract comprises about 5 to 15 g/L of the amino acids.

[0031] In some examples, the extract further comprises maltodextrin, sucrose, mannitol, sorbitol, polyethylene glycol 200, polysorbate 80 (Tween® 80), polyvinylpyrrolidone (PVP or Kollidon 12 PF), or 2-Hydroxypropyl-β-Cyclodextrin.

[0032] In some examples, the spray-dried extract comprises less than or equal to about 15% (w/w) residual water. In some examples, the spray-dried extract comprises less than or equal to about 10% (w/w) residual water. In some examples, the spray-dried extract comprises less than or equal to about 5% (w/w) residual water.

[0033] In some examples, the spray-dried extract is stored at 2°C to 8°C for at least 6 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract. In some examples, the spray-dried extract is stored at 2°C to 8°C for at least 12 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract. In some examples, the spray-dried extract is stored at 2°C to 8°C for at least 18 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract.

[0034] In some examples, the spray-dried extract is stored at about -20°C for at least 6 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract. In some examples, the spray-dried extract is stored at about -20°C for at least 12 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract. In some examples, the spray-dried extract is stored at about -20°C for at least 18 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract.

[0035] In some examples, the spray-dried extract is stored at about room temperature (20°C) for at least 6 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract. In some examples, the spray-dried extract is stored at about room temperature (20°C) for at least 12 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract. In some examples, the spray-dried extract is stored at about room temperature (20°C) for at least 18 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract.

[0036] In some examples, the titer is determined by the Phytip® method. In some examples, the control extract does not comprise trehalose, lactose, leucine, or raffinose. In some examples, the control extract comprises trehalose, lactose, leucine, or raffinose.

[0037] In some examples, the spray-dried extract and the control extract comprise trehalose, and the spray-dried extract is stored at 2°C to 8°C for at least 12 months and following rehydration is able to synthesize the target protein with a titer of at least 80% relative to the control extract rehydrated at time T = zero.

[0038] In some examples, the protein synthesis activity of the rehydrated spray-dried extract decreases at less than about 5% per month when the extracts are stored at 2°C to 8°C prior to rehydration. In some examples, the protein synthesis activity of the rehydrated spray-dried extract is greater than or equal to the protein synthesis activity of rehydrated control spray-dried extract when the extracts are stored at 2°C to 8°C for greater than or equal to 8 months prior to rehydration. In some examples, the spray-dried extracts are stored at 2°C to 8°C for 13 months

prior to rehydration. In some examples, the control spray-dried extract does not comprise trehalose.

[0039] In some examples, the spray-dried extract comprises trehalose or lactose, is stored at 2°C to 8°C for at least 4 months and is able to synthesize the target protein with a titer of at least 75% relative to a control extract stored at -20°C that does not comprise trehalose or lactose. In some examples, the spray-dried extract comprises about 75 g/kg to 105 g/kg trehalose or about 100 g/kg lactose.

[0040] In some examples, the rehydrated extract has greater than or equal to 80% of the initial protein synthesis activity when compared to the protein synthesis activity of rehydrated extract at T = 0 when stored at 2°C to 8°C for at least 18 months prior to rehydration.

[0041] In some examples, the spray-dried bacterial extract has an active oxidative phosphorylation system in cell-free protein synthesis. In some examples, the extract is from an Escherichia species. In some examples, the extract is a powder. In some examples, the extract does not have a cake-like appearance or is not a dried cake.

[0042] In another example, the disclosure provides a method of preparing a spray-dried extract, comprising the steps of: (i) providing a liquid bacterial extract comprising components for cell-free synthesis of a target protein from a template nucleic acid encoding the target protein; (ii) producing droplets of the liquid bacterial extract; (iii) contacting the droplets with a gas to evaporate liquid from the droplets; (iv) separating the dried extract from the gas and smaller particles; and (v) collecting the spray-dried extract.

[0043] In some examples, prior to step (i) the liquid bacterial extract is sterile filtered. In some examples, the sterile filtered liquid bacterial extract is activated by heating.

