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(54) Title: BACTERIAL COMPOSITIONS

(57) Abrégé/Abstract:
This invention provides bacterial compositions. For example, bacterial compositions having a combination of different bacterial strains formulated in a manner to maintain the stability of the bacteria are provided. In some embodiments, methods and materials provided herein can be used to deliver an effective and live dose of probiotics, to protect the probiotics during shelf life storage, and/or to achieve gastrointestinal bioavailability.

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(54) Title: BACTERIAL COMPOSITIONS

(57) Abstract: This invention provides bacterial compositions. For example, bacterial compositions having a combination of different bacterial strains formulated in a manner to maintain the stability of the bacteria are provided. In some embodiments, methods and materials provided herein can be used to deliver an effective and live dose of probiotics, to protect the probiotics during shelf life storage, and/or to achieve gastrointestinal bioavailability.

BACTERIAL COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to co-pending U.S. Provisional Patent Application No. 62/099,410, filed Jan. 2, 2015, and entitled "Bacterial Compositions," which is incorporated herein in its entirety by reference.

BACKGROUND

1. Technical Field

This document relates to the field of bacterial compositions. For example, this document provides bacterial compositions having combinations of different bacterial strains formulated in a manner to maintain the stability of the bacteria.

2. Background Information

Consuming particular microorganisms in the form of a probiotic formulation can provide health benefits to mammals. There are hundreds of different bacterial strains within a human's digestive system. It is believed that some of these different bacteria help maintain a healthy digestive tract and help digest food.

Unfortunately, probiotic strains are extremely sensitive, and some strains may not be able to survive commercial production, storage, or gastrointestinal transit after consumption where they are exposed to heat, moisture, bile, low pH, and digestive enzymes. As a result, an undesirable reduction in the probiotic strain count occurs before the bacteria reach the intestine.

SUMMARY

This document provides bacterial compositions. For example, this document provides bacterial compositions having a combination of different bacterial strains formulated in a manner to maintain the stability of the bacteria. For example, the methods and materials provided herein can be used to deliver an effective and live dose

of probiotics, to protect the probiotics during shelf life storage, and to achieve gastrointestinal bioavailability.

As described herein, a single bacterial composition (e.g., a capsule or tablet or sachet) can be formulated to include at least seven different bacterial strains, a form of silica (e.g., silica powder), fructooligosacchride, magnesium stearate, and a low moisture filler (e.g., a microcrystalline cellulose such as MCC 112) to provide a final composition with a water activity (Aw) less than 0.3 in a manner that helps maintain the stability of the bacteria within the composition and in a manner that delivers the bacteria to the intestines following oral administration. For example, at least about 1 x 10^9 colony forming units (CFUs) of each of *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* can be formulated with silica, fructooligosacchride, magnesium stearate, and a filler with a lower water content to form compositions with an A_w less than 0.3 that maintain the viability of at least about 80 percent of the bacteria for at least about 12 months, at least about 18 months, or at least about 24 months, under standard storage conditions (e.g., room temperature under normal humidity).

As used herein, the term "about" when used to refer to weight % in a composition means ±10% of the reported weight %. As used herein, the term "about" when used to refer to measured characteristics of the composition means ± 20% of the reported value.

The bacterial compositions provided herein can be coated with a coating agent and/or encapsulated to minimize or prevent moisture adsorption and to minimize water activity of the final blend.

In general, one aspect of this document features a bacterial composition comprising the bacteria *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum*, wherein the bacterial composition releases the *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* within an intestine of a mammal following oral administration to the mammal, and wherein the composition has a water activity less than 0.3. For every gram of the

