(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
08 April 2021 (08.04.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/067100 A1

(51) International Patent Classification:
A61K 35/74 (2015.01) *C12N 1/20* (2006.01)
A61K 35/66 (2006.01) *A61K 35/00* (2006.01)
A23L 33/135 (2016.01)

(21) International Application Number:
PCT/US2020/052251

(22) International Filing Date:
23 September 2020 (23.09.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/909,186 01 October 2019 (01.10.2019) US

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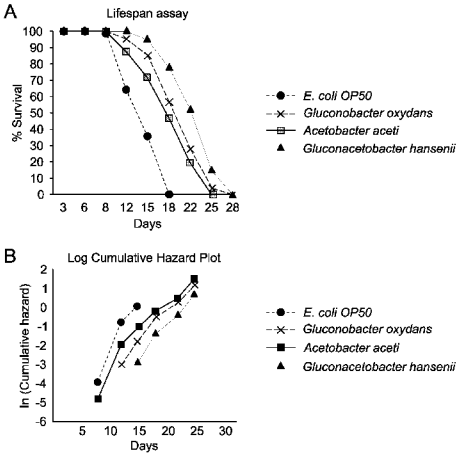
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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: COMPOSITIONS AND METHODS FOR EXTENDING LIFESPAN

(57) Abstract: Compositions are provided that extend the lifespan of a subject, and/or reduce or delay the onset of at least one age-associated symptom or condition. In some embodiments, compositions comprise: at least one bacterial strain or extract(s) or component(s) thereof and an excipient. In some embodiments, at least one bacterial strain comprises Gluconobacter spp., Acetobacter spp., Gluconoacaetobacter spp., Acidomonas spp., Ameyamaea spp., Asaia spp., Granulibacter spp., Kozakia spp., Neoasaia spp., Neokomagataea spp., Saccharibacter spp., Swaminathania spp., Tanticharoenia spp., or a combination thereof. Methods of making and using compositions disclosed herein are also provided.



C

C. elegans administered	Mean ± SE	N	95% C.I.
E. coli OP50	14.86 ± 0.27	104	14.33 ~ 15.40
Gluconobacter oxydans	20.52 ± 0.39	117	19.76 ~ 21.29
Acetobacter aceti	19.17 ± 0.41	124	18.37 ~ 19.98
Gluconacetobacter hansenii	22.95 ± 0.39	103	22.19 ~ 23.70

D

Condition	X^2	pvalue
E. coli OP50 v.s. *Gluconobacter oxydans*	92.48	0.00001
E. coli OP50 v.s. *Acetobacter aceti*	60.58	0.00001
E. coli OP50 v.s. *Gluconacetobacter hansenii*	137.42	0.00001
Gluconobacter oxydans v.s. *Acetobacter aceti*	4.8	0.0285
Gluconobacter oxydans v.s. *Gluconacetobacter hansenii*	14.46	0.0001
Acetobacter aceti v.s. *Gluconacetobacter hansenii*	33.8	0.0001

FIG. 1

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[Continued on next page]

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

COMPOSITIONS AND METHODS FOR EXTENDING LIFESPAN

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to United States Provisional Patent Application No. 62/909,186, filed October 1, 2019, the entire contents of which is hereby incorporated by reference in its entirety.

BACKGROUND

[0002] Aging is a complex process that affects every cellular processes and lead to a wide variety of altered functions.

SUMMARY

[0003] The present disclosure provides compositions comprising at least one bacterial strain or extract(s) or component(s) thereof, and an excipient.

[0004] In some embodiments, at least one bacterial strain comprises a Gluconobacter spp., Acetobacter spp., Gluconoacaetobacter spp., Acidomonas spp., Ameyamaea spp., Asaia spp., Granulibacter spp., Kozakia spp., Neoasaia spp., Neokomagataea spp., Saccharibacter spp., Swaminathania spp., Tanticharoenia spp., or a combination thereof. In some embodiments, at least one bacterial strain comprises Gluconobacter albidus, Gluconobacter cerinus, Gluconobacter frateruii, Gluconobacter japonicus, Gluconobacter kondonii, Gluconobacter nephelii, Gluconobacter oxydans, Gluconoacetobacter diazotrophicus, Gluconoacetobacter hansenii, Gluconoacetobacter saccharivorans, Acetobacter aceti, Acetobacter malorum, or a combination thereof. In some embodiments, at least one bacterial strain comprises Gluconacetobacter hansenii, Gluconobacter oxydans, Acetobacter aceti, or a combination thereof. In some embodiments, at least one bacterial strain comprises Gluconacetobacter hansenii.

[0005] In some embodiments, an excipient is or comprises an inactive (e.g., non-biologically active) agent. An excipient may be included in a composition, for example, to provide or contribute to a desired consistency or stabilizing effect. In some embodiments, excipients may include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, or ethanol.

[0006] In some embodiments, a composition is formulated for oral administration. In some embodiments, a composition is a food, a beverage, a feed composition, or a nutritional supplement. In some embodiments, a composition is a liquid, syrup, tablet, troche, gummy, capsule, powder, gel, or film. In some embodiments, a composition is a pharmaceutical composition. In some embodiments, a composition is an enteric-coated formulation.

[0007] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, an average life span of the *C. elegans* animals in the *C. elegans* culture is extended by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract or component thereof.

[0008] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, average pharyngeal pumping activity of the *C. elegans* animals in the *C. elegans* culture is increased by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract(s) or component(s) thereof.

[0009] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, average locomotion rate of the *C. elegans* animals in the *C. elegans* culture is increased by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract(s) or component(s) thereof.

[0010] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, fertility of the *C. elegans* animals in the *C. elegans* culture is decreased by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract(s) or component(s) thereof.

[0011] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when a *C. elegans* culture comprising *C. elegans* animals is exposed to Ultra Violet irradiation, average survival time of the *C. elegans* animals in the *C. elegans* culture to which the at least one bacterial strain or extract(s) or component(s) thereof has been administered is increased by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract(s) or component(s) thereof.

[0012] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when a *C. elegans* culture comprising *C. elegans* animals is exposed to an elevated temperature, average survival time of *C. elegans* animals in the *C. elegans* culture to which the at least one bacterial strain or extract(s) or component(s) thereof has been administered is increased by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that *C. elegans* animals in of a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract(s) or component(s) thereof. In some embodiments, an elevated temperature is at least 37°C, at least 40°C, at least 45°C, at least 50°C, at least 55°C, at least 60°C, at least 65°C, at least 70°C, at least 75°C, or at least 80°C. In some embodiments, an elevated temperature is 50°C-65°C, 65°C-80°C, or 80°C-120°C.

[0013] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, average amount of intestinal fat observed in the *C. elegans* animals \is decreased by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as

compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract(s) or component(s) thereof.

[0014] In some embodiments, *C. elegans* animals are adult *C. elegans* animals. In some embodiments, *C. elegans* animals are at least 5 days old.

[0015] The present disclosure provides methods comprising administering a composition described herein to a subject a composition.

[0016] In some embodiments, a method is a method of extending lifespan of a subject. In some embodiments, the life span of a subject is extended by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of a comparable subject without administration of the composition.

[0017] In some embodiments, a method is a method of reducing or delaying the onset of at least one age-associated symptom or condition in a subject. In some embodiments, at least one age-associated symptom or condition is reduced or delayed in a subject by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of a comparable subject without administration of the composition. In some embodiments, at least one age-associated symptom or condition is or comprises a decline in muscle and/or neuromuscular function of a subject. In some embodiments, at least one age-associated symptom or condition is or comprises dysregulation of lipid metabolism.

[0018] In some embodiments, a subject is at least 30 years old, at least 35 years old, at least 40 years old, at least 45 years old, at least 50 years old, at least 55 years old, at least 60 years old, at least 65 years old, at least 70 years old, or at least at least 75 years old. In some embodiments, a subject is an elderly subject.

[0019] In some embodiments, a subject is a mammal. In some embodiments, a mammal is a non-human primate (e.g, a higher primate), a sheep, a dog, a rodent (e.g., a mouse or rat), a guinea pig, a goat, a pig, a cat, a rabbit, or a cow. In some embodiments, a mammal is a human.

[0020] In some embodiments, a method comprises administering comprises administering a sufficient amount of the microbes to colonize the subject's microbiome.

[0021] In some embodiments, the step of administering comprises ingesting.