[0044] In some examples, a composition comprising trehalose, lactose, leucine, or raffinose is added to the activated sterile filtered liquid bacterial extract prior to step (ii). In some examples, the composition comprises about 25 to 200 g/kg trehalose, about 25 to 200 g/kg lactose, about 5 to 10 g/L leucine, or about 25 to 200 g/L raffinose.

[0045] In some examples, step (i) further comprises adding one or more amino acids to the activated sterile filtered liquid bacterial extract. In some examples, the one or more amino acids comprise high glass transition temperate (Tg) nonpolar uncharged amino acids selected from the group consisting of L-Valine, L-Tryptophan, L-Isoleucine, L-Leucine, L-Alanine, Glycine, L-Proline, and any combinations thereof. In some examples, the one or more amino acids are selected from leucine, glycine, alanine, valine, isoleucine, proline, tryptophan, serine, threonine, methionine, asparagine, glutamine, cysteine, aspartic acid, glutamic acid, histidine, lysine, and arginine.

[0046] In some examples, one or more of maltodextrin, sucrose, mannitol, sorbitol, polyethylene glycol 200, polysorbate 80 (Tween® 80), polyvinylpyrrolidone (PVP or Kollidon 12 PF), 2-Hydroxypropyl-β-Cyclodextrin, or any combinations thereof, are added to the activated sterile filtered liquid bacterial extract.

[0047] In some examples, step (ii) comprises atomizing the liquid bacterial extract to produce the droplets. In some examples, the atomizing comprises passing the liquid bacterial extract through an atomization device selected from a nozzle or rotary atomizer. In some examples, the median droplet size is from about 20 to 100 microns (Dv50) at an atomized gas pressure of 10 to 50 psig.

[0048] In some examples, step (iii) comprises contacting the droplets with a drying gas through a drying chamber, wherein the drying gas has an outlet temperature of about 60°C to about 90°C. In some examples, step (iv) comprises separating the dried extract from the gas and smaller particles using centrifugal force. In some examples, step (v) comprises collecting the spray-dried extract in a container.

[0049] In some examples, the collected spray-dried extract comprises less than or equal to about 15%, 10% or 5% (w/w) residual water. In some examples, greater than or equal to 85%, 90%, or 95% (w/w) of the liquid is removed from the spray-dried extract.

[0050] In some examples, the protein synthesis activity of the rehydrated spray-dried extract decreases less than about 5% per month when the extracts are stored at 2°C to 8°C prior to

rehydration. In some examples, the protein synthesis activity of the rehydrated spray-dried extract is greater than or equal to the protein synthesis activity of rehydrated control extract when the extracts are stored at 2°C to 8°C for greater than or equal to 8 months prior to rehydration. In some examples, the spray-dried extracts are stored at 2°C to 8°C for 13 months prior to rehydration.

[0051] In some examples, the spray-dried bacterial extract has an active oxidative phosphorylation system in cell-free protein synthesis. In some examples, the liquid extract is from an Escherichia species.

[0052] In another example, the disclosure provides a spray-dried extract for cell-free protein synthesis, the spray dried extract comprising dried, lysed bacterial components and one or more of a stabilizer, wherein the stabilizer has a glass transition temperature (Tg) of at least about 90°C, and wherein the concentration of the stabilizer is between about 5 g/L and 200 g/L or between about 25 g/kg and 200 g/kg in the liquid extract prior to spray-drying.

[0053] In some examples, the stabilizer is selected from trehalose, lactose, and leucine. In some examples, the stabilizer comprises about 25 to 200 g/kg trehalose, about 25 to 200 g/kg lactose, or about 5 to 10 g/L of leucine. In some examples, the trehalose is trehalose dihydrate (TDH) and the lactose is lactose monohydrate (LMH). In some examples, greater than or equal to 85%, 90% or 95% (w/w) of the liquid is removed from the mixture.

