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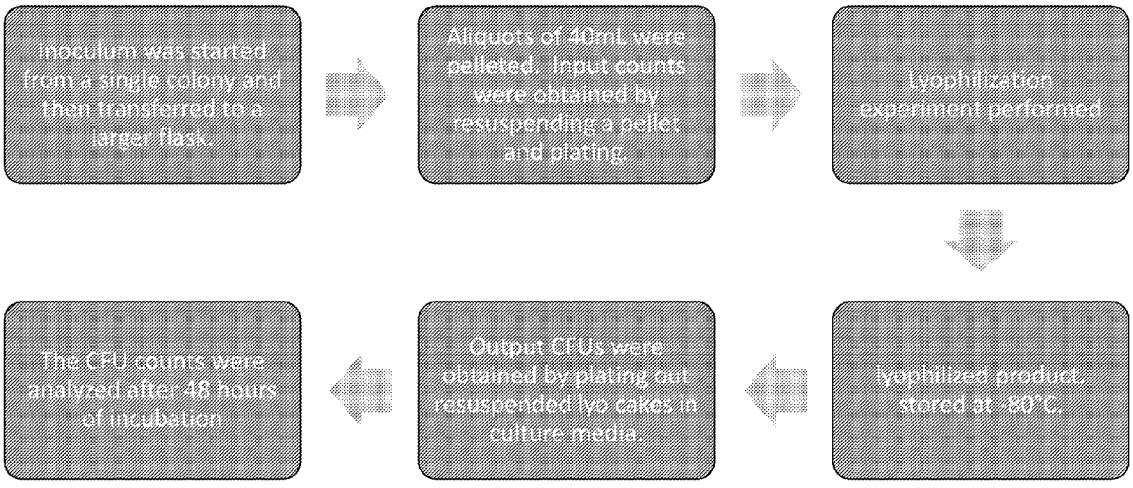
(54) Title: METHODS AND COMPOSITIONS FOR PRESERVING BACTERIA

Figure 1



(57) Abstract: The disclosure provides methods and compositions for the preservation of bacteria.

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METHODS AND COMPOSITIONS FOR PRESERVING BACTERIA

RELATED APPLICATION

This application claims the benefit under 35 U.S.C § 119(e) of U.S. provisional application number 62/414489, filed October 28, 2016, which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

The disclosure provides methods and compositions for the preservation of bacteria.

BACKGROUND

The human intestinal microbiome includes a large number of microorganisms. A significant number of these microorganisms are anaerobic bacteria. Compositions that include anaerobic bacteria that originated from the human intestinal microbiome have shown potential in the treatment of human disease (See *e.g.,* Atarashi et al., *Nature* 500, 232, 2013; Atarashi et al., *Cell* 163, 1, 2015; Mathewson et al., *Nature Immunology* 17, 505, 2016). Anaerobic bacteria are challenging to preserve because of their sensitivity to oxygen. Improved compositions and methods for the preservation of anaerobic bacteria are needed therefore.

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

SUMMARY

Provided herein are compositions and methods for the preservation of bacteria. Bacteria can be preserved through lyophilization (freeze-drying), which allows for the long-term storage of bacteria, including therapeutic amounts of bacteria.

In one aspect, the disclosure provides a composition comprising a disaccharide, yeast extract, cysteine, sodium metabisulfite, and a buffer, wherein the buffer is at a pH of 6.7 to 7.3, wherein the disaccharide is not lactose. In one aspect, the disclosure provides a method for preserving bacteria, the method comprising: adding bacteria from the class Clostridia to a composition of the present invention; and subjecting the composition comprising the bacteria to a lyophilization cycle.

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In one aspect, the disclosure provides methods for the lyophilization of bacterial compositions. In one aspect, the disclosure provides compositions that allow for the lyophilization of anaerobic bacterial strains. Prior to the current disclosure, compositions comprising such bacterial strains would lose all, or most, of their viability upon lyophilization, severely impeding the options for preserving such bacterial strains in amounts sufficient for therapeutic applications. The compositions and methods provided herein allow for the first time the preservation of bacterial strains through lyophilization. The compositions disclosed herein are thought to have these desired preservative properties because of the combination of specific

lyoprotectant (s), nutrient(s), and/or excipient(s). In addition or alternatively, the lyophilization cycle, such as the temperature ramp rate, may also contribute to the beneficial preservation properties of the compositions and methods described herein.

Provided herein are compositions and methods for the preservation of bacteria. In one aspect, the disclosure provides a composition comprising a lyoprotectant, a nutrient, an antioxidant, and a buffer. In some embodiments of the compositions provided herein, the lyoprotectant is a sugar. In some embodiments of the compositions provided herein, the sugar is a disaccharide. In some embodiments of the compositions provided herein, the disaccharide is sucrose. In some embodiments of the compositions provided herein, the sucrose is at a concentration of sucrose is between 6.0% and 10.0%. In some embodiments of the compositions provided herein, the sucrose is at a concentration between 7.0% and 8.0%.

In some embodiments of the compositions provided herein, the disaccharide is trehalose. In some embodiments of the compositions provided herein, the trehalose is at a concentration between 6.0% and 10.0%. In some embodiments of the compositions provided herein, the trehalose is at a concentration between 7.0% and 8.0%.

In some embodiments of the compositions provided herein, the nutrient is yeast extract, Luria-Bertani broth, or plant peptone. In some embodiments of the compositions provided herein, the nutrient is yeast extract. In some embodiments of the compositions provided herein, the concentration of the yeast extract is between 0.5% and 2.0%.

In some embodiments of the compositions provided herein, the antioxidant is inulin, riboflavin, or cysteine. In some embodiments of the compositions provided herein, the antioxidant is cysteine. In some embodiments of the compositions provided herein, the concentration of cysteine is between 0.01% and 0.5%.

In some embodiments of the compositions provided herein, the buffer is a histidine buffer or a tris buffer. In some embodiments of the compositions provided herein, the buffer is a histidine buffer. In some embodiments of the compositions provided herein, the buffer is about pH 7.0. In some embodiments of the compositions provided herein, the buffer is at a concentration between 10 mM and 50 mM.

In some embodiments of the compositions provided herein, the composition has been reduced.

In some embodiments of the compositions provided herein, the composition includes trehalose, yeast extract, cysteine, and a histidine buffer. In some embodiments of the compositions provided herein, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine and 20 mM histidine buffer.

In some embodiments of the compositions provided herein, the composition includes sucrose, yeast extract, cysteine and a histidine buffer. In some embodiments of the compositions provided herein, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine and 20 mM histidine buffer.

Any of the compositions described herein may further comprise an excipient. In some embodiments, the excipient is a stabilizing agent. In some embodiments, the stabilizing agent is a reducing agent. In some embodiments, the reducing agent is sodium metabisulfite. In some embodiments, the sodium metabisulfite is 0.05%.

In some embodiments of the compositions provided herein, the composition includes trehalose, yeast extract, cysteine, a histidine buffer, and an excipient. In some embodiments of the compositions provided herein, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite. In some embodiments of the compositions provided herein, the composition includes sucrose, yeast extract, cysteine, a histidine buffer, and an excipient. In some embodiments of the compositions provided herein, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite.

In some embodiments of the compositions provided herein, the composition includes bacteria of one or more families, classes, genera and/or species. In some embodiments of the compositions provided herein, the bacteria are anaerobic bacteria. In some embodiments of the compositions provided herein, the anaerobic bacteria are strict anaerobic bacteria. In some embodiments of the compositions provided herein, the anaerobic bacteria are facultative anaerobic bacteria.

In some embodiments of the compositions provided herein, the bacteria comprise one or more bacterial strains belonging to the class Clostridia. In some embodiments of the compositions provided herein, the bacteria comprise one or more bacterial strains belonging to the family Clostridiaceae. In some embodiments of the compositions provided herein, the bacteria comprise one or more bacterial strains belonging to the genus *Clostridium*. In some embodiments of the compositions provided herein, the bacteria comprise one or more

bacterial strains selected from the group consisting of *Clostridium bolteae*, *Anaerotruncus colihominis*, *Ruminococcus torques*, *Clostridium symbiosum*, *Blautia producta*, *Dorea longicatena*, *Erysipelotrichaceae bacterium*, and *Subdolinogranulum spp.* In some embodiments, the one or more bacterial strains comprise one or more 16s rRNA sequences having at least 97% sequence identity with the nucleic acid sequences selected from the group consisting of SEQ ID NOs: 1-8. In some embodiments of the compositions provided herein, the composition includes at least 1 x 10^8 colony forming units of bacteria per milliliter of the composition.

In some embodiments of the compositions provided herein, the composition is a stabilizing composition. In some embodiments of the compositions provided herein, a stabilizing composition allows for the recovery of at least 1%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or up to 100% of the colony forming units of the bacteria over a period of time. In some embodiments, the period of time is at least 1 week, at least 2 weeks, at least 4 weeks, at least 2 months, at least 3 months, at least 6 months, or at least 1 year or more.

In some embodiments of the compositions provided herein, a stabilizing composition allows for the recovery of at least 1%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or up to 100% of the colony forming units after a specific event. In some embodiments, the specific event is one or more freeze-thaw cycle or lyophilization cycle.

In one aspect, the disclosure provides methods for preserving bacteria. In some embodiments of the methods provided herein, the method includes adding bacteria to a composition of any of the preceding claims and subjecting the composition including the bacteria to a lyophilization cycle. In some embodiments, the lyophilization cycle comprises one or more steps of a temperature ramp rate between 0.5°C/min to 3°C/min. In some embodiments, the lyophilization cycle comprises one or more steps of a temperature ramp rate is 2.5°C/min. In some embodiments, the composition further comprises assessing the bacteria in a sensitivity assay prior to adding the bacteria to the composition. In some embodiments, the sensitivity assay is a Gram stain or freeze-thaw assay. In some embodiments, if the bacteria are assessed to be sensitive, (i) an excipient is added to the composition; and/or (ii) the lyophilization cycle comprises one or more steps of a temperature ramp rate of 2.5°C/min. In some embodiments, the excipient is a stabilizing agent. In some

embodiments, the stabilizing agent is a reducing agent. In some embodiments, the reducing agent is sodium metabisulfite. In some embodiments, the sodium metabisulfite is 0.05%.

In some embodiments of the methods provided herein, the method further includes measuring the number of colony forming units after subjecting the composition comprising the bacteria to the lyophilization cycle. In some embodiments of the methods provided herein, the method further includes measuring the number of colony forming units prior to subjecting the composition comprising the bacteria to the lyophilization cycle. In some embodiments of the method provided herein, the method further comprises comparing the number of colony forming units prior to subjecting the composition comprising the bacteria to the lyophilization cycle and the number of colony forming units after subjecting the composition comprising the bacteria to the lyophilization cycle, and determining a level of preservation. In some embodiments of the methods provided herein, the method results in the preservation of at least 1%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, up to 100% of the colony forming units.

In one aspect, the disclosure provides methods for generating the compositions provided herein. In some embodiments of the methods provided herein, the method includes creating a mixture by combining the lyoprotectant, the nutrient, the antioxidant, and the buffer, reducing the mixture thereby generating the composition. In some embodiments of the methods provided herein, further comprising adding an excipient to the mixture. In some embodiments of the methods provided herein, the excipient is a stabilizing agent. In some embodiments of the methods provided herein, the stabilizing agent is a reducing agent. In some embodiments of the methods provided herein, the reducing agent is sodium metabisulfite. In some embodiments of the methods provided herein, the method further comprises adding bacteria to the mixture.

In some embodiments of the methods provided herein, the bacteria are strict anaerobic bacteria. In some embodiments of the methods provided herein, the bacteria comprise one or more bacterial strains belonging to the class Clostridia. In some embodiments of the methods provided herein, the bacteria comprise one or more bacterial strains belonging to the family Clostridiaceae. In some embodiments of the methods provided herein, the bacteria comprise one or more bacterial strains belonging to the genus Clostridium.

In some embodiments of the methods provided herein, the bacteria comprise one or more bacterial strains selected from the group consisting of *Clostridium bolteae*, *Anaerotruncus colihominis*, *Ruminococcus torques*, *Clostridium symbiosum*, *Blautia producta*, *Dorea longicatena*, *Erysipelotrichaceae bacterium*, and *Subdolinogranulum spp.*

In some embodiments of the methods provided herein, the bacteria comprise one or more bacterial strains comprise 16S rDNA sequences having at least 97% sequence identity with the nucleic acid sequences selected from the group consisting of SEQ ID NOs: 1-8.

In some embodiments of the methods provided herein, the method further comprises assessing the bacteria in a sensitivity assay prior to adding the bacteria to the mixture. In some embodiments of the methods provided herein, the sensitivity assay is a Gram stain or a freeze-thaw assay. In some embodiments of the methods provided herein, if the bacteria are assessed to be sensitive, an excipient is added to the mixture.

In one aspect, the disclosure provides compositions comprising 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite. In one aspect, the disclosure provides compositions comprising 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite.

In one aspect, the disclosure provides compositions comprising 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and one or more bacterial strain belonging to the class Clostridia. In one aspect, the disclosure provides compositions comprising 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and one or more bacterial strain belonging to the class Clostridia. In one aspect, the disclosure provides compositions comprising 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and one or more bacterial strain belonging to the class Clostridia. In one aspect, the disclosure provides compositions comprising 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and one or more bacterial strain belonging to the class Clostridia.

These and other aspects of the invention, as well as various embodiments thereof, will become more apparent in reference to the drawings and detailed description of the invention.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention.

This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are not intended to be drawn to scale. The figures are illustrative only and are not required for enablement of the disclosure. For purposes of clarity, not every component may be labeled in every drawing. In the drawings:

Figure 1 shows a schematic overview of example experiments described in the Examples.

Figure 2 shows a table including formulations described in Example 1.

Figure 3 shows photographs of representative lyophilization cakes generated in Example 1.

Figure 4 shows a table including example formulations used in Example 1.

Figure 5 shows a table including results described in Example 3.

Figure 6 shows a graph of the pressure during the lyophilization cycle described in Example 5.

DETAILED DESCRIPTION

The preservation of bacterial compositions, including anaerobic bacteria, has been challenging. While bacteria can be frozen down and regrown on plates or in solution, it has been difficult to standardize this process. There is a need to preserve bacteria that can be used for therapeutic purposes. Preservation processes, such as cryopreservation and lyophilization, have been well established for aerobic bacteria, and many factors that affect survival and recovery of aerobic bacteria in the preservation process are understood (Prakash

et al. *FEMS Microbiol Lett* (2013)339:1-9). However, the development of preservation processes for use with anaerobic bacteria are not sufficient. Such development and research using anaerobic bacteria is hindered by the significant difficulties of working with anaerobic bacteria (Mori et al. "The Challenges of Studying the Anaerobic Microbial World" *Microbes Environ.* (2014) 29(4) 335-337). Given that anaerobic bacteria, such as bacterial strains obtained from the human intestinal microbiome have shown potential in the treatment of human disease, improved methods for preserving anaerobic bacteria that allow for high levels of bacterial recovery are needed.

Lyophilization is a recognized process for the preservation of peptides and proteins, and may be used in the preparation of therapeutic compositions to be resuspended and administered to subjects. However, lyophilization of bacterial compositions, in particular anaerobic bacteria, has been challenging. This disclosure, for the first time provides compositions that allow for the lyophilization of anaerobic bacteria without a loss of viability. The disclosure also provides compositions and methods for preserving bacteria that are considered to be sensitive and difficult to preserve without substantial loss of viability. The disclosure teaches that formulations that include certain lyoprotectants, such as disaccharides *e.g.,* trehalose and sucrose, allow for the preservation of anaerobic bacteria, while closely related lyoprotectants, such as mannitol and sorbitol, do not.