[0022] The present disclosure provides uses of compositions disclosed herein for extending the life span of a subject. The present disclosure provides uses of at least one bacterial strain or extract(s) or component(s) thereof for extending the life span of a subject. In some embodiments, at least one bacterial strain comprises *Gluconobacter spp.*, *Acetobacter spp.*, *Gluconoacaetobacter spp.*, *Acidomonas spp.*, *Ameyamaea spp.*, *Asaia spp.*, *Granulibacter spp.*, *Kozakia spp.*, *Neoasaia spp.*, *Neokomagataea spp.*, *Saccharibacter spp.*, *Swaminathania spp.*, *Tanticharoenia spp.*, or a combination thereof. In some embodiments, at least one bacterial strain comprises *Gluconacetobacter hansenii*, *Gluconobacter oxydans*, *Acetobacter aceti*, or a combination thereof. In some embodiments, at least one bacterial strain comprises *Gluconacetobacter hansenii*.

[0023] The present disclosure provides uses of compositions described herein for reducing or delaying the onset of at least one age-associated symptom or condition in a subject. The present disclosure provides uses of at least one bacterial strain or extract(s) or component(s) thereof for reducing or delaying the onset of at least one age-associated symptom or condition in a subject. In some embodiments, at least one bacterial strain comprises *Gluconobacter spp.*, *Acetobacter spp.*, *Gluconoacaetobacter spp.*, *Acidomonas spp.*, *Ameyamaea spp.*, *Asaia spp.*, *Granulibacter spp.*, *Kozakia spp.*, *Neoasaia spp.*, *Neokomagataea spp.*, *Saccharibacter spp.*, *Swaminathania spp.*, *Tanticharoenia spp.*, or a combination thereof. In some embodiments, at least one bacterial strain comprises *Gluconacetobacter hansenii*, *Gluconobacter oxydans*, *Acetobacter aceti*, or a combination thereof. In some embodiments, at least one bacterial strain comprises *Gluconacetobacter hansenii*.

[0024] In some embodiments, at least one age-associated symptom or condition is reduced or delayed in the subject by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of a comparable subject without administration of the composition.

[0025] In some embodiments, at least one age-associated symptom or condition is or comprises a decline in muscle and/or neuromuscular function of the subject. In some embodiments, at least one age-associated symptom or condition is or comprises dysregulation of lipid metabolism.

[0026] In some embodiments, a subject is at least 30 years old, at least 35 years old, at least 40 years old, at least 45 years old, at least 50 years old, at least 55 years old, at least 60 years old, at least 65 years old, at least 70 years old, or at least at least 75 years old. In some embodiments, a subject is an elderly subject.

[0027] In some embodiments, a subject is a mammal. In some embodiments, a mammal is a non-human primate (e.g, a higher primate), a sheep, a dog, a rodent (e.g., a mouse or rat), a guinea pig, a goat, a pig, a cat, a rabbit, or a cow. In some embodiments, a mammal is a human.

[0028] The present disclosure provides uses of compositions described herein for treating a subject who has or is at risk of developing a disease or disorder associated with premature aging. The present disclosure provides uses of at least one bacterial strain or extract(s) or component(s) thereof for treating a subject who has or is at risk of developing a disease or disorder associated with premature aging. In some embodiments, a disease or disorder is Bloom syndrome, Bockayne Syndrome, Hutchinson-Gilford progeria syndrome, mandibuloacral dysplasia with type A lipodystrophy, progeria, progeroid syndrome, Rothmund-Thomson syndrome, Seip syndrome, or Werner syndrome.

[0029] The present disclosure provides methods of characterizing the ability of one or more microbial strains to modify the life span of a subject, an age-associated symptom, and/or an age-associated condition, comprising (a) adding a plurality of microbial strains of a mammalian microbiome to a plurality of *C. elegans* cultures, wherein a different microbial strain is added to each *C. elegans* culture, and wherein each culture comprises *C. elegans* animals of the same *C. elegans* strain, and (b) determining whether each microbial strain of the plurality affects one or more parameters of the *C. elegans* animals of each culture, wherein the one or more parameters are associated with aging, an age-associated symptom, and/or an age-associated condition.

[0030] The present disclosure provides uses of *C. elegans* animals for characterizing the ability of one or more one or more microbial strains to modify the life span of a subject, an age-associated symptom, and/or an age-associated condition.

[0031] The present disclosure provides methods of making a composition as described herein comprising combining at least one bacterial strain or extract(s) or component(s) thereof, and the excipient.

DEFINITIONS

[0032] The scope of the present invention is defined by the claims appended hereto and is not limited by certain embodiments described herein. Those skilled in the art, reading the present specification, will be aware of various modifications that may be equivalent to such described embodiments, or otherwise within the scope of the claims. In general, terms used herein are in accordance with their understood meaning in the art, unless clearly indicated otherwise. Explicit definitions of certain terms are provided below; meanings of these and other terms in particular instances throughout this specification will be clear to those skilled in the art from context.

[0033] Use of ordinal terms such as "first," "second," "third," etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

[0034] The articles "a" and "an," as used herein, should be understood to include the plural referents unless clearly indicated to the contrary. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. In some embodiments, exactly one member of a group is present in, employed in, or otherwise relevant to a given product or process. In some embodiments, more than one, or all group members are present in, employed in, or otherwise relevant to a given product or process. It is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists (e.g., in Markush group or similar format), it is to be understood that each subgroup of the

elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where embodiments or aspects are referred to as "comprising" particular elements, features, etc., certain embodiments or aspects "consist," or "consist essentially of," such elements, features, etc. For purposes of simplicity, those embodiments have not in every case been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification.

[0035] ***Administration***: As used herein, the term "administration" typically refers to the administration of a composition to a subject or system to achieve delivery of an agent to the subject or system. In some embodiments, the agent is, or is included in, the composition; in some embodiments, the agent is generated through metabolism of the composition or one or more components thereof. Those of ordinary skill in the art will be aware of a variety of routes that may, in appropriate circumstances, be utilized for administration to a subject, for example a human. For example, in some embodiments, administration may be ocular, oral, parenteral, topical, etc. In some particular embodiments, administration may be bronchial (e.g., by bronchial instillation), buccal, dermal (which may be or comprise, for example, one or more of topical to the dermis, intradermal, interdermal, transdermal, etc), enteral, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, within a specific organ (e. g. intrahepatic), mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (e.g., by intratracheal instillation), vaginal, vitreal, etc. In many embodiments provided by the present disclosure, administration is oral administration. In some embodiments, administration may involve only a single dose. In some embodiments, administration may involve application of a fixed number of doses. In some embodiments, administration may involve dosing that is intermittent (e.g., a plurality of doses separated in time) and/or periodic (e.g., individual doses separated by a common period of time) dosing. In some embodiments, administration may involve continuous dosing (e.g., perfusion) for at least a selected period of time. Administration of cells can be by any appropriate route that results in delivery to a desired location in a subject where at least a portion of the delivered cells or components of the cells remain viable. A period of viability of cells after administration to a subject can be as short as a few hours, e.g., twenty-four hours, to a few days, to as long as several

years, i.e., long-term engraftment. In some embodiments, administration comprises delivery of a bacterial extract or preparation comprising one or more bacterial metabolites and/or byproducts but lacking fully viable bacterial cells.

[0036] ***Analog***: As used herein, the term "analog" refers to a substance that shares one or more particular structural features, elements, components, or moieties with a reference substance. Typically, an "analog" shows significant structural similarity with the reference substance, for example sharing a core or consensus structure, but also differs in certain discrete ways. In some embodiments, an analog is a substance that can be generated from the reference substance, e.g., by chemical manipulation of the reference substance. In some embodiments, an analog is a substance that can be generated through performance of a synthetic process substantially similar to (e.g., sharing a plurality of steps with) one that generates the reference substance. In some embodiments, an analog is or can be generated through performance of a synthetic process different from that used to generate the reference substance.

[0037] ***Approximately***: As applied to one or more values of interest, includes to a value that is similar to a stated reference value. In certain embodiments, the term "*approximately*" or "*about*" refers to a range of values that fall within ± 10% (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0038] ***Comparable***: As used herein, the term "comparable" refers to two or more agents, entities, situations, sets of conditions, subjects, etc., that may not be identical to one another but that are sufficiently similar to permit comparison therebetween so that one skilled in the art will appreciate that conclusions may reasonably be drawn based on differences or similarities observed. In some embodiments, comparable sets of conditions, circumstances, individuals, or populations are characterized by a plurality of substantially identical features and one or a small number of varied features. Those of ordinary skill in the art will understand, in context, what degree of identity is required in any given circumstance for two or more such agents, entities, situations, sets of conditions, etc. to be considered comparable. For example, those of ordinary skill in the art will appreciate that sets of circumstances, individuals, or populations are comparable to one another when characterized by a sufficient number and type

of substantially identical features to warrant a reasonable conclusion that differences in results obtained or phenomena observed under or with different sets of circumstances, individuals, or populations are caused by or indicative of the variation in those features that are varied.