[0054] In some examples, the extract further comprises one or more high glass transition temperate (Tg) nonpolar uncharged amino acids selected from L-Valine, L-Tryptophan, L-Isoleucine, L-Leucine, L-Alanine, Glycine, L-Proline, and any combinations thereof. In some examples, the extract further comprises one or more, a combination of all, or a subset of amino acids selected from leucine, glycine, alanine, valine, isoleucine, proline, tryptophan, serine, threonine, methionine, asparagine, glutamine, cysteine, aspartic acid, glutamic acid, histidine, lysine, and arginine. In some examples, the extract further comprises maltodextrin, sucrose, mannitol, sorbitol, polyethylene glycol 200, polysorbate 80 (Tween® 80), polyvinylpyrrolidone (PVP or Kollidon 12 PF), 2-Hydroxypropyl-β-Cyclodextrin or any combinations thereof.

[0055] In another example, provided is a method for producing a target protein from a spray dried extract, the method comprising: reconstituting a spray dried extract of the disclosure; providing a template nucleic acid encoding the target protein; and generating the target protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0056] **FIG. 1** shows data from laboratory-scale spray drying. Drying was performed on a lab scale Buchi B-290 spray dryer with respective inlet and outlet temperatures noted in parenthesis for dried samples as (Inlet Temperature/Outlet Temperature). XpressCF® (XCF) testing used batch reactions in a Micro-24 microbioreactor (m24) and Flower Plate (FP) with 30% (by volume) XtractCF® to express product anti-CD74 antibody. Source liquid and dried XtractCF® was from lot DR 3c H4 B5.

[0057] **FIG. 2A** shows data from a pilot-scale spray drying formulation and drying process. Samples were prepared and dried formulated with 100 g/L, 50 g/L, and 25 g/L trehalose. Drying was performed on a pilot scale Mobile Minor PSD-1 spray dryer. XCF testing used batch reactions in a Micro-24 microbioreactor with 30% (by volume) XtractCF® to express product anti-CD74 antibody. Source liquid and dried XtractCF® was from lot ER 15-4.

[0058] **FIG. 2B** shows data from a pilot-scale spray drying formulation and drying process. Samples were prepared and dried formulated with 100 g/L, 50 g/L, and 25 g/L trehalose. Drying was performed on a pilot scale Mobile Minor PSD-1 spray dryer. XCF testing used batch reactions in a Micro-24 microbioreactor with 30% (by volume) XtractCF® to express product anti-CD74 antibody. Source liquid and dried XtractCF® was from lot ER 15-4.

[0059] **FIG. 3A**, **FIG. 3B**, and **FIG. 3C** show long term stability data of spray dried trehalose dihydrate formulated XtractCF® at 2-8°C, -20°C, and room temperature, respectively. Samples were spray dried on a Mobile Minor (PSD-1) with trehalose dihydrate formulation at 25, 50, and 100 g/L amounts and associated residual moisture levels (batch and % residual moisture noted in the legend). The Rates of Titer Loss (%/month) were calculated from linear fits of data from 6 to

WHAT IS CLAIMED IS:

1. A method for producing a stable, spray-dried bacterial extract for cell-free protein synthesis, the method comprising:

i. combining a bacterial extract comprising lysed bacterial components with a composition comprising trehalose, lactose, leucine, and/or raffinose to yield a mixture, wherein the bacterial extract is able to synthesize a target protein from a template nucleic acid encoding the target protein in a cell-free protein synthesis reaction; and

ii. spray-drying the mixture to produce the stable, spray-dried bacterial extract.

2. The method of claim 1, wherein the bacterial extract comprising lysed bacterial components is a liquid or rehydrated bacterial extract.