Further, many proposed methods for generating lyophilized compositions containing bacteria include animal-derived products. For example, Staab et al. report freeze-drying (lyophilizing) anaerobic bacteria using chopped meat carbohydrate broth supplemented with 12% sucrose or double strength skim milk (Staab et al. *Cryobiology* (1987)24:174-178). Phillips et al. demonstrated that many of the anaerobic rumen bacteria tested retained viability after drying in horse serum supplemented with glucose (Philips, et al. *J. Appl. Bact.* (1975)38:319-322). Finally, Šourek recommends a mixture of calf serum or defibrinated sheep blood and lactose for lyophilization of most bacteria (Šourek, *Int. J. Sys. Bacteriol.* (1974) 24(3):358-365). Important for administration of therapeutic products to human subjects, the compositions described herein do not include animal products or animal-derived products.

This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being

carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including," "comprising," or "having," "containing," "involving," and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

Provided herein are compositions and methods for the preservation of bacteria. In one aspect, the disclosure provides a composition comprising a lyoprotectant, a nutrient, an antioxidant, and a buffer. In some embodiments, the compositions also comprise an excipient, such as a stabilizing agent. In some embodiments of the compositions provided herein, the lyoprotectant is a sugar. In some embodiments of the compositions provided herein, the sugar is a disaccharide, such as sucrose, trehalose, lactose, maltose, cellobiose, chitobiose, or lactulose. In some embodiments, the composition does not include mannitol. In some embodiments, the composition does not include sorbitol.

In some embodiments of the compositions provided herein, the disaccharide is sucrose. In some embodiments of the compositions provided herein, the sucrose is at a concentration between 6.0% and 10.0%, inclusive, such as 6.0%, 6.1%, 6.2%, 6.3%, 6.4%, 6.5%, 6.6%, 6.7%, 6.8%, 6.9%, 7.0%, 7.1%, 7.2%, 7.3%, 7.4%, 7.5%, 7.6%, 7.7%, 7.8%, 7.9%, 8.0%, 8.1%, 8.2%, 8.3%, 8.4%, 8.5%, 8.6%, 8.7%, 8.8%, 8.9%, 9.0%, 9.1%, 9.2%, 9.3%, 9.4%, 9.5%, 9.6%, 9.7%, 9.8%, 9.9%, 9.0%, or 10.0%. In some embodiments of the compositions provided herein, the sucrose is at a concentration between 7.0% and 8.0%, inclusive, such as 7.0%, 7.1%, 7.2%, 7.3%, 7.4%, 7.5%, 7.6%, 7.7%, 7.8%, 7.9%, or 8.0%.

In some embodiments of the compositions provided herein, the disaccharide is trehalose. In some embodiments of the compositions provided herein, the trehalose is at a concentration between 6.0% and 10.0%, inclusive, such as 6.0%, 6.1%, 6.2%, 6.3%, 6.4%, 6.5%, 6.6%, 6.7%, 6.8%, 6.9%, 7.0%, 7.1%, 7.2%, 7.3%, 7.4%, 7.5%, 7.6%, 7.7%, 7.8%, 7.9%, 8.0%, 8.1%, 8.2%, 8.3%, 8.4%, 8.5%, 8.6%, 8.7%, 8.8%, 8.9%, 9.0%, 9.1%, 9.2%, 9.3%, 9.4%, 9.5%, 9.6%, 9.7%, 9.8%, 9.9%, 9.0%, or 10.0%. In some embodiments of the compositions provided herein, the trehalose is at a concentration between 7.0% and 8.0%, inclusive, such as 7.0%, 7.1%, 7.2%, 7.3%, 7.4%, 7.5%, 7.6%, 7.7%, 7.8%, 7.9%, or 8.0%.

In some embodiments of the compositions provided herein, the nutrient is yeast extract, Luria-Bertani broth, or plant peptone. In some embodiments of the compositions provided herein, the nutrient is yeast extract. In some embodiments of the compositions provided herein, the concentration of the yeast extract is between 0.5% and 2.0%, inclusive,

such as 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, or 2.0%. In some embodiments, the composition comprises a nutrient that is not an animal product. In some embodiments, the composition comprises a nutrient that is not animal blood. In some embodiments, the composition does not include a nutrient.

In some embodiments of the compositions provided herein, the antioxidant is inulin, riboflavin or cysteine. In some embodiments of the compositions provided herein, the antioxidant is cysteine. In some embodiments of the compositions provided herein, the concentration of cysteine is between 0.01% and 0.5%, inclusive, such as 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.11%, 0.12%, 0.13%, 0.14%, 0.15%, 0.16%, 0.17%, 0.18%, 0.19%, 0.20%, 0.21%, 0.22%, 0.23%, 0.24%, 0.25%, 0.26%, 0.27%, 0.28%, 0.29%,0.30%, 0.31%, 0.32%, 0.33%, 0.34%, 0.35%, 0.36%, 0.37%, 0.38%, 0.39%, 0.40%, 0.41%, 0.42%, 0.43%, 0.44%, 0.45%, 0.46%, 0.47%, 0.48%, 0.49%, or 0.50%.

In some embodiments, an antioxidant other than, or in addition to, cysteine is added to the composition. Antioxidants that can be added to the composition other than, or in addition to, cysteine include inulin, riboflavin, ascorbic acid (vitamin C), tocopherol, tocotrienol, vitamin E, carotenoids, carotene, provitamin A, vitamin A, propyl gallate, tertiary butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene, ubiquinol, glutathione, thiols, polyphenol, catechols, titilazad, NXY-059 (disufenton sodium, Cerovive), oxalic acid, phytic acid, tannins, eugenol, lipoic acid, uric acid, coenzyme Q, melatonin, and combinations thereof. In some embodiments, the composition does not include an antioxidant.

In some embodiments of the compositions provided herein, the buffer is a histidine buffer or a tris buffer (Tris(hydroxymethyl)aminomethane; also known as THAM; 2-Amino-2-(hydroxymethyl)-1,3-propanediol; Tromethamine; or Trometamol). In some embodiments of the compositions provided herein, the buffer is a histidine buffer. In some embodiments of the compositions provided herein, the buffer is about pH 7.0, such as 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, or 7.3. In some embodiments of the compositions provided herein, the concentration of the buffer is between 10 mM and 50 mM, inclusive, such as 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM, 30 mM, 31 mM, 32 mM, 33 mM, 34 mM, 35 mM, 36 mM, 37 mM, 38 mM, 39 mM, 40 mM, 41 mM, 42 mM, 43 mM, 44 mM, 45 mM, 46 mM,

47 mM, 48 mM, 49 mM, or 50 mM. In some embodiments, the composition does not include a buffer.

In some embodiments of the compositions provided herein, the composition includes an excipient. In some embodiments, the excipient is a stabilizing agent. In some embodiments of the compositions provided herein, the stabilizing agent is a reducing agent, chelating agent, acid amino acid, basic amino acid, or neutral surfactant, or polymer. In some embodiments, the excipient is a stabilizing agent. In some embodiments of the compositions provided herein, the stabilizing agent is present in the composition at a concentration between 0.01% and 0.1%, inclusive, such as 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, or 0.1%. In some embodiments, the composition does not include an excipient.

In some embodiments, the stabilizing agent is a reducing agent. In some embodiments, the reducing agent is sodium metabisulfite. In some embodiments, the composition comprises sodium metabisulfite between 0.01% and 0.1%, inclusive, such as 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, or 0.1%. In some embodiments, the composition comprises sodium metabisulfite at 0.05%.

In some embodiments, the reducing agent is ascorbic acid. In some embodiments, the reducing agent is citric acid. In some embodiments, the chelating agent is citric acid.

In some embodiments, the stabilizing agent is an acidic amino acid. In some embodiments, the acidic amino acid is sodium glutamate. In some embodiments, the stabilizing agent is a basic amino acid. In some embodiments, the basic amino acid is arginine.

In some embodiments, the stabilizing agent is a neutral surfactant. In some embodiments, the neutral surfactant is polaxamer. In some embodiments, the stabilizing agent is a polymer. In some embodiments, the polymer is nonionic triblock copolymer. In some embodiments, the polymer is polaxamer. In some embodiments, the polymer is polyvinylpyrrolidone (*e.g.*, KOLLIDON®).

In some embodiments, the excipient is not a polymer.

In some embodiments of the compositions provided herein, the composition has been reduced. Methods for reducing a composition are known in the art and include bringing the composition into an anaerobic environment and exposing the composition to the mixed gas atmosphere in the anaerobic chamber.

In some embodiments of the compositions provided herein, the composition includes sucrose, yeast extract, cysteine, and a histidine buffer. In some embodiments of the compositions provided herein, the composition includes sucrose at a concentration between 7.0% and 8.0%, 1% yeast extract, 0.05% cysteine, and 20 mM histidine buffer. In some embodiments of the compositions provided herein, the composition comprises 7.0% sucrose, 1% yeast extract, 0.05% cysteine, and 20 mM histidine buffer. In some embodiments, the composition also includes a bacterial strain. In some embodiments, the bacterial strain is an anaerobic bacterial strain.

In some embodiments of the compositions provided herein, the composition includes sucrose, yeast extract, cysteine, a histidine buffer, and an excipient. In some embodiments of the compositions provided herein, the composition includes sucrose at a concentration between 7.0% and 8.0%, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite. In some embodiments of the compositions provided herein, the composition comprises 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite. In some embodiments, the composition also includes a bacterial strain. In some embodiments, the bacterial strain is an anaerobic bacterial strain. In some embodiments, the bacterial strain belongs to the class Clostridia.

In some embodiments of the compositions provided herein, the composition includes trehalose, yeast extract, cysteine, and a histidine buffer. In some embodiments of the compositions provided herein, the composition includes trehalose at a concentration between 7.0% and 8.0%, 1% yeast extract, 0.05% cysteine, and 20 mM histidine buffer. In some embodiments of the compositions provided herein, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, and 20 mM histidine buffer. In some embodiments, the composition also includes a bacterial strain. In some embodiments, the bacterial strain is an anaerobic bacterial strain.

In some embodiments of the compositions provided herein, the composition includes trehalose, yeast extract, cysteine, a histidine buffer, and an excipient. In some embodiments of the compositions provided herein, the composition includes trehalose at a concentration between 7.0% and 8.0%, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite. In some embodiments of the compositions provided herein, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite. In some embodiments, the composition also

includes a bacterial strain. In some embodiments, the bacterial strain is an anaerobic bacterial strain.

In one aspect, the compositions provided herein allow for preservation of bacteria. The compositions allow the bacteria to go through a freeze-dry cycle with a minimal loss to viability. In some embodiments of the compositions provided herein, the composition includes bacteria. In some embodiments, the composition includes one or more bacterial strains. In some embodiments of the compositions provided herein, the bacteria are anaerobic bacteria (*e.g.*, strict anaerobic bacteria). In some embodiments of the compositions provided herein, the anaerobic bacteria are strict anaerobic bacteria. In some embodiments of the compositions provided herein, the bacteria are from the class Clostridia. In some embodiments of the compositions provided herein, the bacteria are from the family Clostridiaceae. In some embodiments of the compositions provided herein, the bacteria are from the genus *Clostridium*. In some embodiments of the compositions provided herein, the bacteria belong to Clostridium cluster IV, XIVa, XVI, XVII, or XVIII. In some embodiments of the compositions provided herein, the bacteria belong to Clostridium cluster IV, XIVa, or XVII. In some embodiments of the compositions provided herein, the bacteria belong to Clostridium cluster IV or XIVa.

In some embodiments of the compositions provided herein, the composition includes one or more of the following bacterial strains: *Clostridium bolteae*, *Anaerotruncus colihominis*, *Eubacterium fissicatena*, *Clostridium symbiosum*, *Blautia producta*, *Dorea longicatena, Erysipelotrichaceae bacterium* and *Subdolinogranulum spp*. In some embodiments of the compositions provided herein, the composition includes one or more of the following bacterial strains: *Clostridium bolteae 90A9*, *Anaerotruncus colihominis DSM17241*, *Sellimonas intestinalis, Clostridium bacterium UC5.1-1D4*, *Dorea longicatena CAG:42, Erysipelotrichaceae bacterium 21-3,* and *Clostridium orbiscindens 1_3_50AFAA* In some embodiments of the compositions provided herein, the composition includes two or more (*e.g.*, 2, 3, 4, 5 6, 7, or 8) of the following bacterial strains: *Clostridium bolteae*, *Anaerotruncus colihominis*, *Eubacterium fissicatena*, *Clostridium symbiosum*, *Blautia producta*, *Dorea longicatena, Erysipelotrichaceae bacterium* and *Subdolinogranulum spp.* In some embodiments, the composition includes *Clostridium bolteae.* In some embodiments, the composition includes *Anaerotruncus colihominis.* In some embodiments, the composition includes *Eubacterium fissicatena.* In some embodiments, the composition includes

Clostridium symbiosum. In some embodiments, the composition includes *Blautia producta.* In some embodiments, the composition includes *Dorea longicatena.* In some embodiments, the composition includes *Erysipelotrichaceae bacterium.* In some embodiments, the composition includes *Subdolinogranulum spp.*

In one aspect, as shown herein (*e.g.*, in the Examples) the compositions and methods provided herein allow for the stabilization and preservation of anaerobic bacterial strains. In one aspect, as shown herein (*e.g.*, in the Examples) the compositions and methods provided herein allow for the stabilization and preservation of anaerobic bacterial strains belonging to Clostridium cluster IV, XIVa, or XVII. In one aspect, as shown herein (*e.g.*, in the Examples) the compositions and methods provided herein allow for the stabilization and preservation of anaerobic bacterial strains *Clostridium bolteae*, *Anaerotruncus colihominis*, *Eubacterium fissicatena*, *Clostridium symbiosum*, *Blautia producta*, *Dorea longicatena, Erysipelotrichaceae bacterium* and *Subdolinogranulum spp*. The exemplary bacterial strains of compositions disclosed herein can also be identified by their 16s rRNA sequences (SEQ ID NOs: 1-8). Identifying bacteria by their sequences furthermore allows for the identification of additional bacterial strains that are identical or highly similar to the exemplified bacteria. For instance, the 16s rRNA sequences of bacterial strains were used to identify the closest relative (based on percent identity) through whole genome sequencing and by comparing these sequences with 16S databases (Table 1). In addition, based on whole genome sequencing and comparing of the whole genome to whole genome databases, the bacterial strains having 16S rRNA sequences provided by SEQ ID NOs: 1-8 are most closely related to the following bacterial species: *Clostridium bolteae 90A9*, *Anaerotruncus colihominis DSM 17241*, *Dracourtella massiliensis GD1*, *Clostridium symbiosum WAL-14163*, *Clostridium bacterium UC5.1-1D4*, *Dorea longicatena CAG:42*, *Erysipelotrichaceae bacterium 21_3*, and *Clostridium orbiscindens 1_3_50AFAA* (see, e.g., Table 1). Thus, in one aspect it should be appreciated that each row of Table 1, the bacterial strains are highly similar and/or are identical. In some embodiments, in context of the instant disclosure the names of bacterial strains within a row of Table 1 can be used interchangeably.