composition, the composition can comprise between about 4 x 10^9 colony forming units and about 50 x 10^9 colony forming units of *Lactobacillus acidophilus*. For every gram of the composition, the composition can comprise between about 5 x 10^9 colony forming units and about 60 x 10^9 colony forming units of *Lactobacillus plantarum*. For every gram of the composition, the composition can comprise between about 4 x 10^9 colony forming units and about 50 x 10^9 colony forming units of *Lactobacillus casei*. For every gram of the composition, the composition can comprise between about 4 x 10^9 colony forming units and about 50 x 10^9 colony forming units of *Lactobacillus rhamnosus*. For every gram of the composition, the composition can comprise between about 2 x 10^9 colony forming units and about 40 x 10^9 colony forming units of *Bifidobacterium longum*. For every gram of the composition, the composition can comprise between about 4 x 10^9 colony forming units and about 50 x 10^9 colony forming units of *Bifidobacterium lactis*. For every gram of the composition, the composition can comprise between about 2 x 10^9 colony forming units and about 40 x 10^9 colony forming units of *Bifidobacterium bifidum*. The composition can comprise fructooligosaccharides. For every gram of the composition, the composition can comprise between about 0.5 mg and about 50 mg of fructooligosaccharides. The composition can comprise silicon dioxide. For every gram of the composition, the composition can comprise between about 5 mg and about 20 mg of silicon dioxide. The composition can comprise magnesium stearate. For every gram of the composition, the composition can comprise between about 10 mg and about 20 mg of magnesium stearate. For every gram of the composition, the composition can comprise more than 8 x 10^9 CFUs of bacteria. For every gram of the composition, the composition can comprise more than 10 x 10^9 CFUs of bacteria. For every gram of the composition, the composition can comprise more than 15 x 10^9 CFUs of bacteria. For every gram of the composition, more than 10 x 10^9 CFUs of bacteria of the composition remain viable after about 12 months of storage at room temperature and environmental humidity. For every gram of the composition, more than 10 x 10^9 CFUs of bacteria of the composition remain viable after about 12 months of storage at room temperature and environmental humidity. The *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium*

longum, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* can be lyophilized. The mammal can be a human. The bacterial composition can comprises a coating that prevents release of the *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* until the composition reaches an intestine of the mammal following oral administration to the mammal. The bacterial composition can comprise a capsule housing the *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum*. The composition can comprise a microcrystalline cellulose.

In another aspect, this document features a method for making a bacterial composition. The method comprises, or consists essentially of, (a) coating lyophilized bacteria with silicon dioxide to form coated lyophilized bacteria, (b) adding MCC having a water activity of less than 0.2 and fructooligosaccharides to the coated lyophilized bacteria to form a first mixture, (c) adding magnesium stearate and silicon dioxide to the first mixture to form a blend, and (d) encapsulating the blend into a dosage form, thereby forming the bacterial composition. The lyophilized bacteria can be a mixture of lyophilized *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum*. For every gram of the composition, the composition can comprise (a) between about 4×10^9 colony forming units and about 50×10^9 colony forming units of *Lactobacillus acidophilus*; (b) between about 5×10^9 colony forming units and about 60×10^9 colony forming units of Lactobacillus *plantarum*; (c) between about 4×10^9 colony forming units and about 50×10^9 colony forming units of *Lactobacillus casei*; (d) between about 4×10^9 colony forming units and about 50×10^9 colony forming units of *Lactobacillus rhamnosus*; (e) between about 2×10^9 colony forming units and about 40×10^9 colony forming units of *Bifidobacterium longum*; (f) between about 4×10^9 colony forming units and about 50×10^9 colony forming units of *Bifidobacterium lactis*; and (g) between about 2×10^9 colony forming units and about 40×10^9 colony forming units of *Bifidobacterium bifidum*. For every gram of the composition, the composition can comprise between about 0.5 mg and about 5.0 mg of

fructooligo-saccharides. For every gram of the composition, the composition can comprise between about 5.0 mg and about 20.0 mg of silicon dioxide. For every gram of the composition, the composition can comprise more than 15 x 10^9 CFUs of bacteria. For every gram of the composition, more than 10 x 10^9 CFUs of bacteria of the composition remain viable after about 12 months of storage at room temperature and environmental humidity. The dosage form can be a capsule.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DETAILED DESCRIPTION

This document provides bacterial compositions. For example, this document provides bacterial compositions having a combination of different bacterial strains (e.g., a combination of at least six, seven, eight, nine, or ten different bacterial strains) formulated in a manner to maintain the stability of the bacteria. The bacterial compositions provided herein can be in the form of powders, capsules, pills, tablets, chewing gums, lozenges, candy, or sachets. In some cases, a bacterial composition provided herein can include a coating designed to prevent moisture adsorption and minimize the water activity of the final blend to provide a formulation with good long term stability.