3. The method of claim 1 or 2, wherein the composition comprises trehalose or lactose.

4. The method of any one of claims 1 to 3, wherein the mixture comprises 25 to 200 g/kg trehalose.

5. The method of claim 4, wherein the mixture comprises 50 to 100 g/kg trehalose.

6. The method of claim 4 or 5, wherein the trehalose is trehalose dihydrate (TDH).

7. The method of claim 1, wherein the mixture comprises 25 to 200 g/kg lactose.

8. The method of claim 7, wherein the mixture comprises 50 to 100 g/kg lactose.

9. The method of claim 7 or 8, wherein the lactose is lactose monohydrate (LMH).

10. The method of claim 1, wherein the mixture comprises 5 to 10 g/L of leucine.

11. The method of claim 1, wherein the mixture comprises 25 to 200 g/L raffinose.

12. The method of any one of claims 1-11, wherein the stable, spray-dried bacterial extract comprises 40 to 70 g/L of the bacterial extract solids.

13. The method of any one of claims 1-11, wherein the stable, spray-dried bacterial extract comprises 50 to 60 g/L of the bacterial extract solids.

14. The method of any one of claims 1 to 13, wherein in step (i) the bacterial extract is further combined with one or more high glass transition temperature (T_g), nonpolar, uncharged amino acids.

15. The method of claim 14, wherein the one or more high T_g nonpolar, uncharged amino acids are selected from Valine, Tryptophan, Isoleucine, Leucine, Alanine, Glycine, Proline, and any combinations thereof.

16. The method of claim 14, wherein the one or more high T_g nonpolar, uncharged amino acids are selected from L-Valine, L-Tryptophan, L-Isoleucine, L-Leucine, L-Alanine, Glycine, L-Proline, and any combinations thereof.

17. The method of any one of claims 14 to 16, wherein the mixture comprises 5 to 15 g/kg of the high T_g nonpolar, uncharged amino acids.

18. The method of any one of claims 1 to 17, wherein in step (i) the bacterial extract is further combined with one or more amino acids selected from leucine, glycine, alanine, valine, isoleucine, proline, tryptophan, serine, threonine, methionine, asparagine, glutamine, cysteine, aspartic acid, glutamic acid, histidine, lysine, arginine, and any combinations thereof.

19. The method of claim 18, wherein the mixture comprises 5 to 15 g/kg of the one or more amino acids.

20. The method of any one of claims 1 to 17, wherein in step (i), the mixture further comprises maltodextrin, sucrose, mannitol, sorbitol, polyethylene glycol 200, polysorbate 80 (Tween® 80), polyvinylpyrrolidone (PVP or Kollidon 12 PF), or 2-Hydroxypropyl-β-Cyclodextrin.

21. The method of any one of claims 1 to 20, wherein the spray-dried bacterial extract comprises less than or equal to 15% (w/w) residual water.

22. The method of any one of claims 1 to 20, wherein the spray-dried bacterial extract comprises less than or equal to 10% (w/w) residual water.

23. The method of any one of claims 1 to 20, wherein the spray-dried bacterial extract comprises less than or equal to 5% (w/w) residual water.

24. The method of any one of claims 1 to 23, wherein the spray-dried bacterial extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at 2°C to 8°C for at least 6 months.

25. The method of any one of claims 1 to 23, wherein the spray-dried bacterial extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract, after storage at 2°C to 8°C for at least 12 months.

26. The method of any one of claims 1 to 23, wherein the spray-dried bacterial extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract, after storage at 2°C to 8°C for at least 18 months.

27. The method of any one of claims 1 to 23, wherein the spray-dried bacterial extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract, after storage at about -20°C for at least 6 months.

28. The method of any one of claims 1 to 23, wherein the spray-dried bacterial extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at about -20°C for at least 12 months.

29. The method of any one of claims 1 to 23, wherein the spray-dried bacterial extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at about -20°C for at least 18 months.

30. The method of any one of claims 1 to 23, wherein the spray-dried bacterial extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at about room temperature (20°C) for at least 6 months.

31. The method of any one of claims 1 to 23, wherein the spray-dried bacterial extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at about room temperature (20°C) for at least 12 months.

32. The method of any one of claims 1 to 23, wherein the spray-dried bacterial extract is able to synthesize the target protein with a titer of at least 80% relative to a control extract after storage at about room temperature (20°C) for at least 18 months.