Table 1: Examples of Bacterial species of the compositions disclosed herein

Strain number	SEQ ID NO:	Closest species based on Sanger sequencing of 16S region	Closest species based on Consensus SEQ ID # of 16S region as compared with 16S database	Closest species based on WGS compared versus WG databases	Additional closely related sequences	Clostridium cluster
1	1	Clostridium bolteae	Clostridium bolteae	Clostridium bolteae 90A9		XIVa
2	2	Anaerotruncus colihominis	Anaerotruncus colihominis	Anaerotruncus colihominis DSM 17241		IV
3	3	Eubacterium fissicatena	Dracourtella massiliensis	Dracourtella massiliensis GD1	Ruminococcus torques; Sellimonas intestinalis	XIVa
4	4	Clostridium symbiosum	Clostridium symbiosum	Clostridium symbiosum WAL-14163		XIVa
5	5	Blautia producta	Blautia producta	Clostridium bacterium UC5.1-1D4	Blautia product ATCC 27340	XIVa
6	6	Dorea longicatena	Dorea longicatena	Dorea longicatena CAG:42		XIVa
7	7	Clostridium innocuum	Clostridium innocuum	Erysipelotrichaceae bacterium 21_3		XVII
8	8	Flavinofractor plautii	Flavinofractor plautii	Clostridium orbiscindens 1_3_50AFAA	Subdolinogranulum	IV

In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Clostridium bolteae.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Anaerotruncus colihominis.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Eubacterium fissicatena.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Clostridium symbiosum.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Blautia producta.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Dorea longicatena.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Erysipelotrichaceae bacterium.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer and *Subdolinogranulum spp.*

In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Clostridium bolteae.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Anaerotruncus colihominis.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Eubacterium fissicatena.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Clostridium symbiosum.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Blautia producta.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Dorea longicatena.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer and *Erysipelotrichaceae bacterium.* In some embodiments, the composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Subdolinogranulum spp.*

In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Clostridium bolteae.* In some embodiments, the

composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Anaerotruncus colihominis*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Eubacterium fissicatena*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Clostridium symbiosum*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Blautia producta*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Dorea longicatena*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Erysipelotrichaceae bacterium*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Subdolinogranulum spp.*

In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Clostridium bolteae*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Anaerotruncus colihominis*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Eubacterium fissicatena*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Clostridium symbiosum*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Blautia producta*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Dorea longicatena*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Erysipelotrichaceae bacterium*. In some embodiments, the composition includes 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Subdolinogranulum spp.*

Aspects of the disclosure relate to bacterial strains with 16S rDNA sequences that have homology to a nucleic acid sequence of any one of the sequences of the bacterial strains or species described herein. In some embodiments, the bacterial strain has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% homology relative to any of the strains or bacterial species described herein over a specified region of nucleic acid or amino acid

sequence or over the entire sequence. It would be appreciated by one of skill in the art that the term "homology" or "percent homology," in the context of two or more nucleic acid sequences or amino acid sequences, refers to a measure of similarity between two or more sequences or portion(s) thereof. The homology may exist over a region of a sequence that is at least about 50 nucleotides in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides in length. In some embodiments, the homology exists over the length the 16S rRNA or 16S rDNA sequence, or a portion thereof.

In some embodiments of the compositions provided herein, the composition includes one or more bacterial strains, wherein the one or more bacterial strains include one or more 16s rDNA sequences having at least 97% homology with nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% homology with nucleic acid sequences SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or up to 100% homology with nucleic acid sequences SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8.

In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% homology with nucleic acid sequences SEQ ID NO:1. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% homology with nucleic acid sequences SEQ ID NO:2. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% homology with nucleic acid sequences SEQ ID NO:3. In some embodiments of the compositions

provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% homology with nucleic acid sequences SEQ ID NO:4. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% homology with nucleic acid sequences SEQ ID NO:5. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% homology with nucleic acid sequences SEQ ID NO:6. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% homology with nucleic acid sequences SEQ ID NO:7. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% homology with nucleic acid sequences SEQ ID NO:8.

Additionally, or alternatively, two or more sequences may be assessed for the identity between the sequences. The terms "identical," percent "identity" in the context of two or more nucleic acids or amino acid sequences, refer to two or more sequences or subsequences that are the same. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (*e.g.*, at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity) over a specified region of a nucleic acid or amino acid sequence or over an entire sequence, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides in length. In some embodiments, the identity exists over the length the 16S rRNA or 16S rDNA sequence.

In some embodiments of the compositions provided herein, the composition includes one or more bacterial strains, wherein the one or more bacterial strains include one or more 16s rDNA sequences having at least 97% sequence identity with nucleic acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8. In some embodiments of the compositions provided

herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% sequence identity with nucleic acid sequences SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or up to 100% sequence identity with nucleic acid sequences SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8.

In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% sequence identity with nucleic acid sequences SEQ ID NO: 1. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% sequence identity with nucleic acid sequences SEQ ID NO: 2. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% sequence identity with nucleic acid sequences SEQ ID NO: 3. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% sequence identity with nucleic acid sequences SEQ ID NO:4. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% sequence identity with nucleic acid sequences SEQ ID NO:5. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% sequence identity with nucleic acid sequences SEQ ID NO:6. In some embodiments of the compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% sequence identity with nucleic acid sequences SEQ ID NO:7. In some embodiments of the

compositions provided herein, the composition includes one bacterial strain, wherein the bacterial strain includes one or more 16s rDNA sequences having at least 97% sequence identity with nucleic acid sequences SEQ ID NO:8.

Additionally, or alternatively, two or more sequences may be assessed for the alignment between the sequences. The terms " alignment " or percent " alignment " in the context of two or more nucleic acids or amino acid sequences, refer to two or more sequences or subsequences that are the same. Two sequences are "substantially aligned" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (e.g., at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical) over a specified region of the nucleic acid or amino acid sequence or over the entire sequence, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the alignment exists over a region that is at least about 50 nucleotides in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides in length. In some embodiments, the identity exists over the length the 16S rRNA or 16S rDNA sequence.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. Methods of alignment of sequences for comparison are well known in the art. See, *e.g.*, by the local homology algorithm of Smith and Waterman (1970) *Adv. Appl. Math.* 2:482c, by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* (1970) 48:443, by the search for similarity method of Pearson and Lipman. *Proc. Natl. Acad. Sci. USA* (1998) 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group. Madison. WI), or by manual alignment and visual inspection (see. e.g., Brent et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (Ringbou ed., 2003)). Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., *Nuc. Acids Res.* (1977) 25:3389-3402, and Altschul et al., *J. Mol. Biol.* (1990) 215:403-410, respectively.

It should be appreciated that the terms “bacteria” and “bacterial strains” as used herein are interchangeable.

In some embodiments, the bacterial strains are grown up from a single colony. In some embodiments, the bacterial strains are purified bacterial strains. As used herein, the term "purified" refers to a bacterial strain or composition comprising such that has been separated from one or more components, such as contaminants. In some embodiments, the bacterial strain is substantially free of contaminants. In some embodiments, one or more bacterial strains of a composition may be independently purified from one or more other bacteria produced and/or present in a culture or a sample containing the bacterial strain. In some embodiments, a bacterial strain is isolated or purified from a sample and then cultured under the appropriate conditions for bacterial replication, *e.g.*, under anaerobic culture conditions. The bacteria that is grown under appropriate conditions for bacterial replication can subsequently be isolated/purified from the culture in which it is grown.

The bacterial strains of the composition can be manufactured using fermentation techniques well known in the art. In some embodiments, the active ingredients are manufactured using anaerobic fermenters, which can support the rapid growth of anaerobic bacterial strains. The anaerobic fermenters may be, for example, stirred tank reactors or disposable wave bioreactors. Culture media such as BL media and EG media, or similar versions of these media devoid of animal components, can be used to support the growth of the bacterial species. The bacterial product can be purified and concentrated from the fermentation broth by traditional techniques, such as centrifugation and filtration. Generally, the bacteria are pelleted prior to introducing the bacteria in the composition that already includes the lyoprotectant, nutrient, buffer, and antioxidant. In some embodiments, the bacteria are pelleted prior to introducing the bacteria in the composition that already includes the lyoprotectant, nutrient, buffer, antioxidant, and excipient (*e.g.*, reducing agent).

In some embodiments, the compositions disclosed herein contain about 10, about 10^2, about 10^3, about 10^4, about 10^5, about 10^6, about 10^7, about 10^8, about 10^9, about 10^{10}, about 10^{11}, about 10^{12}, about 10^{13} or more bacteria. In some embodiments, the compositions disclosed herein contain about 10, about 10^2, about 10^3, about 10^4, about 10^5, about 10^6, about 10^7, about 10^8, about 10^9, about 10^{10}, about 10^{11}, about 10^{12}, about 10^{13} or more bacteria per milliliter. It should be appreciated that some of the bacteria may not be viable. In some embodiments, the compositions disclosed herein contain about 10, about 10^2, about 10^3, about 10^4, about 10^5, about 10^6, about 10^7, about 10^8, about 10^9, about 10^{10}, about 10^{11}, about 10^{12}, about 10^{13} or more colony forming units (cfus) of bacteria. In some

embodiments, the compositions disclosed herein contain about 10, about 10^2, about 10^3, about 10^4, about 10^5, about 10^6, about 10^7, about 10^8, about 10^9, about 10^{10}, about 10^{11}, about 10^{12}, about 10^{13} or more colony forming units (cfus) of bacteria per milliliter.

In some embodiments, the compositions disclosed herein contain between 10 and 10^{13}, between 10^2 and 10^{13}, between 10^3 and 10^{13}, between 10^4 and 10^{13}, between 10^5 and 10^{13}, between 10^6 and 10^{13}, between 10^7 and 10^{13}, between 10^8 and 10^{13}, between 10^9 and 10^{13}, between 10^{10} and 10^{13}, between 10^{11} and 10^{13}, between 10^{12} and 10^{13}, between 10 and 10^{12}, between 10^2 and 10^{12}, between 10^3 and 10^{12}, between 10^4 and 10^{12}, between 10^5 and 10^{12}, between 10^6 and 10^{12}, between 10^7 and 10^{12}, between 10^8 and 10^{12}, between 10^9 and 10^{12}, between 10^{10} and 10^{12}, between 10^{11} and 10^{12}, between 10 and 10^{11}, between 10^2 and 10^{11}, between 10^3 and 10^{13}, between 10^4 and 10^{13}, between 10^5 and 10^{13}, between 10^6 and 10^{13}, between 10^7 and 10^{11}, between 10^8 and 10^{11}, between 10^9 and 10^{11}, between 10^{10} and 10^{11}, between 10 and 10^{10}, between 10^2 and 10^{10}, between 10^3 and 10^{10}, between 10^4 and 10^{10}, between 10^5 and 10^{10}, between 10^6 and 10^{10}, between 10^7 and 10^{10}, between 10^8 and 10^{10}, between 10^9 and 10^{10}, between 10 and 10^9, between 10^2 and 10^9, between 10^3 and 10^9, between 10^4 and 10^9, between 10^5 and 10^9, between 10^6 and 10^9, between 10^7 and 10^9, between 10^8 and 10^9, between 10 and 10^8, between 10^2 and 10^8, between 10^3 and 10^8, between 10^4 and 10^8, between 10^5 and 10^8, between 10^6 and 10^8, between 10^7 and 10^8, between 10 and 10^7, between 10^2 and 10^7, between 10^3 and 10^7, between 10^4 and 10^7, between 10^5 and 10^7, between 10^6 and 10^7, between 10 and 10^6, between 10^2 and 10^6, between 10^3 and 10^6, between 10^4 and 10^6, between 10^5 and 10^6, between 10 and 10^5, between 10^2 and 10^5, between 10^3 and 10^5, between 10^4 and 10^5, between 10 and 10^4, between 10^2 and 10^4, between 10^3 and 10^4, between 10 and 10^3, between 10^2 and 10^3, or between 10 and 10^2 total bacteria. In some embodiments, the compositions disclosed herein contain between 10 and 10^{13}, between 10^2 and 10^{13}, between 10^3 and 10^{13}, between 10^4 and 10^{13}, between 10^5 and 10^{13}, between 10^6 and 10^{13}, between 10^7 and 10^{13}, between 10^8 and 10^{13}, between 10^9 and 10^{13}, between 10^{10} and 10^{13}, between 10^{11} and 10^{13}, between 10^{12} and 10^{13}, between 10 and 10^{12}, between 10^2 and 10^{12}, between 10^3 and 10^{12}, between 10^4 and 10^{12}, between 10^5 and 10^{12}, between 10^6 and 10^{12}, between 10^7 and 10^{12}, between 10^8 and 10^{12}, between 10^9 and 10^{12}, between 10^{10} and 10^{12}, between 10^{11} and 10^{12}, between 10 and 10^{11}, between 10^2 and 10^{11}, between 10^3 and 10^{13}, between 10^4 and 10^{13}, between 10^5 and 10^{13}, between 10^6 and 10^{13}, between 10^7 and 10^{11}, between 10^8 and 10^{11}, between 10^9 and

10^{11}, between 10^{10} and 10^{11}, between 10 and 10^{10}, between 10^2 and 10^{10}, between 10^3 and 10^{10}, between 10^4 and 10^{10}, between 10^5 and 10^{10}, between 10^6 and 10^{10}, between 10^7 and 10^{10}, between 10^8 and 10^{10}, between 10^9 and 10^{10}, between 10 and 10^9, between 10^2 and 10^9, between 10^3 and 10^9, between 10^4 and 10^9, between 10^5 and 10^9, between 10^6 and 10^9, between 10^7 and 10^9, between 10^8 and 10^9, between 10 and 10^8, between 10^2 and 10^8, between 10^3 and 10^8, between 10^4 and 10^8, between 10^5 and 10^8, between 10^6 and 10^8, between 10^7 and 10^8, between 10 and 10^7, between 10^2 and 10^7, between 10^3 and 10^7, between 10^4 and 10^7, between 10^5 and 10^7, between 10^6 and 10^7, between 10 and 10^6, between 10^2 and 10^6, between 10^3 and 10^6, between 10^4 and 10^6, between 10^5 and 10^6, between 10 and 10^5, between 10^2 and 10^5, between 10^3 and 10^5, between 10^4 and 10^5, between 10 and 10^4, between 10^2 and 10^4, between 10^3 and 10^4, between 10 and 10^3, between 10^2 and 10^3, or between 10 and 10^2 total bacteria per milliliter.