[0039] ***Conservative:*** As used herein, refers to instances when describing a conservative amino acid substitution, including a substitution of an amino acid residue by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of interest of a protein, for example, the ability of a receptor to bind to a ligand. Examples of groups of amino acids that have side chains with similar chemical properties include: aliphatic side chains such as glycine (Gly, G), alanine (Ala, A), valine (Val, V), leucine (Leu, L), and isoleucine (Ile, I); aliphatic-hydroxyl side chains such as serine (Ser, S) and threonine (Thr, T); amide-containing side chains such as asparagine (Asn, N) and glutamine (Gln, Q); aromatic side chains such as phenylalanine (Phe, F), tyrosine (Tyr, Y), and tryptophan (Trp, W); basic side chains such as lysine (Lys, K), arginine (Arg, R), and histidine (His, H); acidic side chains such as aspartic acid (Asp, D) and glutamic acid (Glu, E); and sulfur-containing side chains such as cysteine (Cys, C) and methionine (Met, M). Conservative amino acids substitution groups include, for example, valine/leucine/isoleucine (Val/Leu/Ile, V/L/I), phenylalanine/tyrosine (Phe/Tyr, F/Y), lysine/arginine (Lys/Arg, K/R), alanine/valine (Ala/Val, A/V), glutamate/aspartate (Glu/Asp, E/D), and asparagine/glutamine (Asn/Gln, N/Q). In some embodiments, a conservative amino acid substitution can be a substitution of any native residue in a protein with alanine, as used in, for example, alanine scanning mutagenesis. In some embodiments, a conservative substitution is made that has a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet, G.H. et al., 1992, Science 256:1443-1445, which is incorporated herein by reference in its entirety. In some embodiments, a substitution is a moderately conservative substitution wherein the substitution has a nonnegative value in the PAM250 log-likelihood matrix.

CONSERVATIVE AMINO ACID SUBSTITUTIONS		
For Amino Acid	**Code**	**Replace With**
Alanine	A	D-ala, Gly, Aib, β-Ala, Acp, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, Aib, β-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, AdaA, AdaG, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, AdaA, AdaG, Leu, D-Leu, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4 or 5-phenylproline, AdaA, AdaG, cis-3,4 or 5-phenylproline, Bpa, D-Bpa
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D-or-L-1-oxazolidine-4-carboxylic acid (Kauer, U.S. Pat. No. (4,511,390)
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met (O), D-Met (O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met (O), D-Met (O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met, AdaA, AdaG

[0040] ***Control***: As used herein, refers to the art-understood meaning of a "*control*" being a standard against which results are compared. Typically, controls are used to augment integrity in experiments by isolating variables in order to make a conclusion about such variables. In some embodiments, a control is a reaction or assay that is performed simultaneously with a test reaction or assay to provide a comparator. A "*control*" also includes a "*control animal*." A "*control animal*" may have a modification as described herein, a modification that is

different as described herein, or no modification (i.e., a wild-type animal). In one experiment, a "*test*" (i.e., a variable being tested) is applied. In a second experiment, the "*control*," the variable being tested is not applied. In some embodiments, a control is a historical control (i.e., of a test or assay performed previously, or an amount or result that is previously known). In some embodiments, a control is or comprises a printed or otherwise saved record. A control may be a positive control or a negative control.

[0041] ***Determining, measuring, evaluating, assessing, assaying*** and ***analyzing***: Determining, measuring, evaluating, assessing, assaying and analyzing are used interchangeably herein to refer to any form of measurement, and include determining if an element is present or not. These terms include both quantitative and/or qualitative determinations. Assaying may be relative or absolute. "Assaying for the presence of" can be determining the amount of something present and/or determining whether or not it is present or absent.

[0042] ***Dosage form***: Those skilled in the art will appreciate that the term "dosage form" may be used to refer to a physically discrete unit of an agent (e.g., a therapeutic agent) for administration to a subject. Typically, each such unit contains a predetermined quantity of agent. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population (i.e., with a therapeutic dosing regimen). Those of ordinary skill in the art appreciate that the total amount of a therapeutic composition or agent administered to a particular subject is determined by one or more attending physicians and may involve administration of multiple dosage forms.

[0043] ***Dosing regimen***: Those skilled in the art will appreciate that the term "dosing regimen" may be used to refer to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which is separated in time from other doses. In some embodiments, individual doses are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit

dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population.

[0044] ***Engineered***: In general, the term "engineered" refers to the aspect of having been manipulated by the hand of man. For example, a cell or organism is considered to be "engineered" if it has been manipulated so that its genetic information is altered (e.g., new genetic material not previously present has been introduced, for example by transformation, mating, somatic hybridization, transfection, transduction, or other mechanism, or previously present genetic material is altered or removed, for example by substitution or deletion mutation, or by mating protocols). As is common practice and is understood by those in the art, progeny of an engineered polynucleotide or cell are typically still referred to as "engineered" even though the actual manipulation was performed on a prior entity.

[0045] ***Functional***: As used herein, a "functional" biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized. A biological molecule may have two functions (i.e., bifunctional) or many functions (i.e., multifunctional).

[0046] ***Gene***: As used herein, refers to a DNA sequence in a chromosome that codes for a product (e.g., an RNA product and/or a polypeptide product). In some embodiments, a gene includes coding sequence (i.e., sequence that encodes a particular product). In some embodiments, a gene includes non-coding sequence. In some particular embodiments, a gene may include both coding (e.g., exonic) and non-coding (e.g., intronic) sequence. In some embodiments, a gene may include one or more regulatory sequences (e.g., promoters, enhancers, etc.) and/or intron sequences that, for example, may control or impact one or more aspects of gene expression (e.g., cell-type-specific expression, inducible expression, etc.). For the purpose of clarity, we note that, as used in the present disclosure, the term "*gene*" generally refers to a portion of a nucleic acid that encodes a polypeptide or fragment thereof; the term may optionally

encompass regulatory sequences, as will be clear from context to those of ordinary skill in the art. This definition is not intended to exclude application of the term "*gene*" to non-protein-coding expression units but rather to clarify that, in most cases, the term as used in this document refers to a polypeptide-coding nucleic acid.

[0047] ***Improve, increase, enhance, inhibit or reduce***: As used herein, the terms "improve," "increase," "enhance," "inhibit," "reduce," or grammatical equivalents thereof, indicate values that are relative to a baseline or other reference measurement. In some embodiments, a value is statistically significantly difference that a baseline or other reference measurement. In some embodiments, an appropriate reference measurement may be or comprise a measurement in a particular system (e.g., in a single individual) under otherwise comparable conditions absent presence of (e.g., prior to and/or after) a particular agent or treatment, or in presence of an appropriate comparable reference agent. In some embodiments, an appropriate reference measurement may be or comprise a measurement in comparable system known or expected to respond in a particular way, in presence of the relevant agent or treatment. In some embodiments, an appropriate reference is a negative reference; in some embodiments, an appropriate reference is a positive reference.

[0048] ***Isolated***: As used herein, refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) designed, produced, prepared, and/or manufactured by the hand of man. In some embodiments, an isolated substance or entity may be enriched; in some embodiments, an isolated substance or entity may be pure. In some embodiments, isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. In some embodiments, as will be understood by those skilled in the art, a substance may still be considered "enriched", "isolated" or even "pure", after having been

combined with certain other components such as, for example, one or more carriers or excipients (e.g., buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without including such carriers or excipients. Those skilled in the art are aware of a variety of technologies for isolating (e.g., enriching or purifying) substances or agents (e.g., using one or more of fractionation, extraction, precipitation, or other separation).