For example, methacrylate coatings, hydroxy propyl methyl cellulose pthalate coatings, cellulose acetate succinate coatings, hydroxy propyl methyl cellulose acetate succinate coatings, polyvinyal acetate pthalate coatings, or cellulose acetate trimellitate sodium alginate coatings can be used to deliver the bacterial contents of a bacterial composition past the stomach. Such coatings can be made and applied as described elsewhere (e.g., U.S. Patent Application Publication No. 2014/370091, Chinese Patent No. CN103976975, and Taiwan Patent No. TW201431561).

In some cases, a bacterial composition provided herein can include any combination of at least six (e.g., at least seven, eight, nine, or ten) different bacterial strains. Examples of different bacterial strains that can be formulated into a bacterial composition provided herein include, without limitation, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, *Bifidobacterium bifidum*, *Bacillus coagulans*, *Lactobacillus paracasei*, *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus bulgaricus*, *Bifidobacterium breve, Lactobacillus brevis, Lactococcus lactis* and *Streptococcus thermophilus*. In some cases, a bacterial composition provided herein can include one or more bacterial species such as *Saccharomyces boulardii*. In some cases, the only bacterial strains present within a particular bacterial composition can be *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum,* or any combination of six selected from *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum*.

Unless otherwise noted, all bacteria amounts in this application are provided on a per gram of total bacterial composition in the composition basis. In some cases, a bacterial composition provided herein can contain at least about 1×10^9 CFUs of each different bacterial strain included in the bacterial composition. In some cases, a bacterial composition provided herein can contain between about 1×10^8 and about 1×10^{11} bacteria of all species (e.g., between about 1×10^9 and about 4×10^{10} bacteria of all species). For example, a bacterial composition provided herein can include between

about 1 x 10^7 CFUs and about 3 x 10^9 CFUs of *Lactobacillus acidophilus*, between about 1 x 10^7 CFUs and about 4 x 10^9 CFUs of *Lactobacillus plantarum*, between about 1 x 10^7 CFUs and about 3 x 10^9 CFUs of *Lactobacillus casei*, between about 1 x 10^7 CFUs and about 3.2 x 10^9 CFUs of *Lactobacillus rhamnosus*, between about 1 x 10^7 CFUs and about 1.6 x 10^9 CFUs of *Bifidobacterium longum*, between about 1 x 10^7 CFUs and about 3.6 x 10^9 CFUs of *Bifidobacterium lactis*, and between about 1 x 10^7 CFUs and about 1.6 x 10^9 CFUs of *Bifidobacterium bifidum*.

Any appropriate method can be used to obtain the bacterial strains to be included within a bacterial composition provided herein. For example, culturing techniques can be used to obtain large amounts of particular bacterial strains. In some cases, the bacterial strains to be included within a bacterial composition provided herein can be obtained commercially. For example, *Lactobacillus acidophilus* can be obtained commercially from Dupont Inc. (Madison, WI; Cat. No. LA-14 200B); *Lactobacillus plantarum* can be obtained commercially from Dupont Inc. (Madison, WI; Cat. No. LP-115 400B); *Lactobacillus casei* can be obtained commercially from Dupont Inc. (Madison, WI; Cat. No. LC-11 300B), *Lactobacillus rhamnosus* can be obtained commercially from Dupont Inc. (Madison, WI; Cat. No. LR-32 200B), *Bifidobacterium longum* can be obtained commercially from Dupont Inc. (Madison, WI; Cat. No. BL-05 100B), *Bifidobacterium lactis*, can be obtained commercially from Dupont Inc. (Madison, WI; Cat. No. BL-04 450B), and *Bifidobacterium bifidum* can be obtained commercially from Dupont Inc. (Madison, WI; Cat. No. BB-06 100B).

A bacterial composition provided herein can include one or more other ingredients. For example, a bacterial composition provided herein can include fructooligosaccharides. In some cases, a bacterial composition provided herein can include between about 0.5 mg and about 50 mg (e.g., between about 1 mg and about 50 mg, between about 5 mg and about 50 mg, between about 10 mg and about 50 mg, between about 0.5 mg and about 25 mg, between about 0.5 mg and about 20 mg, between about 0.5 mg and about 15 mg, between about 0.5 mg and about 10 mg, or between about 5 mg and about 20 mg) of fructooligosaccharides per bacterial composition. For example, a bacterial composition provided herein can contain *Lactobacillus acidophilus*,

Lactobacillus plantarum, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* and between about 5 mg and about 20 mg of fructooligosaccharides.