In some embodiments, the compositions disclosed herein contain between 10 and 10^{13}, between 10^2 and 10^{13}, between 10^3 and 10^{13}, between 10^4 and 10^{13}, between 10^5 and 10^{13}, between 10^6 and 10^{13}, between 10^7 and 10^{13}, between 10^8 and 10^{13}, between 10^9 and 10^{13}, between 10^{10} and 10^{13}, between 10^{11} and 10^{13}, between 10^{12} and 10^{13}, between 10 and 10^{12}, between 10^2 and 10^{12}, between 10^3 and 10^{12}, between 10^4 and 10^{12}, between 10^5 and 10^{12}, between 10^6 and 10^{12}, between 10^7 and 10^{12}, between 10^8 and 10^{12}, between 10^9 and 10^{12}, between 10^{10} and 10^{12}, between 10^{11} and 10^{12}, between 10 and 10^{11}, between 10^2 and 10^{11}, between 10^3 and 10^{13}, between 10^4 and 10^{13}, between 10^5 and 10^{13}, between 10^6 and 10^{13}, between 10^7 and 10^{11}, between 10^8 and 10^{11}, between 10^9 and 10^{11}, between 10^{10} and 10^{11}, between 10 and 10^{10}, between 10^2 and 10^{10}, between 10^3 and 10^{10}, between 10^4 and 10^{10}, between 10^5 and 10^{10}, between 10^6 and 10^{10}, between 10^7 and 10^{10}, between 10^8 and 10^{10}, between 10^9 and 10^{10}, between 10 and 10^9, between 10^2 and 10^9, between 10^3 and 10^9, between 10^4 and 10^9, between 10^5 and 10^9, between 10^6 and 10^9, between 10^7 and 10^9, between 10^8 and 10^9, between 10 and 10^8, between 10^2 and 10^8, between 10^3 and 10^8, between 10^4 and 10^8, between 10^5 and 10^8, between 10^6 and 10^8, between 10^7 and 10^8, between 10 and 10^7, between 10^2 and 10^7, between 10^3 and 10^7, between 10^4 and 10^7, between 10^5 and 10^7, between 10^6 and 10^7, between 10 and 10^6, between 10^2 and 10^6, between 10^3 and 10^6, between 10^4 and 10^6, between 10^5 and 10^6, between 10 and 10^5, between 10^2 and 10^5, between 10^3 and 10^5, between 10^4 and 10^5, between 10 and 10^4, between 10^2 and 10^4, between 10^3 and 10^4, between 10 and 10^3, between 10^2 and 10^3, or

between 10 and 10^2 colony forming units of bacteria. In some embodiments, the compositions disclosed herein contain between 10 and 10^{13}, between 10^2 and 10^{13}, between 10^3 and 10^{13}, between 10^4 and 10^{13}, between 10^5 and 10^{13}, between 10^6 and 10^{13}, between 10^7 and 10^{13}, between 10^8 and 10^{13}, between 10^9 and 10^{13}, between 10^{10} and 10^{13}, between 10^{11} and 10^{13}, between 10^{12} and 10^{13}, between 10 and 10^{12}, between 10^2 and 10^{12}, between 10^3 and 10^{12}, between 10^4 and 10^{12}, between 10^5 and 10^{12}, between 10^6 and 10^{12}, between 10^7 and 10^{12}, between 10^8 and 10^{12}, between 10^9 and 10^{12}, between 10^{10} and 10^{12}, between 10^{11} and 10^{12}, between 10 and 10^{11}, between 10^2 and 10^{11}, between 10^3 and 10^{13}, between 10^4 and 10^{13}, between 10^5 and 10^{13}, between 10^6 and 10^{13}, between 10^7 and 10^{11}, between 10^8 and 10^{11}, between 10^9 and 10^{11}, between 10^{10} and 10^{11}, between 10 and 10^{10}, between 10^2 and 10^{10}, between 10^3 and 10^{10}, between 10^4 and 10^{10}, between 10^5 and 10^{10}, between 10^6 and 10^{10}, between 10^7 and 10^{10}, between 10^8 and 10^{10}, between 10^9 and 10^{10}, between 10 and 10^9, between 10^2 and 10^9, between 10^3 and 10^9, between 10^4 and 10^9, between 10^5 and 10^9, between 10^6 and 10^9, between 10^7 and 10^9, between 10^8 and 10^9, between 10 and 10^8, between 10^2 and 10^8, between 10^3 and 10^8, between 10^4 and 10^8, between 10^5 and 10^8, between 10^6 and 10^8, between 10^7 and 10^8, between 10 and 10^7, between 10^2 and 10^7, between 10^3 and 10^7, between 10^4 and 10^7, between 10^5 and 10^7, between 10^6 and 10^7, between 10 and 10^6, between 10^2 and 10^6, between 10^3 and 10^6, between 10^4 and 10^6, between 10^5 and 10^6, between 10 and 10^5, between 10^2 and 10^5, between 10^3 and 10^5, between 10^4 and 10^5, between 10 and 10^4, between 10^2 and 10^4, between 10^3 and 10^4, between 10 and 10^3, between 10^2 and 10^3, or between 10 and 10^2 colony forming units of bacteria per milliliter.

In some embodiments of the compositions provided herein, the composition includes at least 1 x 10^8 colony forming units of bacteria per milliliter.

Compositions that include bacterial strains can be lyophilized to preserve the bacterial strain. In some embodiments, the composition or the bacterial strains of the composition are lyophilized. Methods of lyophilizing compositions, including compositions comprising bacteria, are known in the art. See, *e.g.*, U.S. Patent 3,261,761; U.S. Patent 4,205,132; PCT Publications WO 2014/029578, WO 2012/098358, WO2012/076665 and WO2012/088261, herein incorporated by reference in their entirety. However, finding conditions that allow for the lyophilization of certain bacteria, such as anaerobic bacteria has been challenging. See *e.g.,* Peiren et al., Appl Microbol Biotechnol (2015) 99: 3559. It should be appreciated that

in one aspect the methods of stabilization and preservation provided herein allow for the ability to generate compositions that allow for the manufacture of bacterial strains, in particular anaerobic bacterial strains. Prior to the instant disclosure, none of the published methods provided for levels of stabilization and preservation that would allow for the manufacture of bacterial strains, particular anaerobic bacterial strains.

Aspects of the disclosure provide methods of preserving bacteria involving subjecting a composition comprising the bacteria to a lyphophilization cycle. In general, lyophilization is a dessication process to preserve a material, such as bacteria, involving freeze-drying. Water is removed from material by freezing the material and then placing the material under a vacuum, during which the ice undergoes sublimation. In some embodiments, the lyophilization cycle involves the steps of freezing, primary drying, and secondary drying. The term "temperature ramp rate" refers to the rate by which the temperature is adjusted between steps of the lyophilization cycle.

In some embodiments, the lyophilization cycle includes one or more steps having a temperature ramp rate between 0.5°C/min to 3°C/min. In some embodiments, the temperature ramp rate is 0.5°C/min, 0.6°C/min ,0.7°C/min, 0.8°C/min, 0.9°C/min, 1.0°C/min, 1.1°C/min, 1.2°C/min, 1.3°C/min, 1.4°C/min, 1.5°C/min, 1.6°C/min, 1.7°C/min, 1.8°C/min, 1.9°C/min, 2.0°C/min, 2.1°C/min, 2.2°C/min, 2.3°C/min, 2.4°C/min, 2.5°C/min, 2.6°C/min, 2.7°C/min, 2.8°C/min, 2.9°C/min, or 3.0°C/min. In some embodiments, the lyophilization cycle includes one or more steps having a temperature ramp rate of 1.0°C/min. In some embodiments, the lyophilization cycle includes one or more steps having a temperature ramp rate of 2.5°C/min.

In some embodiments, each of the steps of the lyophilization cycle have a temperature ramp rate between 0.5°C/min to 3°C/min. In some embodiments, the temperature ramp rate is 0.5°C/min, 0.6°C/min ,0.7°C/min, 0.8°C/min, 0.9°C/min, 1.0°C/min, 1.1°C/min, 1.2°C/min, 1.3°C/min, 1.4°C/min, 1.5°C/min, 1.6°C/min, 1.7°C/min, 1.8°C/min, 1.9°C/min, 2.0°C/min, 2.1°C/min, 2.2°C/min, 2.3°C/min, 2.4°C/min, 2.5°C/min, 2.6°C/min, 2.7°C/min, 2.8°C/min, 2.9°C/min, or 3.0°C/min. In some embodiments, each of the steps of the lyophilization cycle have a temperature ramp rate of 1.0°C/min. In some embodiments, each of the steps of the lyophilization cycle have a temperature ramp rate of 2.5°C/min.

As discussed herein, in some embodiments, a bacteria may be determined to be sensitive, for example in a sensitivity assay, and the temperature ramp rate in the

lyophilization is increased. In some embodiments, a bacteria may be determined to be sensitive and the lyophilization cycle includes one or more steps having a temperature ramp rate of 2.5°C/min.

In one aspect, the compositions provided herein that include bacteria are in solid form. In some embodiments, the solid form is a lyophilized cake (also referred to as a "lyocake"). As used herein, the terms "lyophilization cake" and "lyocake" refer to the solid composition formed by lyophilization of a composition, such as a composition comprising bacteria. The appearance of the lyophilization cake may be evaluated. In embodiments, a lyophilization cake that appears intact and not collapsed is desired.

It should be appreciated that the disclosure embraces solid compositions. The solid compositions may be generated for instance after lyophilization of one of the compositions that include bacteria disclosed herein. The solid form of the composition will have the same components as the liquid formulation used to generate the solid form. Thus, for instance, if a liquid composition included lyoprotectant, a nutrient, an antioxidant, and a buffer and the liquid composition was subject to lyophilization, the lyocake generated would have the same components. The definition of the amount/percentage of each of the components is different when describing the solid formulation. The indicators "mM" and "pH: are not appropriate to describe solid components. However, it should be appreciated that reconstitution of a solid formulation in the same amount of liquid should results in the same composition. In one aspect, the disclosure provides solid compositions that have been generated by the lyophilization of the compositions provided herein.

In some embodiments, the disclosure provides a solid composition that includes bacteria generated by the lyophilization of a liquid composition comprising a lyoprotectant, a nutrient, an antioxidant, and a buffer. In some embodiments, the disclosure provides a solid composition that includes bacteria generated by the lyophilization of a liquid composition comprising a lyoprotectant, a nutrient, an antioxidant, a buffer, and an excipient (*e.g.*, stabilizing agent). In some embodiments, the disclosure provides a solid composition that includes bacteria generated by the lyophilization of a liquid composition comprising sucrose, yeast extract, cysteine, and a histidine buffer. In some embodiments, the disclosure provides a solid composition that includes bacteria generated by the lyophilization of a liquid composition comprising sucrose, yeast extract, cysteine, a histidine buffer, and sodium metabisulfite. In some embodiments, the disclosure provides a solid composition that

includes bacteria generated by the lyophilization of a liquid composition comprising trehalose, yeast extract, cysteine, and a histidine buffer. In some embodiments, the disclosure provides a solid composition that includes bacteria generated by the lyophilization of a liquid composition comprising trehalose, yeast extract, cysteine, a histidine buffer, and sodium metabisulfite. In some embodiments, the disclosure provides a lyocake that includes bacteria generated by the lyophilization of a liquid composition comprising sucrose, yeast extract, cysteine, and a histidine buffer. In some embodiments, the disclosure provides a lyocake that includes bacteria generated by the lyophilization of a liquid composition comprising sucrose, yeast extract, cysteine, a histidine buffer, and sodium metabisulfite. In some embodiments, the disclosure provides a lyocake that includes bacteria generated by the lyophilization of a liquid composition comprising trehalose, yeast extract, cysteine, and a histidine buffer. In some embodiments, the disclosure provides a lyocake that includes bacteria generated by the lyophilization of a liquid composition comprising trehalose, yeast extract, cysteine, a histidine buffer, and sodium metabisulfite.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer and *Clostridium bolteae*. In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Clostridium bolteae*. In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer and *Anaerotruncus colihominis*. In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Anaerotruncus colihominis*. In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Eubacterium fissicatena*. In some embodiments of the compositions provided herein, the composition includes a solid composition generated

by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Eubacterium fissicatena.* In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Clostridium symbiosum.* In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Clostridium symbiosum.* In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer and *Blautia producta.* In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Blautia producta.* In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer and *Dorea longicatena.* In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Dorea longicatena.* In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer and *Erysipelotrichaceae bacterium.* In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Erysipelotrichaceae bacterium.* In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer and *Subdolinogranulum spp.* In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Subdolinogranulum spp.* In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Clostridium bolteae.* In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Clostridium bolteae.* In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Anaerotruncus colihominis.* In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Anaerotruncus colihominis.* In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Eubacterium fissicatena.* n some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Eubacterium fissicatena.* In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Clostridium symbiosum.* In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the

lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Clostridium symbiosum*. In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer and *Blautia producta*. In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Blautia producta*. In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Dorea longicatena*. In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Dorea longicatena*. In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and *Erysipelotrichaceae bacterium*. In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Erysipelotrichaceae bacterium*. In some embodiments, the solid composition is a lyocake.

In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer and *Subdolinogranulum spp.* In some embodiments of the compositions provided herein, the composition includes a solid composition generated by the lyophilization of 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, 0.05% sodium metabisulfite, and *Subdolinogranulum spp.* In some embodiments, the solid composition is a lyocake.

In some embodiments, the solid compositions that include bacterial strains provided herein may be formulated for administration as a pharmaceutical composition, *e.g.*, by reconstitution of a lyophilized product. The term "pharmaceutical composition" as used herein means a product that results from the mixing or combining of a solid formulation provided herein and one or more pharmaceutically acceptable excipient.

An "acceptable" excipient refers to an excipient that must be compatible with the active ingredient (*e.g.,* the bacterial strain) and not deleterious to the subject to which it is administered. In some embodiments, the pharmaceutically acceptable excipient is selected based on the intended route of administration of the composition, for example a composition for oral or nasal administration may comprise a different pharmaceutically acceptable excipient than a composition for rectal administration. Examples of excipients include sterile water, physiological saline, solvent, a base material, an emulsifier, a suspending agent, a surfactant, a stabilizer, a flavoring agent, an aromatic, an excipient, a vehicle, a preservative, a binder, a diluent, a tonicity adjusting agent, a soothing agent, a bulking agent, a disintegrating agent, a buffer agent, a coating agent, a lubricant, a colorant, a sweetener, a thickening agent, and a solubilizer.

In one aspect, the disclosure provides compositions that allow for the preservation of bacteria. In some embodiments, the bacteria are anaerobic bacteria. Compositions useful for the preservations of bacteria are also referred to herein as stabilizing compositions. A composition that allows for the preservation of bacteria (*e.g.*, anaerobic bacteria) or stabilization of bacteria, as used herein, refers to a composition that promotes the viability of the bacteria therein and allows for the recovery of the bacteria following a lyophilization cycle. The stabilization or preservation functionality of the composition can be assessed by comparing the number of viable bacteria (*e.g.*, colony forming units) at two specific time points (*e.g.*, at day 1 and at day 100). In some embodiments, the stabilization or preservation functionality of the composition is assessed by comparing the number of viable bacteria (*e.g.*, colony forming units) at a first time point to the number of viable bacteria (*e.g.*, colony forming units) at a second time point. If the number of colony forming units is the same or substantially the same at the two time points or over a time period, the composition is a perfect stabilizing composition. A large decrease in the number of colony forming units between two time points or over a time period indicates that the composition is not a good stabilizing composition.

The stabilization functionality of the composition can also be assessed by comparing the number of viable bacteria (*e.g.*, colony forming units) before and after a specific event (*e.g.*, lyophilization or a freeze-thaw event). If the number of colony forming units is the same or substantially the same, the composition is a perfect stabilizing composition. A large decrease in the number of colony forming units after a specific event, relative to prior to the specific event, indicates that the composition is not a good stabilizing composition.

In some embodiments of the compositions provided herein, the composition is a stabilizing composition. In some embodiments, the stabilizing composition includes trehalose, yeast extract, cysteine, and a histidine buffer. In some embodiments, the stabilizing composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, and 20 mM histidine buffer. In some embodiments, the stabilizing composition includes trehalose at a concentration between 7.0% and 8.0%, 1% yeast extract, 0.05% cysteine and 20 mM histidine buffer. In some embodiments, the stabilizing composition includes trehalose, yeast extract, cysteine, a histidine buffer, and an excipient (*e.g.*, a stabilizing agent). In some embodiments, the stabilizing composition includes 7.5% trehalose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite. In some embodiments, the stabilizing composition includes trehalose at a concentration between 7.0% and 8.0%, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite.

In some embodiments, the stabilizing composition includes sucrose, yeast extract, cysteine, and a histidine buffer. In some embodiments, the stabilizing composition includes sucrose at a concentration between 7.0% and 8.0%, 1% yeast extract, 0.05% cysteine, and 20 mM histidine buffer. In some embodiments, the stabilizing composition comprises 7.0% sucrose, 1% yeast extract, 0.05% cysteine, and 20 mM histidine buffer. In some embodiments, the stabilizing composition includes sucrose, yeast extract, cysteine, a histidine buffer, and an excipient (*e.g.*, a stabilizing agent). In some embodiments, the stabilizing composition includes sucrose at a concentration between 7.0% and 8.0%, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite. In some embodiments, the stabilizing composition comprises 7.0% sucrose, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite.