[0049] ***Pharmaceutical composition***: As used herein, the term "pharmaceutical composition" refers to a composition in which an active agent is formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, the active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, a pharmaceutical composition may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue, capsules, powders, etc. In some embodiments, an active agent may be or comprise a cell or population of cells (e.g., a culture, for example of an EES microbe); in some embodiments, an active agent may be or comprise an extract or component of a cell or population (e.g., culture) of cells. In some embodiments, an active agent may be or comprise an isolated, purified, or pure compound. In some embodiments, an active agent may have been synthesized in vitro (e.g., via chemical and/or enzymatic synthesis). In some embodiments, an active agent may be or comprise a natural product (whether isolated from its natural source or synthesized in vitro).

[0050] ***Pharmaceutically acceptable***: As used herein, the term "pharmaceutically acceptable" which, for example, may be used in reference to a carrier, diluent, or excipient used to formulate a pharmaceutical composition as disclosed herein, means that the carrier, diluent, or excipient is compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

[0051] ***Pharmaceutically acceptable carrier***: As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in

carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be is "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

[0052] ***Prebiotic***: As used herein, a "prebiotic" refers to an ingredient that allows or promotes specific changes, both in the composition and/or activity in the gastrointestinal microbiota that may (or may not) confer benefits upon the host. In some embodiments, a prebiotic can include one or more of the following: the prebiotic comprises a pome extract, berry extract and walnut extract.

[0053] ***Prevention***: The term "prevention", as used herein, refers to a delay of onset, and/or reduction in frequency and/or severity of one or more symptoms of a particular disease, disorder or condition. In some embodiments, prevention is assessed on a population basis such that an agent is considered to "prevent" a particular disease, disorder or condition if a statistically significant decrease in the development, frequency, and/or intensity of one or more symptoms of the disease, disorder or condition is observed in a population susceptible to the disease, disorder, or condition. In some embodiments, prevention may be considered complete, for example, when onset of a disease, disorder or condition has been delayed for a predefined period of time.

[0054] ***Reference***: As used herein describes a standard or control relative to which a comparison is performed. For example, in some embodiments, an agent, animal, individual, population, sample, sequence or value of interest is compared with a reference or control agent,

animal, individual, population, sample, sequence or value. In some embodiments, a reference or control is tested and/or determined substantially simultaneously with the testing or determination of interest. In some embodiments, a reference or control is a historical reference or control, optionally embodied in a tangible medium. Typically, as would be understood by those skilled in the art, a reference or control is determined or characterized under comparable conditions or circumstances to those under assessment. Those skilled in the art will appreciate when sufficient similarities are present to justify reliance on and/or comparison to a particular possible reference or control. In some embodiments, a reference is a negative control reference; in some embodiments, a reference is a positive control reference.

[0055] ***Risk***: As will be understood from context, "risk" of a disease, disorder, and/or condition refers to a likelihood that a particular individual will develop the disease, disorder, and/or condition. In some embodiments, risk is expressed as a percentage. In some embodiments, risk is from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 up to 100%. In some embodiments risk is expressed as a risk relative to a risk associated with a reference sample or group of reference samples. In some embodiments, a reference sample or group of reference samples have a known risk of a disease, disorder, condition and/or event. In some embodiments a reference sample or group of reference samples are from individuals comparable to a particular individual. In some embodiments, relative risk is 0,1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

[0056] ***Sample***: As used herein, the term "sample" typically refers to an aliquot of material obtained or derived from a source of interest. In some embodiments, a source of interest is a biological or environmental source. In some embodiments, a source of interest may be or comprise a cell or an organism, such as a microbe, a plant, or an animal (e.g., a human). In some embodiments, a source of interest is or comprises biological tissue or fluid. In some embodiments, a biological tissue or fluid may be or comprise amniotic fluid, aqueous humor, ascites, bile, bone marrow, blood, breast milk, cerebrospinal fluid, cerumen, chyle, chime, ejaculate, endolymph, exudate, feces, gastric acid, gastric juice, lymph, mucus, pericardial fluid, perilymph, peritoneal fluid, pleural fluid, pus, rheum, saliva, sebum, semen, serum, smegma, sputum, synovial fluid, sweat, tears, urine, vaginal secretions, vitreous humour, vomit, and/or combinations or component(s) thereof. In some embodiments, a biological fluid may be or

comprise an intracellular fluid, an extracellular fluid, an intravascular fluid (blood plasma), an interstitial fluid, a lymphatic fluid, and/or a transcellular fluid. In some embodiments, a biological fluid may be or comprise a plant exudate. In some embodiments, a biological tissue or sample may be obtained, for example, by aspirate, biopsy (e.g., fine needle or tissue biopsy), swab (e.g., oral, nasal, skin, or vaginal swab), scraping, surgery, washing or lavage (e.g., bronchioalveolar, ductal, nasal, ocular, oral, uterine, vaginal, or other washing or lavage). In some embodiments, a biological sample is or comprises cells obtained from an individual. In some embodiments, a sample is a "primary sample" obtained directly from a source of interest by any appropriate means. In some embodiments, as will be clear from context, the term "sample" refers to a preparation that is obtained by processing (e.g., by removing one or more components of and/or by adding one or more agents to) a primary sample. For example, filtering using a semi-permeable membrane. Such a "processed sample" may comprise, for example nucleic acids or proteins extracted from a sample or obtained by subjecting a primary sample to one or more techniques such as amplification or reverse transcription of nucleic acid, isolation and/or purification of certain components, etc.

[0057] ***Subject***: As used herein, the term "subject" refers to an individual to which a provided treatment is administered. In some embodiments, a subject is animal. In some embodiments, a subject is a mammal, e.g., a mammal that experiences or is susceptible to a disease, disorder, or condition as described herein. In some embodiments, an animal is a vertebrate, e.g., a mammal, such as a non-human primate, (particularly a higher primate), a sheep, a dog, a rodent (e.g. a mouse or rat), a guinea pig, a goat, a pig, a cat, a rabbit, or a cow. Ins some embodiments, an animal is a non-mammal animal, such as a chicken, an amphibian, a reptile, or an invertebrate model *C. elegans*. In some embodiments, a subject is a human. In some embodiments, a patient is suffering from or susceptible to one or more diseases, disorders or conditions as described herein. In some embodiments, a patient displays one or more symptoms of a one or more diseases, disorders or conditions as described herein. In some embodiments, a patient has been diagnosed with one or more diseases, disorders or conditions as described herein. In some embodiments, the subject is receiving or has received certain therapy to diagnose and/or to treat a disease, disorder, or condition. In another embodiment, the subject is an experimental animal or animal substitute as a disease model.

[0058] ***Substantially***: As used herein, refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "*substantially*" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0059] ***Symptoms are reduced***: According to the present invention, "symptoms are reduced" when one or more symptoms of a particular disease, disorder or condition is reduced in magnitude (e.g., intensity, severity, etc.) and/or frequency. For purposes of clarity, a delay in the onset of a particular symptom is considered one form of reducing the frequency of that symptom.

[0060] ***Therapeutic regimen***: A "therapeutic regimen", as that term is used herein, refers to a dosing regimen whose administration across a relevant population may be correlated with a desired or beneficial therapeutic outcome.

[0061] ***Therapeutically effective amount***: As used herein, is meant an amount that produces the desired effect for which it is administered. In some embodiments, the term refers to an amount that is sufficient, when administered to a population suffering from or susceptible to a disease, disorder, and/or condition in accordance with a therapeutic dosing regimen, to treat the disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is one that reduces the incidence and/or severity of, and/or delays onset of, one or more symptoms of the disease, disorder, and/or condition. Those of ordinary skill in the art will appreciate that the term "therapeutically effective amount" does not in fact require successful treatment be achieved in a particular individual. Rather, a therapeutically effective amount may be that amount that provides a particular desired pharmacological response in a significant number of subjects when administered to patients in need of such treatment. In some embodiments, reference to a therapeutically effective amount may be a reference to an amount as measured in one or more specific tissues (e.g., a tissue affected by the disease, disorder or condition) or fluids (e.g., blood, saliva, serum, sweat, tears, urine, etc.). Those of ordinary skill in the art will appreciate that, in some embodiments, a therapeutically effective amount of a particular agent or therapy may be formulated and/or administered in a single dose. In some embodiments, a

therapeutically effective agent may be formulated and/or administered in a plurality of doses, for example, as part of a dosing regimen.

[0062] ***Treatment***: As used herein, the term "treatment" (also "treat" or "treating") refers to any administration of a therapy that partially or completely alleviates, ameliorates, relives, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition. In some embodiments, such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively, or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition.