In some cases, a bacterial composition provided herein can include between about 1 mg and about 50 mg (e.g., between about 1 mg and about 20 mg, between about 2 mg and about 20 mg, or between about 2 mg and about 10 mg) of fructooligosaccharides for every gram of the bacterial composition. For example, a bacterial composition provided herein that weighs 2 grams can contain between about 10 mg and about 100 mg of fructooligosaccharides.

Any appropriate method can be used to obtain fructooligosaccharides that can be included in a bacterial composition provided herein. For example, fructooligosaccharides can be obtained by an inulin degradation process or by a transfructosylation process as described elsewhere (U.S. Patent Application Publication No. 2005/069627). In some cases, fructooligosaccharides can be obtained commercially from Agaviotica Inc. (Monterrey, NL (Mexico); Cat. No. Fructagave PR-95).

In some cases, a bacterial composition provided herein can include a glidant (e.g., silica). Examples of glidants that can be included within a bacterial composition provided herein include, without limitation, silica, stearic acid, calcium stearate, magnesium stearate, sodium stearate, glyceryl behapate (compritol), liquid paraffin, Aerosil® (colloidal silicon dioxide), starch and talc, DL-leucine, and sodium lauryl sulfate. For example, a bacterial composition provided herein can include between about 2.00 mg and about 5.00 mg (e.g., between about 0.005 mg and about 010 mg, between about 0.01 mg and about 0.02 mg, or between about 0.25 mg and about 0.50 mg) of a glidant (e.g., silica) per bacterial composition. For example, a bacterial composition provided herein can contain *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* and between about 0.005 mg and about 0.02 mg of silica.

In some cases, a bacterial composition provided herein can include between about 2.0 mg and about 5.0mg of a glidant (e.g., silica) for every gram of the bacterial

composition. For example, a bacterial composition provided herein that weighs 2 grams can contain between about 4.0 mg and about 10.0 mg of a glidant (e.g., silica).

Any appropriate method can be used to obtain of a glidant (e.g., silica) that can be included in a bacterial composition provided herein. For example, silica can be obtained as described elsewhere (U.S. Patent Application Publication No. 2006/153764). In some cases, silica can be obtained commercially from Grace Davison Inc. (Baltimore, MD; Cat. No. Syloid 244).

In some cases, a bacterial composition provided herein can include a filler having an A_w of less than 0.2. Examples of such fillers include, without limitation, microcrystalline cellulose (MCC 112), rice maltodextrin, lactose anhydrous, mannitol, microcrystalline cellulose (MCC 302), microcrystalline cellulose (MCC 200 LM), microcrystalline cellulose (MCC 101), starch, xylitol, sorbitol, hydroxyl propyl cellulose, a gelatin, polyvinyl pyrrolidone, and dibasic calcium phosphate. In some cases, a bacterial composition provided herein can include between about 300 mg and about 700 mg of a filler having an A_w of less than 0.2 (e.g., MCC 112) per bacterial composition. For example, a bacterial composition provided herein can contain *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* and between about 300 mg and about 700 mg of a filler having an A_w of less than 0.2 (e.g., MCC 112). In some cases, a bacterial composition provided herein can include between about 300 mg and about 700 mg of a filler having an A_w of less than 0.2 (e.g., MCC 112) for every gram of the bacterial composition. For example, a bacterial composition provided herein that weighs 2 grams can contain between about 600 mg and about 1400 mg of a filler having an A_w of less than 0.2 (e.g., MCC 112). As a result, the final composition can have an A_w of less than 0.3.

Any appropriate method can be used to obtain a filler having an A_w of less than 0.2 for use in the bacterial compositions provided herein. For example, a filler having an A_w of less than 0.2 can be obtained as described elsewhere (U.S. Patent Application Publication No. US20140322282, published October 30, 2014). In some cases, a filler

having an A_w of less than 0.2 can be obtained commercially from Mingtai chemical Co Ltd.,Taiwan, Cat. No. M112 D.

In some cases, a bacterial composition provided herein can include magnesium stearate. For example, a bacterial composition provided herein can include between about 10 mg and about 20 mg of magnesium stearate per bacterial composition. For example, a bacterial composition provided herein can contain *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* and between about 10 mg and about 20 mg of magnesium stearate.