Aspects of the disclosure relate to assessing the bacteria in a sensitivity assay. In some embodiments, the bacteria are subjected to a sensitivity assay prior to adding the bacteria to a composition for lyophilization. Sensitivity assays may be used to determine

whether a bacterial may be more sensitive, for example to stresses, such as the presence of oxygen or cell wall/membrane stress. The performance of bacteria in a sensitivity assay may also indicate or predict how the bacteria will survive (remain viable) through a lyophilization cycle. In general, subjecting bacteria that are determined to be sensitive, or more sensitive relative to other bacteria, to a lyophilization cycle may result in reduced recovery of viable bacteria; therefore, in some embodiments, if a bacteria is determined to be sensitive, an excipient may be added to the composition and/or the temperature ramp rate of the lyophilization cycle may be increased. In some embodiments, the sensitivity assay may determine the sensitivity of the bacteria to a stress.

In some embodiments, the sensitivity of a bacteria is compared to the sensitivity of another bacteria or to a reference value. In some embodiments, performance of the bacteria in the sensitivity assay may be compared to the performance of another bacteria or to a reference value. Examples of sensitivity assays include, for example, Gram staining and freeze-thaw assays.

Gram staining is a method typically used to assess the cell wall (peptidoglycan) structure of bacteria. As will be evident to one of ordinary skill in the art, a Gram stain involves subjecting bacteria to a series of steps: first contacting the bacteria with a water-soluble dye (crystal violet) and an iodine solution, then a decolorizing step, and finally a counterstaining step, typically with safanin. The crystal violet binds to peptidoglycan of the bacterial cell wall and forms complexes with the iodine. Bacteria having thick layers of peptidoglycan appear purple in a Gram stain and are referred to as Gram positive (Gram +), whereas bacteria having thinner layers of peptidoglycan or are surrounded with an outer membrane, appear pink in a Gram stain, as these cells are stained with the counterstain and not crystal violet. These cells are referred to as Gram negative (Gram -) bacteria. Bacteria that are characterized as Gram positive (*e.g.*, known in the art to have the peptidoglycan structure of Gram positive bacteria) but do not appear Gram positive in a Gram stain may be considered sensitive or more sensitive compared to other bacteria. In some embodiments, the bacteria are subjected to a Gram stain and are determined to be sensitive.

In some embodiments, the sensitivity assay is a freeze-thaw assay. As will be evident to one of ordinary skill in the art, freeze-thaw assays involve freezing bacteria, *e.g.* in a dry ice/ethanol bath or freezer, and then thawing the bacteria. In some embodiments, the freeze-thaw assay involves one or more cycles of freezing and thawing the bacteria. In some

embodiments, the viability of the bacteria is assessed after the freeze-thaw assay. The freeze-thaw process may cause lysis and therefore reduced viability (reduced recovery) of sensitive bacteria. In some embodiments, reduced viability of bacteria in a freeze-thaw assay may indicated that the bacteria are sensitive or are more sensitive compared to other bacteria.

In some embodiments, the methods described herein involve assessing the bacteria in a sensitivity assay. In some embodiments, if the bacteria are determined to be sensitive or more sensitive compared to other bacteria, an excipient is added to compositions containing the sensitive bacteria. In some embodiments, if the bacteria are determined to be sensitive or more sensitive compared to other bacteria, a stabilizing agent is added to compositions containing the sensitive bacteria. In some embodiments, if the bacteria are determined to be sensitive or more sensitive compared to other bacteria, a reducing agent is added to compositions containing the sensitive bacteria. Without wishing to be bound by any particular theory, the presence of a reducing agent may scavenge oxygen in the composition, thereby improving the viability of sensitive bacteria. In some embodiments, if the bacteria are determined to be sensitive or more sensitive compared to other bacteria, 0.05% sodium metabiulfite is added to compositions containing the sensitive bacteria.

In some embodiments, if the bacteria are determined to be sensitive or more sensitive compared to other bacteria, the composition comprising the sensitive bacteria is subjected to a lyophilization cycle having an increased temperature ramp rate (*e.g.*, greater than 1°C/min). Without wishing to be bound by any particular theory, increasing the temperature ramp rate may reduce the potential exposure of the bacteria to oxygen. In some embodiments, if the bacteria are determined to be sensitive or more sensitive compared to other bacteria, lyophilized compositions comprising the sensitive bacteria are generated by subjecting a composition comprising the sensitive bacteria to a lyophilization cycle having one or more steps of a temperature ramp rate of 2.5°C/min.

In some embodiments of the compositions provided herein, a stabilizing composition is a composition that allows for the recovery of at least 1%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, up to 100% of the colony forming units over a period of time. In some embodiments, the period of time is at least 1 week, at least 2 weeks, at least 4 weeks, at least 2 months, at least 3 months, at least 6 months, or at least 1 year or more. In some embodiments, the percentage of recovered colony forming units or level of preservation is determined by comparing a number

of colony forming units of bacteria (e.g., of a bacterial strain or total bacteria) at a first time point relative to the number of colony forming units of bacteria (e.g., of a bacterial strain or total bacteria) at a second time point over a period of time. For example, a 50% recovery or preservation of 50% of bacteria indicates that half of the bacteria remained viable over the period of time; and a 100% recovery or preservation of 100% of bacteria indicates that all (or substantially all) bacteria remained viable over the period of time.

In some embodiments of the compositions provided herein, a stabilizing composition is a composition that allows for the recovery of at least 1%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, up to 100% of the colony forming units after a specific event. In some embodiments, the specific event is a freeze-thaw cycle or a lyophilization cycle. In some embodiments, the percentage of recovered colony forming units or level of preservation is determined by comparing a number of colony forming units of bacteria (e.g., of a bacterial strain or total bacteria) prior to the specific event relative to the number of colony forming units of bacteria (e.g., of a bacterial strain or total bacteria) after the specific event. For example, a 50% recovery or 50% preservation indicates that half of the bacteria remained viable after the specific event; and a 100% recovery or 100% preservation indicates that all (or substantially all) bacteria remained viable after the specific event.

In one aspect, the disclosure provides methods for preserving bacteria. In some embodiments of the methods provided herein, the method includes adding bacteria to any of the compositions provided herein and subjecting the composition to which the bacteria have been added to a lyophilization cycle. In some embodiments of the methods provided herein, the method includes adding bacteria to a composition including sucrose at a concentration between 7.0% and 8.0%, 1% yeast extract, 0.05% cysteine and 20 mM histidine buffer and subjecting the composition to which the bacteria have been added to a lyophilization cycle. In some embodiments of the methods provided herein, the method includes adding bacteria to a composition including sucrose at a concentration between 7.0% and 8.0%, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite, and subjecting the composition to which the bacteria have been added to a lyophilization cycle. In some embodiments of the methods provided herein, the method includes adding bacteria to a composition including trehalose at a concentration between 7.0% and 8.0%, 1% yeast extract, 0.05% cysteine and 20 mM histidine buffer and subjecting the composition to which the

bacteria have been added to a lyophilization cycle. In some embodiments of the methods provided herein, the method includes adding bacteria to a composition including trehalose at a concentration between 7.0% and 8.0%, 1% yeast extract, 0.05% cysteine, 20 mM histidine buffer, and 0.05% sodium metabisulfite, and subjecting the composition to which the bacteria have been added to a lyophilization cycle.

In some embodiments of the methods provided herein, the method further includes measuring the number of colony forming units after subjecting the composition comprising the bacteria to the lyophilization cycle. In some embodiments of the methods provided herein, the method results in the preservation of at least 1%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, up to 100% of the colony forming units.

In one aspect, the disclosure provides methods for generating the compositions provided herein. In some embodiments of the methods provided herein, the method includes creating a mixture by combining the lyoprotectant, the nutrient, the antioxidant, and the buffer, followed by reduction of the mixture thereby generating the composition. In some embodiments, the method further includes the addition of bacteria.

In some embodiments of the methods provided herein, the method includes creating a mixture by combining trehalose, yeast extract, cysteine and histidine buffer to create a mixture, followed by reduction of the mixture thereby generating the composition. In some embodiments of the methods provided herein, the method includes creating a mixture by combining trehalose, yeast extract, cysteine, histidine buffer, and an excipient to create a mixture, followed by reduction of the mixture thereby generating the composition. In some embodiments of the methods provided herein, the method includes creating a mixture by combining sucrose, yeast extract, cysteine, and histidine buffer to create a mixture, followed by reduction of the mixture thereby generating the composition. In some embodiments of the methods provided herein, the method includes creating a mixture by combining sucrose, yeast extract, cysteine, histidine buffer, and excipient to create a mixture, followed by reduction of the mixture thereby generating the composition.

SEQ ID NO:1 Strain 1 16S ribosomal RNA *Clostridium bolteae*

ATGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACGAAGC
AATTAAAATGAAGTTTTCGGATGGATTTTTGATTGACTGAGTGGCGGACGGGTGAGTAACGCGTGGAT
AACCTGCCTCACACTGGGGGATAACAGTTAGAAATGACTGCTAATACCGCATAAGCGCACAGTACCGC
ATGGTACGGTGTGAAAAACTCCGGTGGTGTGAGATGGATCCGCGTCTGATTAGCCAGTTGGCGGGGTA

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GGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGAC
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CAAGAGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTTATCCTTAGTAGCCAGCAGGTAAAGCTGGGCACTCTAGGGAGACTGCCAGGGATAA
CCTGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGTGCTACA
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ATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTCAGCAACGCCCGAAGTCAGTGA
CCCAACTCGCAAGAGAGGGAGCTGCCGAAGGCGGGGCAGGTAACTGGGGTGAAGTCGTAACAAGGTAG
CCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT

SEQ ID NO:2 Strain 2 16S ribosomal RNA *Anaerotruncus colihominis*

TCAAAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGCGCCTAACACATGCAAGTCGAACGGAG
CTTACGTTTTGAAGTTTTCGGATGGATGAATGTAAGCTTAGTGGCGGACGGGTGAGTAACACGTGAGC
AACCTGCCTTTCAGAGGGGGATAACAGCCGGAAACGGCTGCTAATACCGCATGATGTTGCGGGGGCAC
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TAAAACAGAGGGCGGCGACACCGCGAGGTGAAGCGAATCCCGAAAAAGTGTCTCAGTTCAGATTGCAG
GCTGCAACCCGCCTGCATGAAGTCGGAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGT
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CGCAAGGGGGGCGCTGTCGAAGGTGGGATTGATGACTGGGGTGAAGTCGTAACAAGGTAGCCGTATCG
GAAGGTGCGGCTGGATCACCTCCTTT

SEQ ID NO:3 Strain 3 16S ribosomal RNA *Ruminococcus torques*

TACGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAGCGAAG
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CAACCTGCCTCATACAGGGGGATAACAGTTAGAAATGACTGCTAATACCGCATAAGCGCACAGGACCG
CATGGTGTAGTGTGAAAAACTCCGGTGGTATGAGATGGACCCGCGTCTGATTAGGTAGTTGGTGGGGT
AAAGGCCTACCAAGCCGACGATCAGTAGCCGACCTGAGAGGGTGACCGGCCACATTGGGACTGAGACA
CGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCGA

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GCGTCCGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTTATCTTCAGTAGCCAGCATATAAGGTGGGCACTCTGGAGAGACTGCCAGGGAGAA
CCTGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGGCCAGGGCTACACACGTGCTACA
ATGGCGTAAACAAAGGGAAGCGAGAGGGTGACCTGGAGCGAATCCCAAAAATAACGTCTCAGTTCGGA
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ATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTCAGTAACGCCCGAAGCCAGTGA
CCCAACCTTAGAGGAGGGAGCTGTCGAAGGCGGGACGGATAACTGGGGTGAAGTCGTAACAAGGTAGC
CGTATCGGAAGGTGCGGCTGGATCACCTCCTTT

SEQ ID NO:4 Strain 4 16S ribosomal RNA *Clostridium symbiosum*

ATGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACGAAGC
GATTTAACGGAAGTTTTCGGATGGAAGTTGAATTGACTGAGTGGCGGACGGGTGAGTAACGCGTGGGT
AACCTGCCTTGTACTGGGGGACAACAGTTAGAAATGACTGCTAATACCGCATAAGCGCACAGTATCGC
ATGATACAGTGTGAAAAACTCCGGTGGTACAAGATGGACCCGCGTCTGATTAGCTAGTTGGTAAGGTA
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GGACTGCTTTGGAAACTGTTTAACTGGAGTGTCGGAGAGGTAAGTGGAATTCCTAGTGTAGCGGTGAA
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TACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGGAGTCAGTAACGCCCGAAGTCAGTGAC
CCAACCGCAAGGAGGGAGCTGCCGAAGGCGGGACCGATAACTGGGGTGAAGTCGTAACAAGGTAGCCG
TATCGGAAGGTGCGGCTGGATCACCTCCTTT

SEQ ID NO:5 Strain 5 16S ribosomal RNA *Blautia producta*

ATCAGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGAA
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GTAACCTGCCTCATACAGGGGGATAACAGTTAGAAATGGCTGCTAATACCGCATAAGCGCACAGGACC
GCATGGTCTGGTGTGAAAAACTCCGGTGGTATGAGATGGACCCGCGTCTGATTAGCTAGTTGGAGGGG
TAACGGCCCACCAAGGCGACGATCAGTAGCCGGCCTGAGAGGGTGAACGGCCACATTGGGACTGAGAC
ACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCG
ACGCCGCGTGAAGGAAGAAGTATCTCGGTATGTAAACTTCTATCAGCAGGGAAGAAAATGACGGTACC
TGACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCG

GATTTACTGGGTGTAAAGGGAGCGTAGACGGAAGAGCAAGTCTGATGTGAAAGGCTGGGGCTTAACCC
CAGGACTGCATTGGAAACTGTTTTTCTAGAGTGCCGGAGAGGTAAGCGGAATTCCTAGTGTAGCGGTG
AAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTACTGGACGGTAACTGACGTTGAGG
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GACCCAACCTTACAGGAGGGAGCTGCCGAAGGCGGGACCGATAACTGGGGTGAAGTCGTAACAAGGTA
GCCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT

SEQ ID NO:6 Strain 6 16S ribosomal RNA *Dorea Longicatena*

AACGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGAAG
CACTTAAGTTTGATTCTTCGGATGAAGACTTTTGTGACTGAGCGGCGGACGGGTGAGTAACGCGTGGG
TAACCTGCCTCATACAGGGGGATAACAGTTAGAAATGACTGCTAATACCGCATAAGACCACGGTACCG
CATGGTACAGTGGTAAAAACTCCGGTGGTATGAGATGGACCCGCGTCTGATTAGGTAGTTGGTGGGGT
AACGGCCTACCAAGCCGACGATCAGTAGCCGACCTGAGAGGGTGACCGGCCACATTGGGACTGAGACA
CGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGAGGAAACTCTGATGCAGCGA
CGCCGCGTGAAGGATGAAGTATTTCGGTATGTAAACTTCTATCAGCAGGGAAGAAAATGACGGTACCT
GACTAAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTATCCGG
ATTTACTGGGTGTAAAGGGAGCGTAGACGGCACGGCAAGCCAGATGTGAAAGCCCGGGGCTCAACCCC
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AATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTGCTGGACGATGACTGACGTTGAGGC
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CAAGAATGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAG
CAACGCGAAGAACCTTACCTGATCTTGACATCCCGATGACCGCTTCGTAATGGAAGCTTTTCTTCGGA
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AATGGCGTAAACAAAGAGAAGCGAACTCGCGAGGGTAAGCAAATCTCAAAAATAACGTCTCAGTTCGG
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CGTATCGGAAGGTGCGGCTGGATCACCTCCTTT