33. The method of any one of claims 24 to 32, wherein the titer is determined by the Phytip® method.

34. The method of any one of claims 24 to 33, wherein the control extract does not comprise trehalose, lactose, leucine, or raffinose.

35. The method of any one of claims 24 to 33, wherein the control extract comprises trehalose, lactose, leucine, or raffinose.

36. The method of any one of claims 1 to 23, wherein the spray-dried extract and the control extract comprise trehalose, and the spray-dried extract is stored at 2°C to 8°C for at least 12 months and following reconstitution is able to synthesize the target protein with a titer of at least 80% relative to the control extract reconstituted at time zero.

37. The method of any one of claims 1 to 23, wherein the protein synthesis activity of the spray-dried extract decreases less than 5% per month compared to a control extract when the spray-dried extract is stored at 2°C to 8°C and is then rehydrated.

38. The method of claim 37, wherein the control extract does not comprise trehalose, lactose, leucine or raffinose.

39. The method of claim 37, wherein the control extract comprises trehalose, lactose, leucine or raffinose.

40. The method of any one of claims 1 to 38, wherein the spray-dried bacterial extract comprises an active oxidative phosphorylation system in cell-free protein synthesis.

41. The method of any one of claims 1 to 40, wherein the bacterial extract is from an Escherichia species.

42. The method of any one of claims 1 to 41, wherein prior to step (i), the bacterial extract is heated at 20°C to 45°C for 30 minutes to 10 hours.

43. The method of any one of claims 1 to 41, wherein the spray-drying in step (ii) comprises:

atomizing the mixture to produce droplets;

contacting the droplets with a gas to evaporate liquid from the droplets;

separating the dried extract from the gas and smaller particles; and

collecting the spray-dried extract.

44. The method of claim 43, wherein greater than or equal to 90% (w/w) of the liquid is removed from the mixture.

45. The method of claim 43, wherein greater than or equal to 95% (w/w) of the liquid is removed from the mixture.

46. The method of any one of claims 1 to 45, further comprising (iii) rehydrating the spray-dried bacterial extract; and (iv) synthesizing the target protein under conditions that support a cell-free protein synthesis reaction.

47. The method of claim 46, wherein the rehydrated bacterial extract comprises 20% to 60% (by volume) of the cell-free protein synthesis reaction.

48. The method of claim 47, wherein the rehydrated bacterial extract comprises 30% to 40% (by volume) of the cell-free protein synthesis reaction.

49. A spray-dried bacterial extract for cell-free protein synthesis comprising:

dried, lysed bacterial components; and

a composition comprising trehalose, lactose, leucine, or raffinose,

wherein upon rehydration, the extract is able to synthesize a target protein from a template nucleic acid encoding the target protein.

50. The spray-dried extract of claim 49, wherein the composition comprises trehalose or lactose.

51. The spray-dried extract of claim 49, wherein the composition comprises 25 to 200 g/kg of trehalose.

52. The spray-dried extract of claim 49, wherein the composition comprises 50 to 100 g/kg of trehalose.

53. The spray-dried extract of claim 51 or 52, wherein the trehalose is trehalose dihydrate (TDH).

54. The spray-dried extract of claim 49, wherein the composition comprises 25 to 200 g/kg of lactose.

55. The spray-dried extract of claim 54, wherein the composition comprises 50 to 100 g/kg of lactose.

56. The spray-dried extract of claim 54 or 55, wherein the lactose is lactose monohydrate (LMH).

57. The spray-dried extract of claim 49, wherein the composition comprises 5 to 10 g/L of leucine.

58. The spray-dried extract of claim 49, wherein the composition comprises 25 to 200 g/L raffinose.

59. The spray-dried extract of any one of claims 49 to 58, wherein the extract further comprises one or more high glass transition temperate (T_g) nonpolar uncharged amino acids.

60. The spray-dried extract of claim 59, wherein the one or more high T_g nonpolar, uncharged amino acids are selected from Valine, Tryptophan, Isoleucine, Leucine, Alanine, Glycine, Proline, and any combinations thereof.