In some cases, a bacterial composition provided herein can include between about 10 mg and about 20 mg of magnesium stearate for every gram of the bacterial composition. For example, a bacterial composition provided herein that weighs 2 grams can contain between about 20 mg and about 40 mg of magnesium stearate.

Any appropriate method can be used to obtain magnesium stearate that can be included in a bacterial composition provided herein. For example, magnesium stearate can be obtained as described elsewhere (e.g., Chinese Patent No. CN103880645, dated June 25, 2014; Chinese Patent No. CN103524324, dated January 22, 2014, and Chinese Patent No. CN10319361, dated July 10, 2013). In some cases, magnesium stearate can be obtained commercially from Peter Greven Asia, Malaysia; Cat. No. Palmstar MGST 200. A bacterial composition provided herein (e.g., a capsule or tablet) can be formulated to have a particular dose. For example, a bacterial composition provided herein can be in the form of a capsule or tablet with a total weight that is between about 150 mg and about 800 mg (e.g., between about 200 mg and about 800 mg, between about 300 mg and about 800 mg, between about 350 mg and about 800 mg, between about 150 mg and about 700 mg, between about 150 mg and about 650 mg, or between about 350 mg and about 700 mg).

As described herein, a single bacterial composition (e.g., a capsule or tablet) can be formulated to include at least six different bacterial strains and a filler having an A_w less than 0.2 (e.g., MCC 112) in a manner that helps maintain the stability of the bacteria within the composition. In some cases, a single bacterial composition provided herein

can be coated with at coating or placed into a capsule having the ability to deliver the contents to the intestines of a mammal following oral administration. For example, a bacterial composition provided herein can be designed to release its contents when the composition reaches a location of the intestines having a pH above about 6.8. In some cases, a bacterial composition that includes at least seven different bacterial strains and a filler having an A_w less than 0.2 (e.g., MCC 112) can include fructooligosaccharides, magnesium stearate, and/or silica. In some cases, the bacterial strains of a bacterial composition provided herein can be lyophilized to form a dried powder containing viable bacteria. In some cases, a single bacterial composition (e.g., a capsule or tablet or sachet) can be formulated to include at least seven different lyophilized bacterial strains, a filler having an A_w less than 0.2 (e.g., MCC 112), fructooligosaccharides, magnesium stearate, and silica.

In some cases, a bacterial composition provided herein can maintain the viability of at least about 80 percent (e.g. at least about 90, 95, or 99 percent) of the bacteria for at least 12 months (e.g., at least 40, 50, 60, 70, 80, or 90 days or at least 3, 6, 9, or 12 months) under standard storage conditions (e.g., room temperature under normal humidity). In some cases, a bacterial composition provided herein can maintain the viability of at least about 80 percent of the bacteria for at least 18 months or for at least 24 months.

The bacterial composition provided herein can be administered to a mammal (e.g., a human). In some cases, a human can be instructed to self-administer a number (e.g., one, two, three, four, five, or more) bacterial compositions provided herein (e.g., capsules or tablets or sachets) per unit time (e.g., per day, per week, or per month). For example, a human can be instructed to self-administer one or two bacterial compositions provided herein (e.g., a capsule of Example 1) per day.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 – Stability of bacterial compositions

Two bacterial compositions were produced (Prototype trial 1 and Prototype trial 2) as follows. The final formulations were stored in various bottles as described below:

(i) MCC 101 and MCC 112 were used as diluent in prototype trials.

(ii) PET bottle, HDPE bottle, and sealed Aluminum foil packet were used in storing the final capsule.

Prototype trial 1:

Component	Potency of raw ingredient (billion CFU/g)	% of ingredients	Final Potency (Billion CFU/g)
Lactobacillus acidophilus	200	7.58	15.00
Bifidobacterium lactis	450	2.07	9.00
Fructooligosaccharides		3.70	
Dicalcium Phosphate		1.85	
MCC 101		81.90	
Calcium Stearate		1.40	
Silicon Dioxide		1.48	
Total		100.0%	24

270 mg of above blend was filled into a single capsule.