BRIEF DESCRIPTION OF THE DRAWING

[0063] **FIG. 1** includes data showing that administration of *Acetobacteraceae* increased the lifespan of *C. elegans*. **Panel (A)** shows a lifespan assay on *C. elegans* animals administered either *E. coli OP50*, *Gluconobacter oxydans*, *Acetobacter aceti* or *Gluconacetobacter hansenii*. Compared to the animals administered *E. coli OP50*, *C. elegans* animals administered either *Gluconobacter oxydans*, *Acetobacter aceti* or *Gluconacetobacter hansenii* lived longer. **Panel (B)** includes a cumulative hazard plot of *C. elegans* animals administered either *E. coli OP50*, *Gluconobacter oxydans*, *Acetobacter aceti* or *Gluconacetobacter hansenii*. **Panel (C)** includes a Restricted Mean Lifespan (RMLS) of *C. elegans* animals administered either *E. coli OP50*, *Gluconobacter oxydans*, *Acetobacter aceti* or *Gluconacetobacter hansenii*. **Panel (D)** includes a statistical analysis of data shown in Fig. 1, panels (A)-(C).

[0064] **FIG. 2** includes data showing that administration of *Acetobacteraceae* improved muscle function/activity. **Panel (A)** includes data obtained by measuring pharyngeal pumping in *C. elegans* animals administered either *E. coli OP50*, *Gluconobacter oxydans*, *Acetobacter aceti*

or *Gluconacetobacter hansenii*. Compared to pharyngeal pumping rates of *C. elegans* animals administered *E. coli* OP50, the pumping rates in *C. elegans* animals administered either *Gluconobacter oxydans*, *Acetobacter aceti* or *Gluconacetobacter hansenii* were significantly higher in Day 6 and Day 12 *C. elegans* animals. The number of *C. elegans* animals scored are indicated on the top of each bar. Each bar shows Mean±s.d. **Panel (B)** includes data obtained by measuring body bends/minute in *C. elegans* animals administered either *E. coli OP50*, *Gluconobacter oxydans*, *Acetobacter aceti* or *Gluconacetobacter hansenii*. Compared to the number of body bends/minute in *C. elegans* animals administered *E. coli* OP50, the body bends/minute rates in animals administered either *Gluconobacter oxydans*, *Acetobacter aceti* or *Gluconacetobacter hansenii* were significantly higher in Day 6 and Day 12 animals. NS indicates no significant difference. The number of *C. elegans* animals scored are indicated on the top of each bar. Each bar shows Mean±s.d.

[0065] **FIG. 3** includes data showing that administration of *Acetobacteraceae* improved stress resistance. **Panel (A)** includes data obtained from a UV resistance assay on *C. elegans* animals administered either *E. coli OP50*, *Gluconobacter oxydans*, *Acetobacter aceti* or *Gluconacetobacter hansenii*. Compared to UV-irradiated animals administered *E. coli* OP50, UV-irradiated *C. elegans* animals administered either *Gluconobacter oxydans*, *Acetobacter aceti* or *Gluconacetobacter hansenii* lived longer. Mean±s.d. for each measurement is plotted. **Panel (B)** includes data obtained from a thermotolerance assay on *C. elegans* animals administered either *E. coli OP50*, *Gluconobacter oxydans*, *Acetobacter aceti* or *Gluconacetobacter hansenii*. Compared to *C. elegans* animals shifted to 37°C administered *E. coli* OP50, *C. elegans* animals shifted to 37°C administered either *Gluconobacter oxydans*, *Acetobacter aceti* or *Gluconacetobacter hansenii* lived longer. Mean±s.d. for each measurement is plotted.

[0066] **FIG. 4**. includes data showing that administration of *Acetobacteraceae* decreased fat deposition. Compared to the animals administered *E. coli* OP50, *C. elegans* animals administered *Gluconacetobacter hansenii* had decreased fat levels as revealed by Oil Red O staining.

[0067] **FIG. 5** includes data showing that *prx-5* was needed for the *G. hansenii*-induced lifespan extension. **Panel (A)** includes data obtained from a lifespan assay on wildtype or *prx-*

5(0) animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*. Compared to the *C. elegans* animals administered *E. coli* OP50, *C. elegans* animals administered either *Gluconacetobacter hansenii* lived longer. Lifespan curves of *prx-5(0)* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii* were similar. **Panel (B)** includes data obtained from a Restricted Mean Lifespan (RMLS) of wildtype or *prx-5(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*. **Panel (C)** includes data obtained from a cumulative hazard plot of wildtype or *prx-5(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*.

[0068] **FIG. 6** includes data showing that *tcer-1* and *aak-2* were needed for the *G. hansenii*-induced lifespan extension. **Panel (A)** includes data obtained from a lifespan assay on wildtype or *tcer-1(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*. Compared to the *C. elegans* animals administered *E. coli* OP50, *C. elegans* animals administered either *Gluconacetobacter hansenii* lived longer. Lifespan curves of *tcer-1(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii* are similar. **Panel (B)** includes data obtained from a Restricted Mean Lifespan (RMLS) of wildtype or *tcer-1(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*. **Panel (C)** includes data obtained from a lifespan assay on wildtype or *aak-2(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*. Compared to the *C. elegans* animals administered *E. coli* OP50, animals administered either *Gluconacetobacter hansenii* lived longer. Lifespan curves of *aak-2(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii* are similar. **Panel (D)** includes data obtained from a Restricted Mean Lifespan (RMLS) of wildtype or *aak-2(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*.

[0069] **FIG. 7** includes data showing that *daf-16* was not required for the *G. hansenii*-induced lifespan extension. **Panel (A)** includes data obtained from a lifespan assay on wildtype or *daf-16(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*. **Panel (B)** includes data obtained from a Restricted Mean Lifespan (RMLS) of wildtype or *daf-16 (0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*.

[0070] **FIG. 8** includes data showing that *hsf-1* was needed for the *G. hansenii*-induced thermotolerance phenotype. **Panel (A)** includes data showing *G. hansenii* administration does

not affect heat shock protein expression. **Panel (B)** includes data obtained from a thermotolerance assay on wildtype or *hsf-1(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*.

[0071] **FIG. 9** includes data showing that an analysis of the genetic pathways required for *G. hansenii*-induced thermotolerance phenotype. **Panel (A)** includes data obtained from a thermotolerance assay on wildtype or *tcer-1(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*. **Panel (B)** includes data obtained from a thermotolerance assay on wildtype or *prx-5(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*. **Panel (C)** includes data obtained from a thermotolerance assay on wildtype or *aak-2(0) C. elegans* animals administered either *E. coli OP50* or *Gluconacetobacter hansenii*.

DETAILED DESCRIPTION

[0072] Aging is a complex process that affects numerous cellular processes and can lead to a wide variety of altered functions. In some instances, aging is accompanied by a gradual decline of tissue structure and cellular functions, which can lead to increased morbidity and mortality risk. Over the past century, human life expectancy has dramatically increased throughout the world (Beltrán-Sánchez et al., 2015). Increased life expectancy poses new challenges in terms of healthcare and well-being of our aging population (Knickman and Snell, 2002). Chronic human ailments often associated with aging population, such as cardiovascular diseases, cancer, arthritis, diabetes, and neurodegenerative diseases, are increasing at an alarming rate throughout the world (Franceschi et al., 2018) (Lunenfeld and Stratton, 2013) (Frasca et al., 2017). Thus, a goal of aging research is to identify therapeutic interventions that can delay age-related decline in cellular function and promote longevity.

[0073] The present disclosure provides the recognition that microbial species present in the microbiome of a subject can impact the life span of the subject. The present disclosure provides the insight that certain microbes, particularly those in a microbiome (e.g., a human microbiome) can be modulated to modify the expected life span of a subject. For example, among other things, the present disclosure provides the recognition that the certain microbes can

be administered to a subject and can extend the life span of the subject, and/or reduce or delay the onset of age-associated symptoms or conditions in a subject.

[0074] The present disclosure further provides that *C. elegans* are a powerful tool in determining which microbes of a microbiome can extend the life span of a subject, and/or reduce or delay the onset of age-associated symptoms or conditions in a subject. As such, the present disclosure provides technologies for identifying such microbes.