61. The spray-dried extract of claim 59, wherein the one or more high T_g nonpolar, uncharged amino acids are selected from the group consisting of L-Valine, L-Tryptophan, L-Isoleucine, L-Leucine, L-Alanine, Glycine, L-Proline, and any combinations thereof.

62. The spray-dried extract of any one of claims 59 to 61, wherein the extract comprises 5 to 15 g/kg of amino acids.

63. The spray-dried extract of any one of claims 49 to 62, wherein the extract further comprises one or more, a combination of all, or a subset of amino acids selected from leucine, glycine, alanine, valine, isoleucine, proline, tryptophan, serine, threonine, methionine, asparagine, glutamine, cysteine, aspartic acid, glutamic acid, histidine, lysine, and arginine.

64. The spray-dried extract of claim 63, wherein the extract comprises 5 to 15 g/L of the amino acids.

65. The spray-dried extract of any one of claims 49 to 64, wherein the extract further comprises maltodextrin, sucrose, mannitol, sorbitol, polyethylene glycol 200, polysorbate 80 (Tween® 80), polyvinylpyrrolidone (PVP or Kollidon 12 PF), or 2-Hydroxypropyl-β-Cyclodextrin.

66. The spray-dried extract of any one of claims 49 to 65, wherein the spray-dried extract comprises less than or equal to 15% (w/w) residual water.

67. The spray-dried extract of any one of claims 49 to 65, wherein the spray-dried extract comprises less than or equal to 10% (w/w) residual water.

68. The spray-dried extract of any one of claims 49 to 65, wherein the spray-dried extract comprises less than or equal to 5% (w/w) residual water.

69. The spray-dried extract of any one of claims 49 to 68, wherein the spray-dried extract is stored at 2°C to 8°C for at least 6 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract.

70. The spray-dried extract of claim 69, wherein the spray-dried extract is stored at 2°C to 8°C for at least 12 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract.

71. The spray-dried extract of claim 69, wherein the spray-dried extract is stored at 2°C to 8°C for at least 18 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract.

72. The spray-dried extract of any one of claims 49 to 68, wherein the spray-dried extract is stored at about -20°C for at least 6 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract.

73. The spray-dried extract of claim 72, wherein the spray-dried extract is stored at about -20°C for at least 12 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract.

74. The spray-dried extract of claim 72, wherein the spray-dried extract is stored at about -20°C for at least 18 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract.

75. The spray-dried extract of any one of claims 49 to 68, wherein the spray-dried extract is stored at about room temperature (20°C) for at least 6 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract.

76. The spray-dried extract of claim 75, wherein the spray-dried extract is stored at about room temperature (20°C) for at least 12 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract.

77. The spray-dried extract of claim 75 or 76, wherein the spray-dried extract is stored at about room temperature (20°C) for at least 18 months and is able to synthesize the target protein with a titer of at least 80% relative to a control extract.

78. The spray-dried extract of any one of claims 69 to 74, wherein the titer is determined by the Phytip® method.

79. The spray-dried extract of any one of claims 69 to 78, wherein the control extract does not comprise trehalose, lactose, leucine, or raffinose.

80. The spray-dried extract of any one of claims 69 to 78, wherein the control extract comprises trehalose, lactose, leucine, or raffinose.

81. The spray-dried extract of any one of claims 69 to 74, wherein the spray-dried extract and the control extract comprise trehalose, and the spray-dried extract is stored at 2°C to 8°C for at least 12 months and following rehydration is able to synthesize the target protein with a titer of at least 80% relative to the control extract rehydrated at time (T) = zero.

82. The spray-dried extract of any one of claims 49 to 68, wherein the protein synthesis activity of the rehydrated spray-dried extract decreases at less than 5% per month when the extracts are stored at 2°C to 8°C prior to rehydration.