Potency of 270 mg of blend=24/1000*270=6.48 billion CFUs/capsule

Prototype trial 2:

Component	Potency of raw ingredient (billion CFU/g)	% of ingredients	Final Potency (Billion CFU/g)
Lactobacillus acidophilus	200	7.58	15.00
Bifidobacterium lactis	450	2.07	9.00
Fructooligosaccharides		3.70	
MCC 112		83.75	
Calcium Stearate		1.40	
Silicon Dioxide		1.48	
Total		100.0%	24

270 mg of above blend was filled into a single capsule.

Potency of 270 mg of blend=24/1000*270=6.48 billion CFUs/capsule

5

Prototype trial 3 is made as follows:

Component	Potency of raw ingredient (billion CFUs/g)	% of ingredients	Final Potency (Billion CFU/g)
Lactobacillus acidophilus	200	7.58	15.00
Bifidobacterium lactis	450	2.07	9.00
Fructooligosaccharides		3.70	
Dicalcium Phosphate		1.85	
MCC 112		81.90	
Calcium Stearate		1.40	
Silicon Dioxide		1.48	
Total		100.0%	24

270 mg of above blend is used to fill a single capsule.

Potency of 270 mg of blend=24/1000*270=6.48 billion CFUs/capsule

Once produced, the two bacterial compositions were tested for stability (Table 1).

Table 1. Probiotics count (CFUs/capsule) and water activity.

Formulation filler	MCC 101		MCC 112		MCC 112		MCC 112	
Packaging type	PET (Polyethylene terephthalate) bottle		PET bottle		High Density Polyethylene (HDPE) bottle		Aluminum foil packet	
	CFU/ capsule	A_w	CFU/ capsule	A_w	CFU/ capsule	A_w	CFU/ capsule	A_w
Initial	7.3 billion	NA	5.4 billion	0.165	5.0 billion	0.130	5.8 billion	0.130
after 3 months at 25°C/60 relative humidity	NA	NA	8.4 billion	0.169	8.4 billion	0.133	8.1 billion	0.141
after 3 months at 30°C/75 relative humidity	0.0016 billion	NA	2.9 billion	0.171	8.7 billion	0.125	7.8 billion	0.141

These results demonstrate that, using microcrystalline cellulose in bacterial compositions having at least two strains, MCC 112 improved stability over MCC 101 and that using a High Density Polyethylene (HDPE) bottle or Aluminum foil packet improved stability over using a PET bottle.

Example 2 – Stability of bacterial compositions

A bacterial composition was produced as follows.

Component	Potency of strain	% of ingredients, by weight	Final Potency in Billion CFU
Lactobacillus acidophilus	200	3.75	7.5
Lactobacillus plantarum	400	2.5	10
Lactobacillus casei	300	2.5	7.5
Lactobacillus rhamnosus	200	4.0	8.0
Bifidobacterium longum	100	4.0	4.0
Bifidobacterium lactis	450	2.0	9.0
Bifidobacterium bifidum	100	4.0	4.0
Fructooligosaccharides		5.0	
MCC 112		70.25	
Magnesium Stearate (Palmstar MGST 325)		1.50	
Silicon Dioxide (Syloid 244)		0.50	
Total		100.0%	50

400 mg of the above blend was filled into a single DR capsule. The potency of the 400 mg of blend was 20 billion CFUs/capsule.

Once produced, the bacterial composition was tested for stability (Table 2).

Table 2. Probiotics count (CFUs/capsule) and water activity.

Formulation filler	MCC 112
Bottle type	HDPE
Initial CFUs/capsule	12.7 billion
Initial water activity (A_w)	0.139

CFUs/capsule after 3 months at 25°C/60 relative humidity	14.0 billion
Water activity (A_w) after 3 months at 25°C/60 relative humidity	0.133

These results confirm that using microcrystalline cellulose MCC 112 and a High Density Polyethylene (HDPE) bottle maintained stability in a bacterial composition having seven strains of bacteria.

Example 3 – Bacterial Composition

A bacterial composition was prepared as follows (Table 3). Briefly, lyophilized bacteria of varying CFUs of each of *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* and silicon dioxide (silica; 2mg) were blended for two minutes to coat the dried bacteria uniformly. The silica can adsorb moisture, keeping the lyophilized bacteria dry with minimal water activity and improving the stability of final formulation. MCC 112 (grade of microcrystalline cellulose with A_w of less than 0.2 (700mg), and fructooligosaccharides (50 mg) were then added, and the mixture was blended for an additional 20 minutes. After blending, additional silica (3 mg) as a glidant and magnesium stearate (15 mg) as lubricant, were added, and the mixture was blended for another three minutes. The final mixture was inserted into capsules (e.g., DR capsules). DR capsules are vegetarian capsules made with a hypromellose (HPMC) formulation that can help protect sensitive ingredients from the low pH environment of the stomach. By protecting against early disintegration, disintegration generally starts approximately 45 minutes later than a typical immediate release capsule of about 5 minutes, and the ingredients are released in the intestine in alkaline pH.