SEQ ID NO:7 Strain 7 16S ribosomal RNA *Erysipelotrichaceae bacterium*

ATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGAAG
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ATGTGTCCGGGATAACTGCTGGAAACGGTAGCTAAAACCGGATAGGTATACAGAGCGCATGCTCAGTA
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CGCGTGAGTGAAGAAGGTCTTCGGATCGTAAAGCTCTGTTGTAAGTGAAGAACGGCTCATAGAGGAAA
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AGCCGGTGGCATAACCGTAAGGAGTGAGCCGTCGAAGGTAGGACCGATGACTGGGGTTAAGTCGTAAC
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SEQ ID NO:8 Strain 8 16S ribosomal RNA *Subdoligranulum spp*

TATTGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGGG
GTGCTCATGACGGAGGATTCGTCCAACGGATTGAGTTACCTAGTGGCGGACGGGTGAGTAACGCGTGA
GGAACCTGCCTTGGAGAGGGGAATAACACTCCGAAAGGAGTGCTAATACCGCATGATGCAGTTGGGTC
GCATGGCTCTGACTGCCAAAGATTTATCGCTCTGAGATGGCCTCGCGTCTGATTAGCTAGTAGGCGGG
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CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGGCAATGGGCGCAAGCCTGACCCAGC
AACGCCGCGTGAAGGAAGAAGGCTTTCGGGTTGTAAACTTCTTTTGTCGGGGACGAAACAAATGACGG
TACCCGACGAATAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTA
TCCGGATTTACTGGGTGTAAAGGGCGTGTAGGCGGGATTGCAAGTCAGATGTGAAAACTGGGGGCTCA
ACCTCCAGCCTGCATTTGAAACTGTAGTTCTTGAGTGCTGGAGAGGCAATCGGAATTCCGTGTGTAGC
GGTGAAATGCGTAGATATACGGAGGAACACCAGTGGCGAAGGCGGATTGCTGGACAGTAACTGACGCT
GAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGGATA
CTAGGTGTGGGGGGTCTGACCCCCTCCGTGCCGCAGTTAACACAATAAGTATCCCACCTGGGGAGTAC
GATCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGTTTAATT
CGAAGCAACGCGAAGAACCTTACCAGGGCTTGACATCCCACTAACGAAGCAGAGATGCATTAGGTGCC
CTTCGGGGAAAGTGGAGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAG
TCCCGCAACGAGCGCAACCCTTATTGTTAGTTGCTACGCAAGAGCACTCTAGCGAGACTGCCGTTGAC
AAAACGGAGGAAGGTGGGGACGACGTCAAATCATCATGCCCCTTATGTCCTGGGCCACACACGTACTA
CAATGGTGGTTAACAGAGGGAGGCAATACCGCGAGGTGGAGCAAATCCCTAAAAGCCATCCCAGTTCG
GATTGCAGGCTGAAACCCGCCTGTATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGT
GAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTCGGGAACACCCGAAGTCCGT
AGCCTAACCGCAAGGAGGGCGCGGCCGAAGGTGGGTTCGATAATTGGGGTGAAGTCGTAACAAGGTAG
CCGTATCGGAAGGTGCGGCTGGATCACCTCCTTT

This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including," "comprising," or "having," "containing," "involving," and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms hall include the singular. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art. Generally, nomenclatures used in connection with, and techniques of biochemistry, enzymology, molecular and cellular biology, microbiology, virology, cell or tissue culture, genetics and protein and nucleic chemistry described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated.

The present invention is further illustrated by the following Examples, which in no way should be construed as further limiting. The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference, in particular for the teaching that is referenced hereinabove. However, the citation of any reference is not intended to be an admission that the reference is prior art.

EXAMPLES

EXAMPLE 1

Overview

A variety of lyophilization formulations were assessed for the lyophilization of the anaerobic bacterium *Dorea longicatena.* The experimental design is depicted in Figure 1.

Bacterial culture

An inoculum of *Dorea langicatena* was started from a single colony until it reached an OD600 of about 0.68, corresponding to late log phase, and then transferred to a larger flask.

Aliquots of 40 mls of bacteria were pelleted. The pelleted bacteria were at 1.6 X 10^7 cfu/ml. (cfu = colony forming units)

Lyophilization buffer preparation

The formulations depicted in Figure 2 and Table 2 were prepared in an anaerobic chamber. The mannitol and sorbitol were crystalline, while the sucrose and trehalose were amorphous. In addition to the formulation components shown in Figure 2, all formulations included 1% yeast extract, and 0.05% cysteine. The concentrations of the histidine and tris-base buffer was 20 mM. Formulations were filtered through a 0.22 microM filter. Sixteen 50 ml conical tubes contain the bacterial pellets were stored in a chamber at 2-8˚C were provided The number of bacteria in each tube was 1.6 X 10^7 cfu/ml. Pellets were washed with 20 ml lyophilization formulation twice and spun at 3900 rpm for 10 minutes. The osmolality of the formulations ranged from 292 to 329 mosmole. The washed pellets were re-suspended with 25ml lyophilization buffer and 5 vials were prepared for each lyophilization formulation. Four vials were used in the lyophilization cycle and one vial was kept at -80°C as a control. The vials were partially stoppered with 20-mm diameter Type I elastomeric chlorobutyl stoppers. One of the sixteen 50 ml conical tubes was washed with culture media and used as a control for the lyophilization cycle.

Bacterial pellets used as controls were washed with 12 ml cell culture medium twice, and spun at 3900 rpm for 10 minutes. The cells were subsequently re-suspended with 25 ml cell culture medium and a total of 5 vials were filled: 5 ml fill in a 20 ml vial. Four vials were lyophilized and one was kept at -80°C as a control. The vials were partially stoppered with 20mm diameter Type I elastomeric chlorobutyl stoppers.

Table 2: Formulations used in Example 1.

Formulation #	Buffer/pH	Mannitol	Trehalose	Sorbitol	Sucrose	Lactose	Osmolality
1	His / 6.5	4%					318
2	His / 7.0	4%					314
3	Tris / 7.0	4%					307
4	Tris / 7.5	4%					300
5	His / 6.5		8%				304
6	His / 7.0		8%				302
7	Tris / 7.0		8%				312
8	Tris / 7.5		8%				318
9	His / 6.5				8%		316

10	His / 7.0				8%		317
11	Tris / 7.0				8%		308
12	His / 7.0				8%		309
13	His / 7.0			4%			304
14	Tris / 7.0			4%			292
15	His / 7.0	4%				4%	329
16	Culture Media						

Lyophilization cycle:

The lyophilization cycle was performed with the lyophilization parameters shown in Table 3 below.

Table 3. Lyophilization cycle

Step	Temperature	Temperature Ramp (°C/min)	Hold in hrs	Pressure
Loading	4˚C	1˚C/min	2	N/A
Freeze	-50°C	1°C/min.	2	N/A
Primary Drying	-25°C	1°C/min	67	100 mTorr
Secondary Drying	+20°C	1°C/min	20.9	100 mTorr

Upon completion of the lyophilization cycle, back fill of nitrogen was performed to reach 600,000 mTorr and then vials were stoppered to keep N_2 in the vials in vacuum. Once the vials were stoppered, the back fill was completed to reach 760,000 mtorr. The lyophilized products in glass vials were retrieved from the lyophilizer and promptly sealed with the aluminum crimp-caps to prevent the atmospheric air contamination and to prevent the N_2 releasing from the vial. The completion of primary and secondary drying stages was determined based on the Pirani pressure. All lyophilized containers were stored at -20°C for storage prior to viability testing.

The samples were cooled to 4˚C for 2hrs and frozen at -50°C for 2 hrs. As the 16 formulation will have different Tg', the primary drying was set to -25°C. It is known the amorphous sugars such as sucrose have a transition of -32°C and the crystalline mannitol has the eutectic temperature of about 15°C. The primary drying at -25°C was selected so that the majority of the formulations would stay below transition or eutectic temperature during

lyophilization. The pressure profile suggests that Pirani pressure reached 100 mtorr at about 63 hrs of lyophilization. The primary drying was extended to 73 hrs to ensure the ending of primary drying. The bound water was removed during the secondary drying. Based on the Pirani pressure profile, the secondary drying was completed at 81 hrs of lyophilization. The lyophilization was extended to 88 hrs to ensure the complete removal of the bound water.

Results of lyophilization cycle

The physical appearance of the lyophilized formulations are shown in Figure 3. The data indicates that except the formulation containing the crystalline sorbitol and formulation containing culture media, all the cakes of other 13 formulations are not collapsed and are intact. The cake of the formulation containing sorbitol were collapsed and raised with the bubbling. Similarly, the formulation containing culture media alone also collapsed.

Based on the cake appearances, this lyophilization run looks acceptable for the formulation containing amorphous trehalose and sucrose and crystalline mannitol. The cakes of the formulation containing sorbitol and culture media collapsed after lyophilization.

Viability of lyophilized samples.

The lyophilized cakes were moved into the anaerobic chamber, opened and resuspended in 5 ml of culture media as the cakes were representative of a 5 ml sample. (resuspended in culture media HiVeg media). Dilutions were prepared from 10^1 to 10^6 to 10^7 and 100 microliter of the each dilution was dispensed on an Eggerth Gagnon plate and spread with help of sterile glass beads. Plating was done on pre-reduced Eggerth Gagnon plates enriched with 5% horse blood. Incubation was done at 37°C in the incubator in the anaerobic chamber. Colony forming units were counted 48-72 hours after plating.

The combined results of the lyophilization experiment and the viability of the assessed samples is shown in Figure 4 and Table 4. The best results are obtained with His pH 7.0 or Tris pH 7.5 as the buffer and sucrose or trehalose as the sugar.

Table 4: Results of formulation experiment

Formulation #	Buffer/pH	Mannitol	Trehalose	Sorbitol	Sucrose	Lactose	Viability (CFU)
1	His / 6.5	4%					0
2	His / 7.0	4%					0
3	Tris / 7.0	4%					0

4	Tris / 7.5	4%					0
5	His / 6.5		8%				2.8 x 10^3
6	His / 7.0		8%				9.5 x 10^4
7	Tris / 7.0		8%				1.0 x 10^4
8	Tris / 7.5		8%				1.7 x 10^5
9	His / 6.5				8%		~150
10	His / 7.0				8%		3.1 x 10^5
11	Tris / 7.0				8%		2.9 x 10^4
12	Tris / 7.5				8%		2.3 x 10^5
13	His / 7.0			4%			6.4 x 10^4
14	Tris / 7.0			4%			2.3 x 10^5
15	His / 7.0	4%				4%	0
16	Media						~350

EXAMPLE 2

Overview

This study assessed lyophilization formulations and components for the lyophilization of the anaerobic bacterium *Dorea longicatena*..

Bacterial culture

An inoculum of *Dorea longicatena* was started from a single colony in 50 mL centrifuge tubes containing 40mL of Vegitone media. The inoculum was allowed to grow overnight and two tubes were used to inoculate 750 mL of Vegitone in a 1 Liter bottle at a starting OD of 0.025. This was allowed to grow for twenty hours and was harvested at an OD of 0.68. Aliquots of 40 mL were added to sixteen 50 mL centrifuge tubes. The tubes were spun down at 3560 RCF for 10 minutes and the supernatants were discarded. The tubes were sealed and placed in an BD EZPak Anaerobic Container with a BD EZPak Gas Generating Pouch (Becton, Dicksinon and Company; Franklin Lakes, NY) to ensure an anaerobic environment. The box was placed in a 2 - 8°C refrigerator prior to viability testing.

Lyophilization buffer preparation and lyophilization cycle

The formulations presented in Table 5 were prepared to assess lyophilization of the bacterial cultures. Each formulation tested had 1% yeast extract and 0.05% L-Cysteine added. Yeast extract was added to provide each strain animal-free nutrients. L-Cysteine was added as a reducing agent to mitigate oxygen exposure.

Prior to viability testing, the vials were resuspended in 25 mL of media and plated to determine starting viabilities.

Each sample was washed twice using the formulation buffers shown in Table 5. A volume of 25mL was used for the final resuspension, to concentrate the starting sample. Each formulation shown in Table 5 had aliquots of 5mL added to separate 20mL vials and were then lyophilized using a primary drying of -25°C at 100mTorr and using a secondary drying of 20°C at 100mTorr.

Viability of lyophilized samples

After lyophilization, the final viable cell counts were determined by resuspending each vial in 5mL of media and plating on EGHB (Table 6). The data indicates that bacteria that were lyophilized in formulations containing sucrose or trehalose as a lyoprotectant were able to be recovered. Formulations with mannitol results in no recoverable, viable bacteria, and using culture media only for lyophilization resulted in poor recovery. Formulations with sorbitol had poor lyophilization cake formation. Histidine was selected as the buffer, as it resulted in viability with both sucrose and trehalose. Formulations 6 and 10 were further evaluated.

Table 5: Lyophilization Formulations

Formulation	20mM Histidine (pH)	20mM Tris (pH)	Mannitol (%)	Trehalose (%)	Sorbitol (%)	Sucrose (%)	Lactose (%)	Culture Medium (mL)	mOsmo /kg
1	6.5		4						318
2	7		4						314
3		7	4						307
4		7.5	4						300
5	6.5			7.5					304
6	7			7.5					302
7		7		7.5					312
8		7.5		7.5					318
9	6.5					7			316
10	7					7			317
11		7				7			308
12	7					7			309
13	7				4				304
14		7			4				292
15	7		4				4		329
16								25	N/A

Table 6: Viability of lyophilized samples

Formulation*	Formulation	Count A	Count B	Average	SD
1	4% mannitol-His pH 6.5	0	0	0	0
2	4% mannitol-His pH 7.0	N/A^	0	0	
3	4% mannitol-Tris-pH 7.0	0	0	0	0
4	4% mannitol-Tris-pH 7.5	0	0	0	0
5	7.5% Trehalose His pH 6.5	$3.30x\ 10^3$	$2.30x10^3$	$2.80x10^3$	$7.07x10^2$
6	7.5% Trehalose His pH 7.0	$8.00x10^4$	$1.10x10^5$	$9.50x10^4$	$2.12x10^4$
7	7.5% Trehalose Tris-pH 7.0	$1.00x10^3$	$1.90x10^4$	$1.00x10^4$	$1.27x10^4$
8	7.5% Trehalose Tris-pH 7.5	$5.20x10^4$	$2.80x10^5$	$1.66x10^5$	$1.61x10^5$
9	7% Sucrose-His pH 6.5	$3.00x10^2$	0	150	212
10	7% Sucrose-His pH 7.0	$1.50x10^4$	$6.00x10^5$	$3.08x10^5$	$4.14x10^5$
11	7% Sucrose-Tris-pH 7.0	$5.70x10^4$	0	$2.85x10^4$	$4.03x10^4$
12	7% Sucrose-Tris-pH 7.5	$1.90x10^5$	$2.60x10^5$	$2.25x10^5$	$4.95x10^4$
13	4% sorbitol His-pH 7.0	$8.40x10^4$	$4.30x10^4$	$6.35x10^4$	$2.90x10^4$
14	4% sorbitol Tris-pH 7.0	$1.20x10^5$	$3.40x10^5$	$2.30x10^5$	$1.56x10^5$
15	4% mannitol+4% Lactose-His pH 7.0	0	0	0	0
16	Culture Medium	100	600	350	353

*Formulations correspond to the formulations presented in Table 5.

^sample contaminated; no value determinable.

EXAMPLE 3

Overview

A selected number of optimized lyophilization formulations were assessed for the lyophilization of eight different anaerobic strains. The experimental design is depicted in Figure 1.