83. The spray-dried extract of any one of claims 49 to 68, wherein the protein synthesis activity of the rehydrated spray-dried extract is greater than or equal to the protein synthesis activity of rehydrated control spray-dried extract when the extracts are stored at 2°C to 8°C for greater than or equal to 8 months prior to rehydration.

84. The spray-dried extract of claim 83, wherein the spray-dried extracts are stored at 2°C to 8°C for 13 months prior to rehydration.

85. The spray-dried extract of claim 83 or 84, wherein the control spray-dried extract does not comprise trehalose.

86. The spray-dried extract of any one of claims 49 to 68, wherein the spray-dried extract comprises trehalose or lactose, is stored at 2°C to 8°C for at least 4 months and is able to synthesize the target protein with a titer of at least 75% relative to a control extract stored at -20°C that does not comprise trehalose or lactose.

87. The spray-dried extract of claim 84, wherein the spray-dried extract comprises 75 g/kg to 105 g/kg trehalose or about 100 g/kg lactose.

88. The spray-dried extract of any one of claims 49 to 68, wherein the rehydrated extract has greater than or equal to 80% of the initial protein synthesis activity when compared to the protein synthesis activity of rehydrated extract at T = 0 when stored at 2°C to 8°C for at least 18 months prior to rehydration.

89. The spray-dried extract of any one of claims 49 to 88, wherein the spray-dried bacterial extract has an active oxidative phosphorylation system in cell-free protein synthesis.

90. The spray-dried extract of any one of claims 49 to 89, wherein the extract is from an Escherichia species.

91. The spray-dried extract of any one of claims 49 to 90, wherein the extract is a powder.

92. The spray-dried extract of any one of claims 49 to 90, wherein the extract does not have a cake-like appearance or is not a dried cake.

93. A spray-dried extract for cell-free protein synthesis, the spray dried extract comprising:

dried, lysed bacterial components; and

one or more of a stabilizer,

wherein the stabilizer(s) comprise trehalose, lactose, leucine, raffinose, or any combination thereof; and each of the stabilizer(s) has a glass transition temperature (Tg) of at least 90 °C, and

wherein the concentration of the stabilizer(s) is between 5 g/L and 200 g/L or between 25 g/kg and 200 g/kg in the liquid extract prior to spray-drying.

94. The spray-dried extract of claim 93, wherein the stabilizer(s) are selected from trehalose, lactose, and leucine.

95. The spray-dried extract of claim 94, wherein the stabilizer(s) comprise 25 to 200 g/kg trehalose, 25 to 200 g/kg lactose or 5 to 10 g/L of leucine.

96. The spray-dried extract of claim 95, wherein the trehalose is trehalose dihydrate (TDH) and the lactose is lactose monohydrate (LMH).

97. The spray-dried extract of any one of claims 93 to 96, wherein greater than or equal to 85%, 90% or 95% (w/w) of the liquid is removed from the mixture.

98. The spray-dried extract of any one of claims 93 to 97, wherein the extract further comprises one or more high glass transition temperate (T_g) nonpolar uncharged amino acids selected from L-Valine, L-Tryptophan, L-Isoleucine, L-Leucine, L-Alanine, Glycine, L-Proline, and any combinations thereof.

99. The spray-dried extract of any one of claims 93 to 97, wherein the extract further comprises one or more, a combination of all, or a subset of amino acids selected from leucine, glycine, alanine, valine, isoleucine, proline, tryptophan, serine, threonine, methionine, asparagine, glutamine, cysteine, aspartic acid, glutamic acid, histidine, lysine, and arginine.

100. The spray-dried extract of any one of claims 93 to 99, wherein the extract further comprises maltodextrin, sucrose, mannitol, sorbitol, polyethylene glycol 200, polysorbate 80 (Tween® 80), polyvinylpyrrolidone (PVP or Kollidon 12 PF), 2-Hydroxypropyl-β-Cyclodextrin, or any combinations thereof.

101. A method for producing a target protein from a spray dried extract, the method comprising:

reconstituting the spray dried extract of claims 93-100;

providing a template nucleic acid encoding the target protein; and

generating the target protein.