[0075] C. elegans

[0076] The free-living nematode *C. elegans* has been used extensively as a model system. *C. elegans* are inexpensive to cultivate, easy to physically manipulate, and has a multitude of genetic and molecular tools available for study. *C. elegans* are simple multicellular organisms: adults contain approximately 1,000 somatic cells yet have a variety of tissue types such as muscles, nerves, and intestinal cells. *C. elegans* have a short generation time, which allows for rapid experimentation. *C. elegans* generally progress from egg to larva to fertile adult in 3 days at room temperature. A single adult *C. elegans* can have between 300 and 1,000 progenies, which allows for a significant number of animals to be used and then quickly replenished in a relatively short amount of time. Due to the sexual dimorphism, *C. elegans* are useful for genetics. Self-fertilizing hermaphrodites can be maintained as homozygous mutations without the need for mating and males can be used for genetic crosses. *C. elegans* are transparent at every stage of their life cycle, which provides the ability to see inside the organism. This permits the observation of cellular events. It also permits the use of phosphorescent, luminescent, and fluorescent reporters. Manipulation of protein expression in *C. elegans* can also be performed using RNA-mediated interference (RNAi), which can allow for rapid assessment of gene function. Another advantage of using *C. elegans* a model system is the ability to freeze and recover the animals, thereby allowing long-term storage.

[0077] *C. elegans* can be genetically modified using a number of techniques to generate *C. elegans* strains. The sexual dimorphism of *C. elegans* allows for genetic manipulations to be performed with relative ease and according to know procedures. For example, if a strain needs to be propagated, single hermaphrodites can be used to self-fertilize and generate a population of

offspring. Even if a mutation renders an animal unable to mate, it remains possible for a hermaphrodite to produce progeny. Another aspect of *C. elegans* reproduction that makes *C. elegans* an effective genetic tool is the animal's ability to cross males with hermaphrodites. For example, mating experiments allow genetic markers such as mutations causing visible phenotypes to be placed together in a single organism along with an unknown mutation in order to facilitate mapping of that mutation. Hermaphrodites make only a limited number of sperm and can typically have approximately 300 self-progeny. Mating increases the number of offspring produced by a single hermaphrodite to approximately 1,000 due to the addition of the male-produced sperm. The relatively large number of progeny coupled with the short life span of *C. elegans* allows for rapid and inexpensive analyses to be performed on the animals.

[0078] In addition to genetic modifications via reproduction, *C. elegans* can be genetically modified via injection of transgenes. Microinjection is an effective method for creating animals and for introducing various types of molecules directly to cells. For DNA transformation, one approach is to inject DNA into a distal arm of a *C. elegans* gonad. A distal germline of *C. elegans* contains a central core of cytoplasm that is shared by many germ cell nuclei. Therefore, DNA injected into a distal arm of a *C. elegans* gonad can be delivered to many progeny. Microinjection directly into oocyte nuclei can induce chromosomal integration of transgenes, but this technique can be more difficult to perform. *C. elegans* can also incorporate genetic material that is administered to them.

[0079] *C. elegans* are relatively simple to culture. *C. elegans* can be cultivated in either liquid culture or on the Nematode Growth Medium (NGM) agar plates in the presence of bacteria. It is possible to grow the animals in a chemically defined medium without the addition of bacteria, which can be useful because the components of a medium can be altered in order to study the nutrient or other chemical requirements of the animals. In some embodiments, *C. elegans* are grown on the agar plates. *C. elegans* can be grown on Nematode Growth Medium (NGM) agar plates. Bacteria can be spread on the NGM plates as a food source for the animals. For example, OP50, a leaky *E. coli* uracil auxotroph can be used. OP50 will grow slowly and provide nutrients for the animals without overgrowing them. Once the animals have eaten all of the food on a plate they will burrow into the agar and can be maintained on the "starved" plate for weeks at a time in a 15°C incubator. The animals can be transferred to an agar plate with

fresh bacteria by either cutting and moving a small block of agar from the starved plate with a sterile instrument such as a micropipette tip, or washing the animals off the surface of the plate with sterile water, or by picking one or more individuals onto a fresh plate, which will cause the *C. elegans* to reemerge. At any time, *C. elegans* can be cryogenically preserved. *C. elegans* prefer to grow between 15°C and 25°C, but the temperature can vary depending on the strain of *C. elegans* and conditions being tested. In some embodiments, a *C. elegans* culture can be cultured at a temperature of at least 5°C, at least 10°C, at least 15°C, at least 20°C, at least 25°C, at least 30°C, at least 35°C, or at least 40°C. In some embodiments, a *C. elegans* culture can be cultured at a temperature of at most 65°C, at most 60°C, at most 55°C, at most 50°C, at most 55°C, at most 40°C, at most 35°C, at most 30°C, at most 25°C, or at most 20°C. Standard protocols for *C. elegans* manipulation and culture are known, e.g., as described by Stiernagle T. Maintenance of *C. elegans*. Wormbook, ed. The C. elegans Research Community, WormBook. (February 11,2006), which is incorporated herein by reference.

[0080] The bacterivorous nematode *Caenorhabditis elegans* is an outstanding model organism aging studies because of a short lifespan (~15 days). *C. elegans* is a powerful model for studying genetic pathways that modulate the aging process (Knickman and Snell, 2002) (Johnson, 2003) (Antebi, 2007) (Wilkinson et al., 2012). *C. elegans* is well-suited for forward and reverse genetic approaches as well as for identifying and characterizing small-molecule compounds that influence aging (Antebi, 2007) (Collins et al., 2006) (Denzel et al., 2019) (Arey and Murphy, 2017). Studies in *C. elegans* have discovered conserved genetic pathways that modulate aging and which correspond to pathways involved in human longevity (Bitto et al., 2015) (Collins et al., 2006) (Arey and Murphy, 2017) (Finch and Ruvkun, 2001). These include the insulin/IGF-1 like signaling (IIS) pathway (Tissenbaum and Ruvkun, 1998) (Kenyon, 2011), the target of rapamycin (TOR) (Robida-Stubbs et al., 2012) (Johnson et al., 2013), Nrf2/Antioxidant stress response pathway (Blackwell et al., 2015), TGF beta signaling (Kaplan et al., 2015) (Luo et al., 2010), Sirtuins (Dang, 2014) (Guarente, 2007) (Longo and Kennedy, 2006), autophagy(Gelino et al., 2016) (Hansen et al., 2008) (Chang et al., 2017), and the AMP-activated protein kinase (AMPK) pathways (Burkewitz et al., 2014) (Curtis et al., 2006) (Onken and Driscoll, 2010). In view of similarities between animals, mechanistic pathways that influence lifespan may be conserved throughout animal evolution.

[0081] Interventions that have been demonstrated to delay aging and extend lifespan in *C. elegans*, include perturbations in nutrient sensing, dietary restriction, mutations affecting mitochondrial metabolism, mutations affecting ribosomal function and drugs such as rapamycin (Kapahi et al., 2017) (Finch and Ruvkun, 2001) (Srivastava, 2017) (Pan and Finkel, 2017) (Bansal et al., 2015) (Kenyon, 2005) (Wilkinson et al., 2012). Thus, *C. elegans* can represent a powerful model to identify and characterize interventions that promote healthy aging and may be beneficial in humans (Johnson, 2003).

[0082] Several recent studies implicate a major role for the human gut microbiome in regulating various aspects of human development including aging (Vaiserman et al., 2017) (Zapata and Quagliarello, 2015) (Bischoff, 2016). The microbiome directly affects host development by, among other things, providing nutrients and essential metabolic compounds (Choi et al., 2018). Dramatic changes in the microbiome composition were observed between infants and adults, and between middle-aged and older adults (Choi et al., 2018)(An et al., 2018)(Claesson et al., 2012)(Kim and Jazwinski, 2018) (Gerber, 2014) (Maffei et al., 2017). Also, changes in microbiome composition have been suggested as an important factor in several age-related conditions, including metabolic syndrome and cancer (Tilg and Kaser, 2011). Although changes in microbiome composition are likely to result in altered microbial metabolism, how these changes affect aging is not understood. The majority of metabolites in human plasma are microbe-derived and the gut microbiome is a likely source. Whether these microbiome-derived metabolic factors might affect aging process is not known.

[0083] Technologies provided in the present disclosure can be used to for the identification of microbes, extracts, or microbiome-derived components (e.g., factors, metabolites, etc.) that modulate the aging processes, define the conserved signaling pathways through which these microbes or microbiome-derived factors influence aging and to develop novel therapeutics based on these factors for beneficial impacts on overall human health in old age. Since both *C. elegans* and bacteria are genetically tractable, it is possible to use technologies described herein to assess how diet affects aging in an unbiased fashion.