CA 02972457 2017-06-27

Table 3. Bacterial composition.

		example formula 1		example formula 2		example formula 3		example formula 4		example formula 5	
Component	raw ingr. level (billion CFUs/g)	% of ingr., by weight	finished product (billion CFUs/g)	% of ingr., by weight	finished product (billion CFUs/g)	% of ingr., by weight	finished product (billion CFUs/g)	% of ingr., by weight	finished product (billion CFUs/g)	% of ingr., by weight	finished product (billion CFUs/g)
Lactobacillus acidophilus	200	3.75%	7.5	3.75%	7.5	3.75%	7.5	7.50%	15.0	7.50%	15
Lactobacillus plantarum	400	2.50%	10	2.50%	10	2.50%	10	1.00%	4.0	2.00%	8
Lactobacillus casei	300	2.50%	7.5	2.50%	7.5	2.50%	7.5	1.80%	5.40	0.90%	2.7
Lactobacillus rhamnosus	200	4.00%	8	4.00%	8	4.00%	8	0.90%	1.80	0.90%	1.8
Bifidobacterium longum	100	4.00%	4	4.00%	4	4.00%	4	1.00%	1.00	0.10%	0.1
Bifidobacterium lactis	450	2.00%	9	2.00%	9	2.00%	9	15.00 %	67.5	7.50%	33.75
Bifidobacterium bifidum	100	4.00%	4	4.00%	4	4.00%	4	0.20%	0.20	0.10%	0.1

CA 02972457 2017-06-27

Fructooligo-saccharides		5.00%		5.00%		5.00%		5.00%		5.00%	
MCC 112		70.25%									
MCC 101						30		65.6			
MCC 302										74%	
Rice Maltodextrin				70.25		40.25					
Magnesium Stearate (Palmstar MGST 325)		1.50%		1.00%		0.75%		1.5%			
Calcium Stearate				0.25		0.5				1.50%	
colloidal silicon dioxide (AEROSIL® R 972 Pharma)								0.25%			
Silicon Dioxide (Syloid 244)		0.50%		0.75%		0.75%		0.25		0.50%	
Total		100%	50					100.0	94.9	100%	61.45

Ingr. = ingredient

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A bacterial composition comprising *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum*, wherein said bacterial composition releases said *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* within an intestine of a mammal following oral administration to said mammal, and wherein said composition has a water activity less than 0.3.

2. The composition of claim 1, wherein for every gram of the composition said composition comprises between about 4 x 10^9 colony forming units and about 50 x 10^9 colony forming units of *Lactobacillus acidophilus*.

3. The composition of claim 1 or claim 2, wherein for every gram of the composition said composition comprises between about 5 x 10^9 colony forming units and about 60 x 10^9 colony forming units of *Lactobacillus plantarum*.

4. The composition of one of claims 1-3, wherein for every gram of the composition said composition comprises between about 4 x 10^9 colony forming units and about 50 x 10^9 colony forming units of *Lactobacillus casei*.

5. The composition of one of claims 1-4, wherein for every gram of the composition said composition comprises between about 4 x 10^9 colony forming units and about 50 x 10^9 colony forming units of *Lactobacillus rhamnosus*.

6. The composition of one of claims 1-5, wherein for every gram of the composition said composition comprises between about 2 x 10^9 colony forming units and about 40 x 10^9 colony forming units of *Bifidobacterium longum*.

7. The composition of one of claims 1-6, wherein for every gram of the composition said composition comprises between about 4 x 10^9 colony forming units and about 50 x 10^9 colony forming units of *Bifidobacterium lactis*.

8. The composition of one of claims 1-7, wherein for every gram of the composition said composition comprises between about 2 x 10^9 colony forming units and about 40 x 10^9 colony forming units of *Bifidobacterium bifidum*.