Bacterial cultures

An inoculum of each of the bacterial strains shown in Table 7 was started from a single colony until it reached the OD shown in Table 8 and then transferred to a larger flask. Aliquots 40 mls of bacteria were pelleted. The cfus of the pelleted bacteria are shown in Figure 5.

Table 7. Bacterial strains used in Example 3

Strain number	Closest known relative
1	*Clostridium bolteae*
2	*Anaerotruncus colihominis*
3	*Ruminococcus torques*
4	*Clostridium symbiosum*
5	*Blautia producta*
6	*Dorea longicatena*
7	*Erysipelotrichaceae bacterium*
8	*Subdoligranulum spp*

Bacterial strains were identified by the closest known relative as identified by sequence homology/identity.

Table 8: OD600 of bacterial strains used for Example 3

Strain	OD (600nm)
1	2.73
2	0.55
3	0.84
4	1.93
5	0.95
6	0.61
7	1.79
8	0.272

Lyophilization buffer preparation

Two lyophilization formulations were assessed:

- Formulation A: 20 mM Histidine pH 7.0, 1% yeast extract, 0.05% cysteine and 7.5 % trehalose with osmolality of 305 mOsmo/kg

- Formulation B: 20 mM Histidine pH 7.0, 1% yeast extract, 0.05% cysteine and 7.0 % sucrose with osmolality of 315 mOsmo/kg.

The formulations were prepared in the anaerobic chamber. Sixteen 50 ml conical tubes containing the bacterial pellets were used. The number of bacteria in each tube varied per strain and varied between 1.4 x 10^7 and 2.75 x 10^9 cfus/ml (See Figure 5). The bacterial pellets were washed with 20 ml lyophilization formulation twice, and spun at 3900 rpm for 10 minutes. The osmolality of the formulations ranged from 305-315 mosmole. The pellets were re-suspended with 20 ml lyophilization formulation buffer and 4 vials were filled for each (5 ml fill in a 20 ml). Three vials were lyophilized and one vial was kept at -80°C as a control. The vials were partially stoppered with 20-mm diameter Type I elastomeric chlorobutyl stoppers.

Lyophilization cycle:

The lyophilization run was performed with the lyophilization parameters shown in Table 9 below.

Table 9: Lyophilization cycle

Step	Temperature	Temperature Ramp (°C/min)	Hold in hrs	Pressure
Loading	4°C	1°C/min	0.1	NA
Freeze	-50°C	1°C/min.	2	NA
Primary Drying	-25°C	1°C/min	66	100 mTorr
Secondary Drying	+20°C	1°C/min	9.0	100 mTorr

Upon completion of the lyophilization cycle, back fill of nitrogen was performed to reach 600,000 mTorr and then vials were stoppered to keep N_2 in the vials in vacuum. Once the vials were stoppered, the back fill was completed to reach 760,000 mtorr. The lyophilized samples in glass vials were retrieved from the lyophilizer and promptly sealed with the aluminum crimp-caps to prevent the atmospheric air contamination and to prevent the N_2 releasing from the vial. The completion of primary and secondary drying stages was

determined based on the Pirani pressure reaching the set shelf pressure. All lyophilized containers were stored at -20C prior to viability testing.

The samples were cooled to 4˚C for 10 minutes and frozen at -50°C for 2 hrs. As the 2 formulation will have different Tg', the primary drying was set to -25°C. It is known that the amorphous sugars such as sucrose have a transition of -32°C while the Trehalose transition temperature (-29°C) is about 3˚C higher than sucrose. The primary drying at -25°C was selected so that the majority of the formulations will stay below transition during lyophilization. The pressure profile suggests that Pirani pressure reached 100 mtorr at about 56 hrs of lyophilization. The primary drying was extended to 66 hrs to ensure the ending of primary drying. The bound water was removed during the secondary drying. Based on the Pirani pressure profile, the secondary drying was completed at 76 hrs of lyophilization.

Results of lyophilization cycle

The physical appearance of each lyophilized formulation data showed that none of the cakes are collapsed. The color of the lyophilization cakes varied slightly between bacterial strains.

Viability of lyophilized samples.

The lyophilized caked were moved into the anaerobic chamber, opened and resuspended in 5 ml of culture media as the cakes were representative of a 5ml sample. (Resuspended in peptone yeast extract glucose media with tween). Dilutions were prepped from 10^1 to 10^6 to 10^7 and 100 microliter of the each dilution was dispensed on an Eggerth Gagnon plate and spread with help of sterile glass beads. Plating was done on pre-reduced Eggerth Gagnon plates enriched with 5% horse blood. Incubation was done at 37 degrees in the incubator in the anaerobic chamber. Colony forming units were counted 48-72 hours after plating.

The results of the lyophilization experiment and the viability of the assessed samples is shown in Figure 5 and Table 10. Formulations A and B provide a good recovery for bacterial strains 2-5 and 7-8.

Assessment of lyophilization (freeze-dry) versus freeze only)

The impact of the lyophilization cycle on bacterial strains in Formulations A and B was assessed by comparing the lyophilization of samples versus freezing the samples only (i.e., freezing the samples at -80°C, but not exposing the samples to a vacuum "freeze-thaw"). Viability of the freeze-thaw samples was assessed in the same way as the viability for the sample that went through the lyophilization cycle.

The results of the freeze-thaw cycle experiment are shown in Figure 5 and Table 10. Formulations A and B provide a good stability for bacterial strains 2-5 and 7-8. Strains 1 and 6 were further evaluated.

Table 10: Viability Results of Example 3

Bacterial Strain	Initial CFU	Post Lyo CFU (Condition A)	Post Freeze Thaw (Condition A)	Post Lyo CFU (Condition B)	Post Freeze Thaw (Condition B)
01	1.04×10^8	5×10^7	3×10^7	2×10^6	8×10^7
02	2.16×10^8	1×10^8	7×10^9	1.5×10^8	1.4×10^{10}
03	8.1×10^8	8×10^9	3.1×10^{10}	7.5×10^9	1×10^{10}
04	9.6×10^8	3×10^8	3.2×10^9	3.05×10^9	1.2×10^9
05	6.7×10^7	1.2×10^9	2.5×10^{10}	1×10^9	2×10^{10}
06	1.4×10^7	4.4×10^5	N/A	1×10^6	8×10^5
07	2.75×10^9	4×10^9	5×10^9	4×10^{10}	Lawn on 10^7
08	1.65×10^8	3.1×10^8	3.1×10^9	4.1×10^8	2.4×10^{10}

EXAMPLE 4

Lyophilization formulations for bacterial strains 1 and 6 (*Clostridium bolteae* and *Dorea longicatena*) were further evaluated using additional excipients (Tables 11 and 12) to improve the yield post lyophilization. Strains 1 and 6 were harvested at an OD of 2.83 and 1.27, respectively. The cultures were aliquoted into centrifuge tubes and pelleted at 3560RCF for 10 minutes. The supernatants were discarded and the pellets were placed in an BD EZPak Anaerobic Container with a BD EZPak Gas Generating Pouch until use in the studies.

Prior to viability testing, the pellets were washed twice using the formulation buffers to be tested, aliquoted out 5 mL from the final resuspension into vials and lyophilized them. The lyophilization used a primary drying of -25°C at 100 mTorr and used a secondary drying of 20°C at 100mTorr. The temperature ramp rate used in the lyophilization cycle was

increased to 2.5°C/min between conditions, as compared to the 1.0°C/min used in Examples 1-3.

To assess viability of the bacterial strains, the final lyophilized bacteria were resuspended in 5 mL of media and plated to determine the viable cell count after lyophilization. The excipients in the formulations and the results for strain 1 and 6 are shown in Tables 11 and 12, respectively. The initial viable cell counts prior to lyophilization for strain 1 and 6 were of 2.14×10^9 CFU/mL and 5.15×10^7 CFU/mL, respectively. The post-lyophilization average is the average of results from two vials.

Table 11: Lyophilization Formulation Excipients and Viability of Strain 1

Condition Formulation #	Additional Excipients	Post Lyo Average	Viability Average
1	None	$4.40x10^8$	20.59%
2	0.05% sodium meta bisulfite	$5.30x10^8$	24.80%
3	0.05% Ascorbic acid	$4.40x10^8$	20.59%
4	0.05% Citric acid	$7.60x10^8$	35.57%
5	0.5% sodium glutamate	$3.75x10^8$	17.55%
6	0.5% Arginine	$5.75x10^8$	26.91%
7	5% poloxamer 188	$8.50x10^5$	0.04%
8	5% Kollidon 30	$3.40x10^6$	0.16%
9	Poloxamer + Kollidon 30	$1.03x10^7$	0.48%

Table 12: Lyophilization Formulation Excipients and Viability of Strain 6

Condition Formulation #	Additional Excipients	Post-Lyo Average	Viability Average
1	None	$6.23x10^6$	11.96%
2*	0.05% sodium meta bisulfite	$4.32x10^7$	84.22%
3	0.05% Ascorbic acid	$3.56x10^6$	6.92%
4*	0.05% Citric acid	$1.31x10^7$	25.35%
5*	0.5% sodium glutamate	$1.89x10^7$	36.72%
6	0.5% Arginine	$9.25x10^6$	17.97%
7	5% poloxamer 188	$2.93x10^6$	5.69%
8	5% Kollidon 30	$2.76x10^5$	0.54%
9	Poloxamer + Kollidon 30	$7.00x10^3$	0.01%

EXAMPLE 5

This study was performed to evaluate lyophilization parameters in the freeze-drying trays (*e.g.*, GORE® Lyoguard® freeze-drying trays) that would be used for scaling up the lyophilization process for manufacturing. The previous studies described in Examples 1-4

were performed in 20mL vials. An engineering batch of Strain 3 (*Ruminococcus torques*) was compared to a tray of formulation buffer in separate freeze-drying trays.

The bacterial culture was grown in a 10 Liter fermenter volume which was diafiltered into the 7% Sucrose, 1% Yeast Extract, 0.05% L-Cysteine, 20mM Histidine, pH 7.0 formulation buffer. The freeze-drying trays were filled with a volume of 750 mL. The lyophilization cycle was run with the ramp rate of 1°C per minute. The lyophilization cycle data is shown in Figure 6. The pressure profile shows the pirani pressure reached 70 mTorr at 48 hrs. The bound water was removed during the secondary drying and the pirani reaches 70 mTorr at 57 hours. The appearance of the freeze-drying trays after lyophilization showed lyophilization cakes that were intact and not collapsed (data not shown), demonstrating the adequacy of the cycle. The primary drying step using 70 mTorr and -10°C along with a secondary drying step using 70 mTorr and 20°C successfully lyophilized strain 3 in the freeze-draying trays. This scaled up process for lyophilizing bacterial compositions can be applied to the other bacterial strains.

EXAMPLE 6

Additional studies were performed to evaluate bacterial recovery using 0.05% sodium metabisulfite as an excipient in the formulation and an increased temperature ramp of 2.5°C/min. A culture of bacterial strain 2 (*Anaerotruncus colihominis*) was inoculated in a 500 mL centrifuge bottle with a 1 mL cryovial. The culture was grown overnight and harvested at an OD of 0.325. This bacteria were spun down and washed with the a lyophilization formulation buffer containing 7% sucrose, 1% yeast extract, 0.05% cysteine, 0.05% sodium metabisulfite, and 20mM histidine. The bacteria were pelleted again, the supernatant was discarded, and the pellet was resuspended in a final volume of 100 mL of the same lyophilization formulation buffer. Seven milliliters of the resuspension were aliquoted into 20 mL vials and lyophilized using a temperature ramp rate of 2.5°C/min between temperature hold points.

The viability of strain 2 before lyophilization was measured as 6.4×10^8 CFU/mL. The viability after lyophilization was 1.93×10^8 CFU/mL, resulting in a viability of 30%. This was an improved yield from previous runs, so the formulation buffer selected for strain 2 included 0.05% sodium metabisulfite as an excipient. The freezing rate of 2.5°C/min was selected for

strain 2 based on this run, which was used as the rate of temperature ramp rate between all steps during lyophilization.

Formulations and lyophilization cycle conditions for each of bacterial strains 1-8 are shown in Table 13.

Table 13: Lyophilization formulations and conditions

Bacterial Strain*	Formulation	Temperature Ramp Rate	Primary Drying	Secondary Drying
1	7% Sucrose, 1% Yeast Extract, 0.05% L-Cysteine, 0.05% Sodium Metabisulfite, 20mM Histidine, pH 7.0	2.5°C/min	-10°C / 70mTorr	20°C / 70mTorr
2	7% Sucrose, 1% Yeast Extract, 0.05% L-Cysteine, 0.05% Sodium Metabisulfite, 20mM Histidine, pH 7.0	2.5°C/min	-10°C / 70mTorr	20°C / 70mTorr
3	7% Sucrose, 1% Yeast Extract, 0.05% L-Cysteine, 20mM Histidine, pH 7.0	1°C/min	-10°C / 70mTorr	20°C / 70mTorr
4	7% Sucrose, 1% Yeast Extract, 0.05% L-Cysteine, 20mM Histidine, pH 7.0	1°C/min	-10°C / 70mTorr	20°C / 70mTorr
5	7% Sucrose, 1% Yeast Extract, 0.05% L-Cysteine, 20mM Histidine, pH 7.0	1°C/min	-10°C / 70mTorr	20°C / 70mTorr
6	7% Sucrose, 1% Yeast Extract, 0.05% L-Cysteine, 0.05% Sodium Metabisulfite, 20mM Histidine, pH 7.0	2.5°C/min	-10°C / 70mTorr	20°C / 70mTorr
7	7% Sucrose, 1% Yeast Extract, 0.05% L-Cysteine, 20mM Histidine, pH 7.0	1°C/min	-10°C / 70mTorr	20°C / 70mTorr
8	7% Sucrose, 1% Yeast Extract, 0.05% L-Cysteine, 20mM Histidine, pH 7.0	1°C/min	-10°C / 70mTorr	20°C / 70mTorr

*Strain numbering corresponds to Table 7.

CLAIMS

1. A composition comprising a disaccharide, yeast extract, cysteine, sodium metabisulfite, and a buffer, wherein the buffer is at a pH of 6.7 to 7.3, wherein the disaccharide is not lactose.

2. The composition of claim 1, wherein the disaccharide is sucrose.

3. The composition of claim 2, wherein the sucrose is at a concentration between 6.0% and 10.0%.

4. The composition of claim 3, wherein the sucrose is at a concentration between 7.0% and 8.0%.

5. The composition of claim 1, wherein the disaccharide is trehalose.

6. The composition of claim 5, wherein the trehalose is at a concentration between 6.0% and 10.0%.

7. The composition of claim 5 or 6, wherein the trehalose is at a concentration between 7.0% and 8.0%.

8. The composition of any one of the preceding claims, wherein the concentration of the yeast extract is between 0.5% and 2.0%.

9. The composition of any one of the preceding claims, wherein the concentration of cysteine is between 0.01% and 0.5%.

10. The composition of any one of the preceding claims, wherein the buffer is at about pH 7.0, wherein the buffer is a histidine buffer or a Tris buffer.

11. The composition of any one of the preceding claims, wherein the buffer is at a concentration between 10 mM and 50 mM.

12. The composition of claim 5, wherein the composition comprises 7.5% trehalose, 1% yeast extract, 0.05% cysteine, and 20 mM histidine buffer.

13. The composition of claim 2, wherein the composition comprises 7.0% sucrose, 1% yeast extract, 0.05% cysteine, and 20 mM histidine buffer.

14. The composition of any one of the preceding claims, wherein the concentration of sodium metabisulfite is 0.05%.

15. The composition of any one of the preceding claims, wherein the composition comprises bacteria belonging to the class Clostridia.