[0084] *C. elegans* is a bacterivore nematode that feeds on various bacterial species growing on rotting fruits and vegetation. Many of these microbes also colonize the *C. elegans* gut to serve as its microbiome. In the laboratory, *C. elegans* are administered exclusively *E. coli*

OP50. *E. coli* act as nutrition for the animal, providing essential nutrients that the nematode cannot synthesize *de novo*. *C. elegans* is emerging as a powerful model to study the effects of diet on aging because it is possible to easily replace the standard diet *of E. coli* with other microbes (MacNeil and Walhout, 2013). Recent studies in *C. elegans* suggest that bacteria-derived diffusible metabolites can directly impact *C. elegans* aging (Ezcurra, 2018) (Smith et al., 2008). Animals administered *E. coli* mutants that were unable to synthesize coenzyme Q were found to live longer (Jonassen et al., 2001). The *C. elegans* lifespan extending effect of Metformin (widely used for diabetes treatment) was found to be due to alterations in bacterial folate and methionine metabolism (Cabreiro et al., 2013) (Onken and Driscoll, 2010). Genetic or pharmacological inhibition of *E. coli* folate synthesis leads to an increase in *C. elegans* lifespan (Maynard et al., 2018). Strain-specific effects of *E. coli* on *C. elegans* lifespan were found to be due to structural differences in Lipopolysaccharides (Maier et al., 2010). *Bacillus subtilis*-derived NO was found to extend lifespan via modulation of the DAF-16/FOXO and heat shock factor 1 (HSF-1) pathways (Donato et al., 2017). Probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* can enhance immunity and extend lifespan in *C. elegans* (Zhao et al., 2013)(Fasseas et al., 2013) (Grompone et al., 2012) (Komura et al., 2013) (Martorell et al., 2016) (Sugawara and Sakamoto, 2018) (Zhao et al., 2017). Studies in *C. elegans* have also uncovered that the effect of genetic mutations on lifespan can depend upon the type of specific bacterial diet (Maier et al., 2010) (Brooks et al., 2009) (Heintz and Mair, 2014). TOR complex-2-specific factor *Rictor* mutants are short-lived when grown on *E. coli* OP50 bacteria but long-lived when cultured on *E. coli* HT115 (Soukas et al., 2009). *C. elegans alh-6* (aldehyde dehydrogenase gene) mutants are short-lived when cultured on *E. coli* OP50 but not when cultured in HT115 (Pang and Curran, 2014). The underlying mechanism(s) involved in these differences are not known; however, it is possible that metabolites or signals produced by these *E. coli* strains could be one of the contributing factors. In summary, these studies represent the beginning of an era of exploration into how the microbiome influence host longevity. Studies from several labs have identified a core set of microbes that constitute the natural microbiome of *C. elegans* (Dirksen et al., 2016) (Félix and Braendle, 2010). Animals sampled directly from their native habitats carry a variety of bacteria, dominated by *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* (Samuel et al., 2016). A *C. elegans* microbiome was found to be distinct from its

natural habitat suggesting a selective or preferential gating of microbes. Although the effects of feeding individual bacterial species of the *C. elegans* microbiome on animal development have been investigated, a systematic analysis of the effects of the microbiome on *C. elegans* aging has not previously been conducted.

[0085] Compositions

[0086] The present disclosure provides compositions comprising at least one bacterial strain or extract(s) or component(s) thereof, and an excipient. While the present disclosure provides exemplary microbes (e.g., bacterial strains) that affect aging, the present disclosure also provides methods for identifying additional microbes that may be used in accordance with the compositions and methods described herein.

[0087] In some embodiments, at least one bacterial strain comprises a Gluconobacter spp., Acetobacter spp., Gluconoacaetobacter spp., Acidomonas spp., Ameyamaea spp., Asaia spp., Granulibacter spp., Kozakia spp., Neoasaia spp., Neokomagataea spp., Saccharibacter spp., Swaminathania spp., Tanticharoenia spp., or a combination thereof. In some embodiments, at least one bacterial strain comprises Gluconobacter albidus, Gluconobacter cerinus, Gluconobacter frateruii, Gluconobacter japonicus, Gluconobacter kondonii, Gluconobacter nephelii, Gluconobacter oxydans, Gluconoacetobacter diazotrophicus, Gluconoacetobacter hansenii, Gluconoacetobacter saccharivorans, Acetobacter aceti, Acetobacter malorum, or a combination thereof. In some embodiments, at least one bacterial strain comprises Gluconacetobacter hansenii, Gluconobacter oxydans, Acetobacter aceti, or a combination thereof. In some embodiments, at least one bacterial strain comprises Gluconacetobacter hansenii.

[0088] In some embodiments, a composition comprises at least one bacterial strain. In some embodiments, a composition comprises at least 2 bacterial strains, at least 3 bacterial strains, at least 4 bacterial strains, at least 5 bacterial strains, at least 6 bacterial strains, at least 7 bacterial strains, at least 8 bacterial strains, at least 9 bacterial strains, at least 10 bacterial strains, at least 15 bacterial strains, or at least 20 bacterial strains. In some embodiments, a composition comprises at most 100 bacterial strains, at most 90 bacterial strains, at most 80 bacterial strains,

at most 70 bacterial strains, at most 60 bacterial strains, at most 50 bacterial strains, at most 40 bacterial strains, at most 30 bacterial strains, at most 20 bacterial strains, at most 10 bacterial strains, or at most 5 bacterial strains.

[0089] In some embodiments, extract(s) of at least one bacterial strain comprise one or more extracts of the at least one bacterial strain. In some embodiments, component(s) of at least one bacterial strain comprise one or more extracts of the at least one bacterial strain. Accordingly, comprising at least one bacterial strain or extract(s) or component(s) thereof as described herein can include, e.g., two extracts from *Gluconobacter oxydans*, a component from *Acetobacter aceti*, and *Gluconacetobacter hansenii*.

[0090] Compositions described herein can include an excipient. In some embodiments, an excipient is or comprises an inactive (e.g., non-biologically active) agent. An excipient may be included in a composition, for example, to provide or contribute to a desired consistency or stabilizing effect. In some embodiments, excipients may include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, or ethanol.

[0091] In some embodiments, compositions for use in accordance with the present disclosure are pharmaceutical compositions, e.g., for administration (e.g., oral administration) to a mammal (e.g., a human). Pharmaceutical compositions typically include an active agent (*e.g.*, individual microbial strains or combinations of microbial strains), and an excipient. An excipient can be a pharmaceutically acceptable carrier, for instance saline, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration.

[0092] In some embodiments, a composition or a pharmaceutical composition for use in accordance with the present disclosure may include and/or may be administered in conjunction with, one or more supplementary active compounds; in certain embodiments, such supplementary active agents can include ginger, curcumin, probiotics (e.g, probiotic strains of one or more of the following genera: *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Bacillus*, and/or *Escherichia coli* (see

Fijan, Int J Environ Res Public Health. 2014 May; 11(5): 4745–4767, which is incorporated herein by reference); prebiotics (nondigestible food ingredients that help support growth of probiotic microbes, e.g., fructans such as fructooligosaccharides (FOS) and inulins, galactans such as galactooligosaccharides (GOS), dietary fibers such as resistant starch, pectin, beta-glucans, and xylooligosaccharides (Hutkins et al., Curr Opin Biotechnol. 2016 Feb; 37: 1–7, which is incorporated herein by reference) and combinations thereof.

[0093] Compositions or pharmaceutical compositions are typically formulated to be compatible with their intended route of administration. Examples of routes of administration include oral administration. Methods of formulating suitable compositions have been reported, see, e.g., *Remington: The Science and Practice of Pharmacy*, 21st ed., 2005; and the books in the series *Drugs and the Pharmaceutical Sciences: a Series of Textbooks and Monographs* (Dekker, NY). Oral compositions generally include an inert diluent or an edible carrier. To give but a few examples, in some embodiments, an oral formulation may be or comprise a syrup, a liquid, a tablet, a troche, a gummy, a capsule, e.g., gelatin capsules, a powder, a gel, a film, *etc.*

[0094] In some embodiments, compatible binding agents, and/or adjuvant materials can be included as part of a composition (e.g., pharmaceutical composition). In some particular embodiments, a composition can contain, e.g., any one or more of the following inactive ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. In some embodiments, compositions can be taken as-is or sprinkled onto or mixed into a food or liquid (such as water). In some embodiments, a composition that may be administered to subjects as described herein may be or comprise an ingestible item (e.g., a food or drink) that comprises (e.g., is supplemented) with an individual microbial strain or combinations of microbial strains (e.g., from a mammalian microbiome), extracts thereof, and/or components thereof.