9. The composition of one of claims 1-8, wherein said composition comprises fructooligosaccharides.

10. The composition of one of claims 1-9, wherein for every gram of the composition said composition comprises between about 0.5 mg and about 50 mg of fructooligosaccharides.

11. The composition of one of claims 1-10, wherein said composition comprises silicon dioxide.

12. The composition of one of claims 1-11, wherein for every gram of composition said composition comprises between about 5 mg and about 20 mg of silicon dioxide.

13. The composition of one of claims 1-12, wherein said composition comprises magnesium stearate.

14. The composition of one of claims 1-13, wherein for every gram of composition said composition comprises between about 10 mg and about 20 mg of magnesium stearate.

15. The composition of one of claims 1-14, wherein for every gram of the composition said composition comprises more than 8 x 10^9 CFUs of bacteria.

16. The composition of one of claims 1-14, wherein for every gram of the composition said composition comprises more than 10 x 10^9 CFUs of bacteria.

17. The composition of one of claims 1-14, wherein for every gram of the composition said composition comprises more than 15 x 10^9 CFUs of bacteria.

18. The composition of claim 17, wherein for every gram of the composition more than 10 x 10^9 CFUs of bacteria of said composition remain viable after about 12 months of storage at room temperature and environmental humidity.

19. The composition of claim 17, wherein for every gram of the composition more than 10 x 10^9 CFUs of bacteria of said composition remain viable after about 12 months of storage at room temperature and environmental humidity.

20. The composition of one of claims 1-19, wherein said *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* are lyophilized.

21. The composition of one of claims 1-20, wherein said mammal is a human.

22. The composition of one of claims 1-21, wherein said bacterial composition comprises a coating that prevents release of said *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum* until said composition reaches an intestine of said mammal following oral administration to said mammal.

23. The composition of one of claims 1-22, wherein said bacterial composition comprises a capsule housing said *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium*

lactis, and *Bifidobacterium bifidum*.

24. The composition of one of claims 1-23, wherein said composition comprises a microcrystalline cellulose.

25. A method for making a bacterial composition, wherein said method comprises:

(a) coating lyophilized bacteria with silicon dioxide to form coated lyophilized bacteria,

(b) adding MCC having a water activity of less than 0.2 and fructooligosaccharides to said coated lyophilized bacteria to form a first mixture,

(c) adding magnesium stearate and silicon dioxide to said first mixture to form a blend, and

(d) encapsulating said blend into a dosage form, thereby forming said bacterial composition.

26. The method of claim 25, wherein said lyophilized bacteria is a mixture of lyophilized *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Bifidobacterium lactis*, and *Bifidobacterium bifidum*.

27. The method of claim 25 or claim 26, wherein for every gram of the composition said composition comprises:

(a) between about 4×10^9 colony forming units and about 50×10^9 colony forming units of *Lactobacillus acidophilus*;

(b) between about 5×10^9 colony forming units and about 60×10^9 colony forming units of Lactobacillus *plantarum*;

(c) between about 4×10^9 colony forming units and about 50×10^9 colony forming units of *Lactobacillus casei*;

(d) between about 4×10^9 colony forming units and about 50×10^9 colony forming units of *Lactobacillus rhamnosus*;

(e) between about 2 x 10^9 colony forming units and about 40 x 10^9 colony forming units of *Bifidobacterium longum*;

(f) between about 4 x 10^9 colony forming units and about 50 x 10^9 colony forming units of *Bifidobacterium lactis*; and

(g) between about 2 x 10^9 colony forming units and about 40 x 10^9 colony forming units of *Bifidobacterium bifidum*.

28. The method of one of claims 25-27, wherein for every gram of the composition said composition comprises between about 0.5 mg and about 5.0 mg of fructooligo-saccharides.

29. The method of one of claims 25-28, wherein for every gram of the composition said composition comprises between about 5.0 mg and about 20.0 mg of silicon dioxide.

30. The method of one of claims 25-29, wherein for every gram of the composition said composition comprises more than 15 x 10^9 CFUs of bacteria.

31. The method of one of claims 25-30, wherein for every gram of the composition more than 10 x 10^9 CFUs of bacteria of said composition remain viable after about 12 months of storage at room temperature and environmental humidity.

32. The method of one of claims 25-31, wherein said dosage form is a capsule.