16. The composition of any one of the preceding claims, wherein the composition comprises bacteria belonging to *Clostridium* cluster IV, XIVa, XVI, XVII, and/or XVIII.

17. The composition of any one of the preceding claims, wherein the composition comprises bacteria, wherein the bacteria comprise one or more bacterial strains selected from the group consisting of *Clostridium bolteae*, *Anaerotruncus colihominis*, *Sellimonas intestinalis*, *Clostridium symbiosum*, *Blautia producta*, *Dorea longicatena*, *Clostridium innocuum*, and*Flavonifractor plautii*.

18. The composition of any one of the preceding claims, wherein the composition comprises bacteria, wherein the bacteria comprise one or more bacterial strains comprising 16S rDNA sequences having at least 97% sequence identity with nucleic acid sequences selected from the group consisting of SEQ ID NOs: 1-8.

19. A method for preserving bacteria, the method comprising:

adding bacteria belonging to the class Clostridia to a composition of any one of the preceding claims; and

subjecting the composition comprising the bacteria to a lyophilization cycle.

20. The method of claim 19, wherein the bacteria belong to *Clostridium* cluster IV, XIVa, XVI, XVII, and/or XVIII.

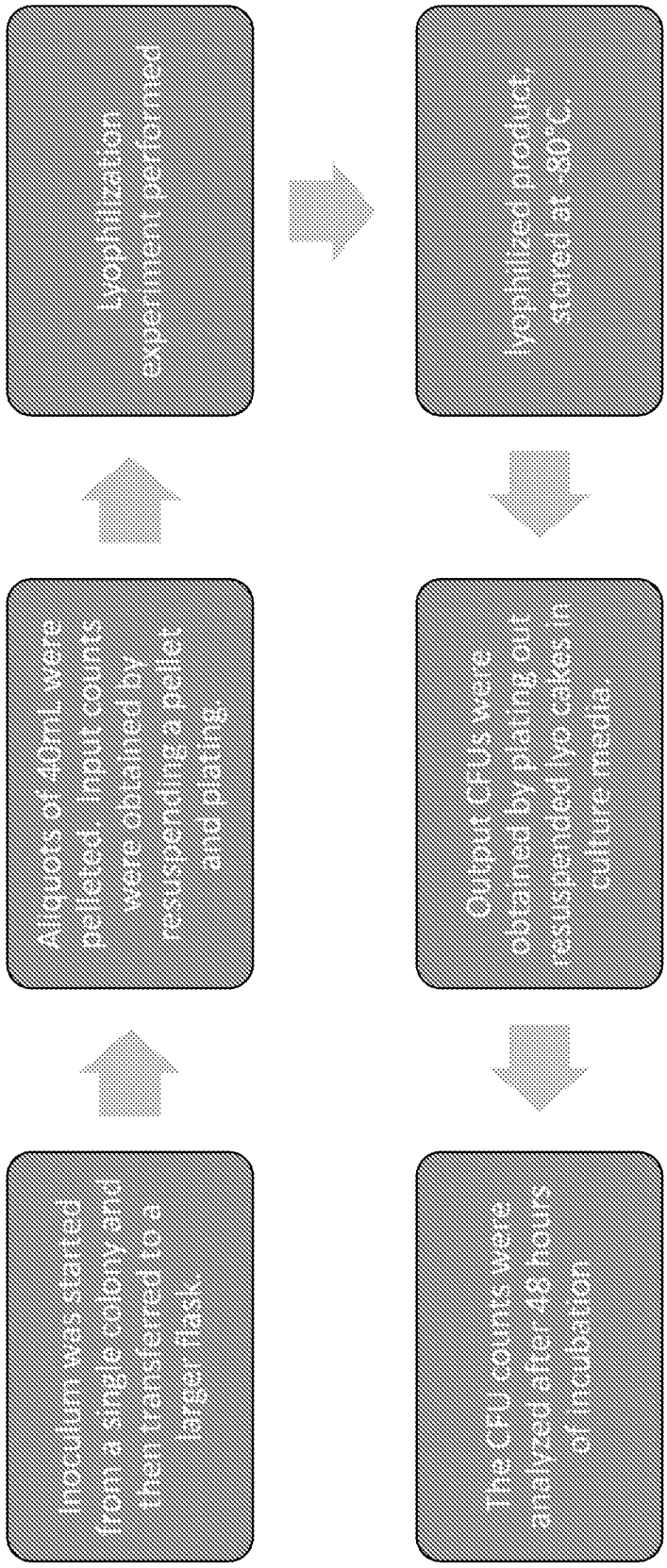
Inoculum was started from a single colony and then transferred to a larger flask
Aliquots of 40mL were pelleted. Input counts were obtained by resuspending a pellet and plating.
Lyophilization experiment performed
The CFU counts were analyzed after 48 hours of incubation
Output CFUs were obtained by plating out resuspended lyo cakes in culture media
lyophilized product stored at -80°C

Figure 1

Figure 2

Formulation #	Buffer / pH	Mannitol	Trehalose	Sorbitol	Sucrose	Lactose	Osmolality
1	His / 6.5	4%					318
2	His / 7.0	4%					314
3	Tris / 7.0	4%					307
4	Tris / 7.5	4%					300
5	His / 6.5		8%				304
6	His / 7.0		8%				302
7	Tris / 7.0		8%				312
8	Tris / 7.5		8%				318
9	His / 6.5				8%		316
10	His / 7.0				8%		317
11	Tris / 7.0				8%		308
12	His / 7.0				8%		309
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14	Tris / 7.0			4%			292
15	His / 7.0	4%				4%	329
16	Culture media						

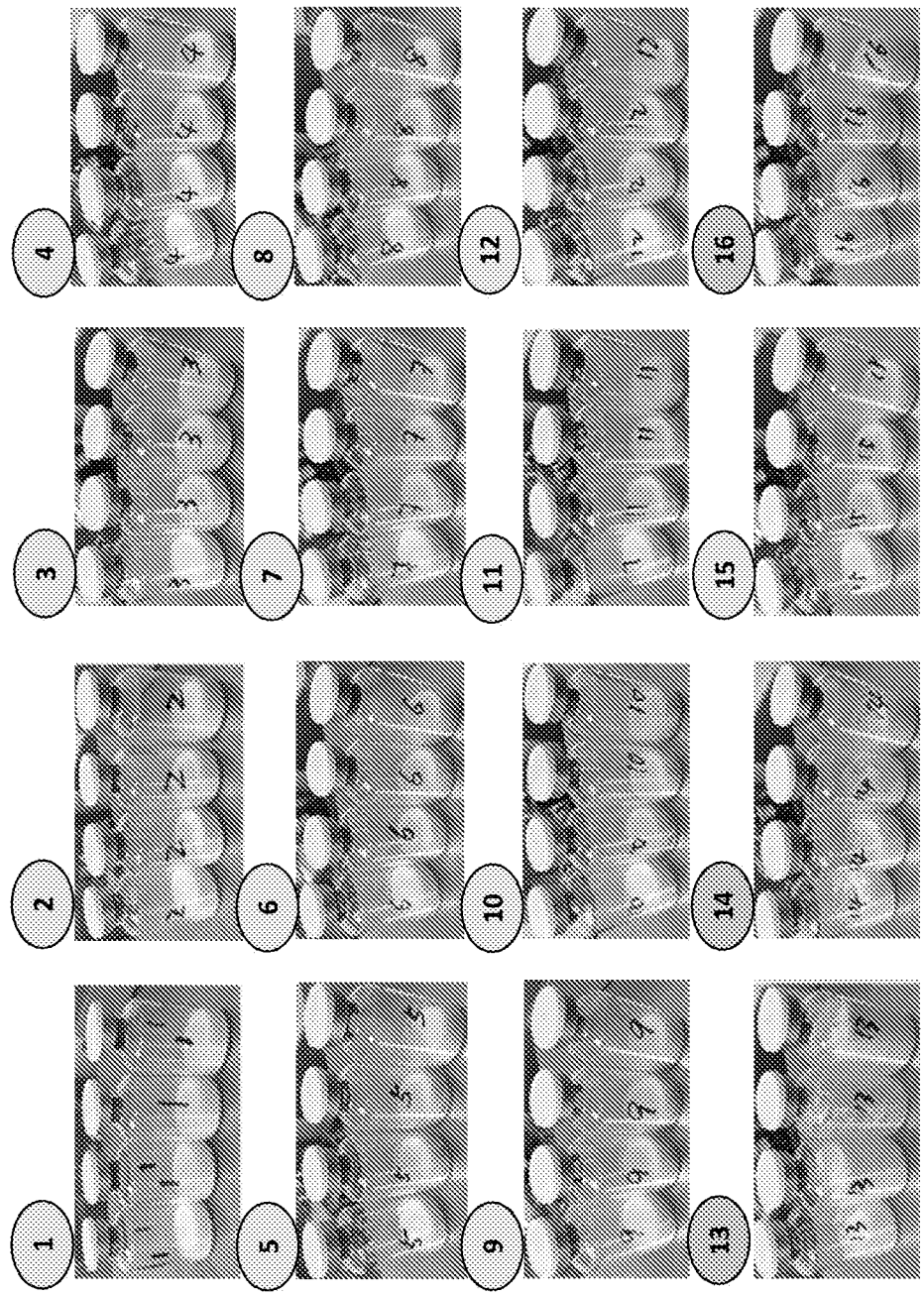
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Figure 3

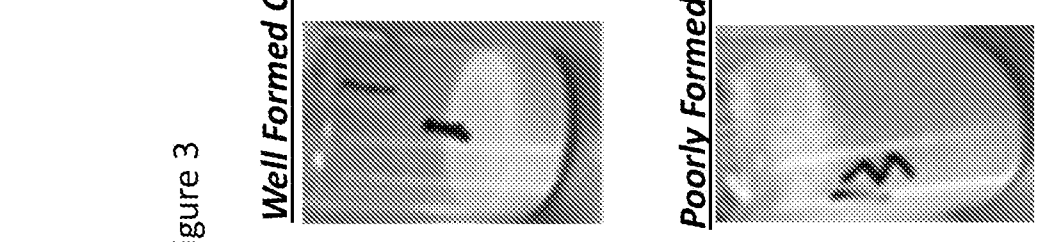
Well Formed Cake
Poorly Formed Cake

Figure 4

Formulation #	Buffer / pH	Mannitol	Trehalose	Sorbitol	Sucrose	Lactose	Viability (CFU)
1	His / 6.5	4%					0
2	His / 7.0	4%					0
3	Tris / 7.0	4%					0
4	Tris / 7.5	4%					0
5	His / 6.5		8%				2.8 x 10^3
6	His / 7.0		8%				9.5 x 10^4
7	Tris / 7.0		8%				1.0 x 10^4
8	Tris / 7.5		8%				1.7 x 10^5
9	His / 6.5				8%		~150
10	His / 7.0				8%		3.1 x 10^5
11	Tris / 7.0				8%		2.9 x 10^4
12	Tris / 7.5				8%		2.3 x 10^5
13	His / 7.0			4%			6.4 x 10^4
14	Tris / 7.0			4%			2.3 x 10^5
15	His / 7.0	4%				4%	0
16	media						~350

Poor Cake

Figure 5

Formulation Conditions

A - **Trehalose 7.5%**, 20 mM Histidine, pH 7.0, 1% Yeast extract, 0.05% Cysteine, 300 mOsmo/kg

B - **Sucrose 7%**, 20 mM Histidine, pH 7.0, 1% Yeast extract, 0.05% Cysteine, 300 mOsmo/kg

Bacterial Strain	Initial CFU	Post Lyo CFU (Condition A)	Post Freeze Thaw (Condition A)	Post Lyo CFU (Condition B)	Post Freeze Thaw (Condition B)
01	1.04×10^8	5×10^7	3×10^7	2×10^6	8×10^7
02	2.16×10^8	1×10^8	7×10^9	1.5×10^8	1.4×10^{10}
03	8.1×10^8	8×10^9	3.1×10^{10}	7.5×10^9	1×10^{10}
04	9.6×10^8	3×10^8	3.2×10^9	3.05×10^9	1.2×10^9
05	6.7×10^7	1.2×10^9	2.5×10^{10}	1×10^9	2×10^{10}
06	1.4×10^7	4.4×10^5	N/A	1×10^6	8×10^5
07	2.75×10^9	4×10^9	5×10^9	4×10^{10}	Lawn on 10^7
08	1.65×10^8	3.1×10^8	3.1×10^9	4.1×10^8	2.4×10^{10}

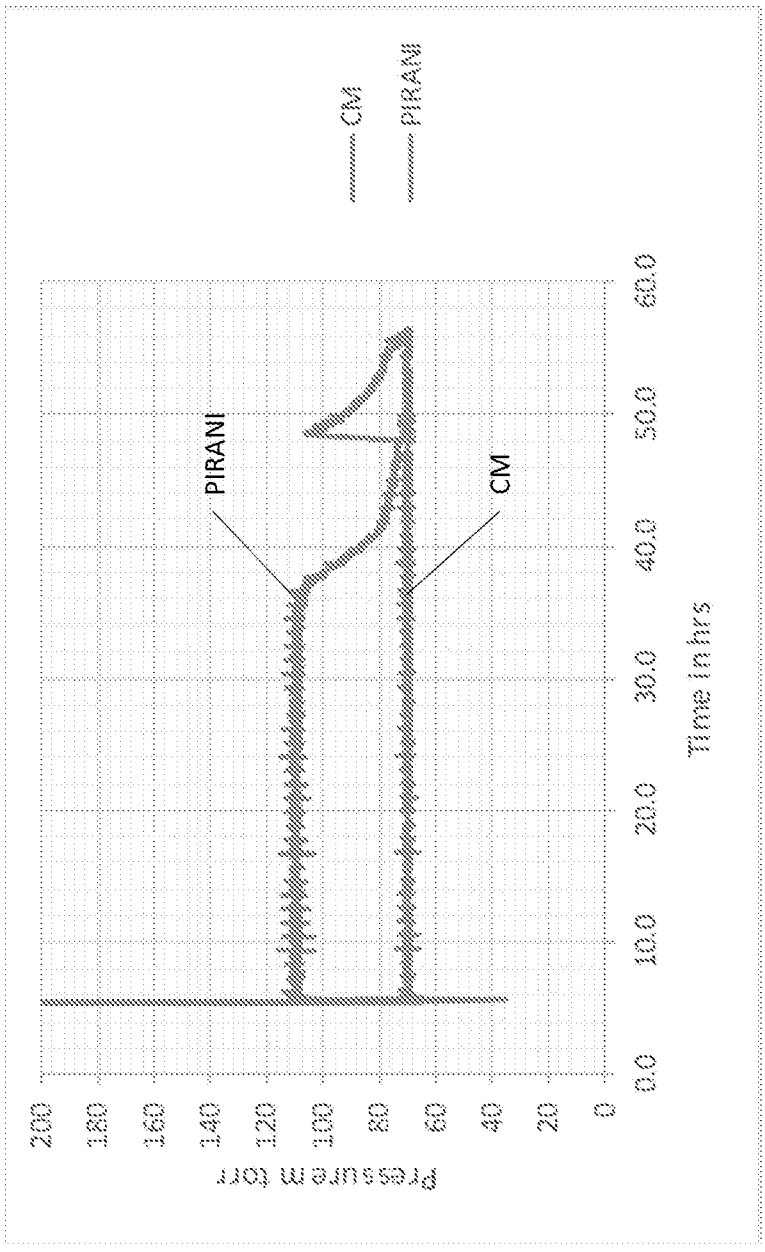
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Figure 6

SEQUENCE LISTING

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