[0095] In some embodiments, a food can be or comprise one or more of bars, candies, baked goods, cereals, salty snacks, pastas, chocolates, and other solid foods, as well as liquid or semi-solid foods including yogurt, soups and stews, and beverages such as smoothies, shakes,

juices, and other carbonated or non-carbonated beverages. In some embodiments, foods are prepared by a subject by mixing in individual microbial strains or combinations of microbial strains (e.g., from a mammalian microbiome), extracts thereof, and/or components thereof.

[0096] Compositions can be included in a kit, container, pack, or dispenser, together with instructions for administration or for use in a method described herein.

[0097] In some embodiments, at least one microbial (e.g., bacterial) strain that have been killed (*e.g.,* heat killed). Alternatively, in some embodiments, at least one microbial (e.g., bacterial) strains may include cells that are viable or alive.

[0098] In some embodiments, methods of treatment as described herein involve administering at least one viable or living microbial (e.g., bacterial) strain. In some such embodiments, at least one viable or living microbial (e.g., bacterial) strain is administered according to a regimen that achieves population of the subject's microbiome with administered cells.

[0099] In some embodiments, at least one microbial (e.g., bacterial) strain as described herein comprises and/or is formulated through use of one or more cell cultures and/or supernatants or pellets thereof, and/or a powder formed therefrom.

[0100] In some embodiments, a pharmaceutical composition provided herein can promote the colonization of at least one microbial (e.g., bacterial) strain, particularly microbial strain(s) that have been identified, characterized, or assessed as extending life span, or reducing or delaying the onset of at least one age-associated symptom or condition in a subject. In some embodiments, a pharmaceutical composition provided herein can promote the colonization of at least one microbial (e.g., bacterial) strain, particularly microbial strain(s) that have been identified, characterized, or assessed as extending life span, or reducing or delaying the onset of at least one age-associated symptom or condition in a subject.

[0101] In some embodiments, a pharmaceutical composition is tailored to a specific mammal (e.g., a specific human subject) based on that mammal's (e.g., human's) microbiome. In some embodiments, a pharmaceutical composition is specific for a microbiome of a mammalian subject (e.g., human). In some embodiments, a pharmaceutical composition is specific for microbiomes of a population of mammals (e.g., humans). Populations of mammals

can include, but are not limited to: families, mammals in the same regional location (e.g., neighborhood, city, state, or country), mammals with the same disease or condition, mammals of a particular age or age range, mammals that consume a particular diet (e.g., food, food source, or caloric intake).

[0102] In some embodiments, a composition described herein is formulated for oral administration. In some embodiments, a composition is a food, a beverage, a feed composition, or a nutritional supplement. In some embodiments, a composition is a liquid, syrup, tablet, troche, gummy, capsule, powder, gel, or film. In some embodiments, a composition is a pharmaceutical composition. In some embodiments, a composition is an enteric-coated formulation.

[0103] Compositions described herein can affect aging or sign of aging. As discussed above, a model system by which to characterize the ability of a microbe (e.g., bacterial strain) in a composition can be *C. elegans*. For example, in some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, an average life span of the *C. elegans* animals in the *C. elegans* culture is extended by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract or component thereof. A life span is a time period between the birth of a subject and the death of a subject. An average life span can be the average time period between the birth and the death of a plurality of subjects (e.g., *C. elegans*, mammals, humans).

[0104] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, average pharyngeal pumping activity of the *C. elegans* animals in the *C. elegans* culture is increased by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract(s) or component(s) thereof. Pharyngeal pumping activity can be measured, e.g., by counting grinder movements, e.g., a single contraction and relaxation of a *C. elegans* corpus and/or terminal bulb. In some embodiments, pharyngeal pumping activity can be measure in pumps (or grinder movements)

per minute (ppm). An average pharyngeal pumping activity can be the average number of pumps (e.g., per minute) of a plurality of subjects (e.g., *C. elegans*, mammals, humans).

[0105] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, average locomotion rate of the *C. elegans* animals in the *C. elegans* culture is increased by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract(s) or component(s) thereof. In some embodiments, a locomotion rate can be calculated by animal bends per minute. An average locomotion rate can be the average number of animal bends (e.g., per minute) of a plurality of subjects (e.g., *C. elegans*, mammals, humans).

[0106] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, fertility of the *C. elegans* animals in the *C. elegans* culture is decreased by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract(s) or component(s) thereof. In some embodiments, fertility can be determined by the number of reproductive events (e.g., births) that occur. In some embodiments, fertility can be determined by the number of progeny. An average fertility rate can be the average number of reproductive events or the average number of progeny for a plurality of animals, e.g., *C. elegans*.

[0107] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when a *C. elegans* culture comprising *C. elegans* animals is exposed to Ultra Violet irradiation, average survival time of the *C. elegans* animals in the *C. elegans* culture to which the at least one bacterial strain or extract(s) or component(s) thereof has been administered is increased by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract(s) or component(s) thereof. In some embodiments, survival time is measured from the time the animal is exposed to UV irradiation until the time the animal dies.

[0108] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when a *C. elegans* culture comprising *C. elegans* animals is exposed to an elevated temperature, average survival time of *C. elegans* animals in the *C. elegans* culture to which the at least one bacterial strain or extract(s) or component(s) thereof has been administered is increased by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that *C. elegans* animals in of a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract(s) or component(s) thereof. In some embodiments, an elevated temperature is at least 37°C, at least 40°C, at least 45°C, at least 50°C, at least 55°C, at least 60°C, at least 65°C, at least 70°C, at least 75°C, or at least 80°C. In some embodiments, an elevated temperature is 50°C-65°C, 65°C-80°C, or 80°C-120°C. In some embodiments, survival time is measured from the time the elevated temperature is reached until the time the animal dies.

[0109] In some embodiments, at least one bacterial strain or extract(s) or component(s) thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, average amount of intestinal fat observed in the *C. elegan* animals in the *C. elegans* culture is decreased by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extract(s) or component(s) thereof. In some embodiments, an amount of intestinal fat is determined by visual observation following staining, e.g., Oil red O staining. In some embodiments, the area stained by, e.g., Oil red O stain, can be measured.

[0110] In some embodiments, *C. elegans* animals are adult *C. elegans* animals. In some embodiments, *C. elegans* animals are at least 5 days old.

[0111] Methods

[0112] The present disclosure provides the recognition that compositions described herein can be useful in extending life span, or reducing or delaying an age-associated symptom or condition in a subject. The present disclosure provides methods comprising administering a

composition described herein to a subject a composition. As discussed above, a composition can be formulated to be compatible with their intended route of administration.

[0113] In some embodiments, a method is a method of extending lifespan of a subject. In some embodiments, the life span of a subject is extended by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of a comparable subject without administration of the composition.

[0114] In some embodiments, a method is a method of reducing or delaying the onset of at least one age-associated symptom or condition in a subject. In some embodiments, at least one age-associated symptom or condition is reduced or delayed in a subject by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50%, as compared to that of a comparable subject without administration of the composition. In some embodiments, at least one age-associated symptom or condition is or comprises a decline in muscle and/or neuromuscular function of a subject. In some embodiments, at least one age-associated symptom or condition is or comprises dysregulation of lipid metabolism. In some embodiments, at least one age-associated symptom or condition is or comprises, e.g., a level of mitosis, organ function, organ wall thickness, variability in core body temperature, bone density, a level of peristalsis, retinal thickness, eardrum thickness, hearing loss, vision loss, or a combination thereof.

[0115] In some embodiments, a subject is at least 30 years old, at least 35 years old, at least 40 years old, at least 45 years old, at least 50 years old, at least 55 years old, at least 60 years old, at least 65 years old, at least 70 years old, or at least at least 75 years old. In some embodiments, a subject is an elderly subject. A subject could be younger than 30 years old, however, if, e.g., the subject suffers from a disease or condition associate with premature aging.

[0116] In some embodiments, a method is a method of treating a subject who has or is at risk of developing a disease or disorder associated with premature aging. In some embodiments, a disease or disorder is Bloom syndrome, Bockayne Syndrome, Hutchinson-Gilford progeria syndrome, mandibuloacral dysplasia with type A lipodystrophy, progeria, progeroid syndrome, Rothmund-Thomson syndrome, Seip syndrome, or Werner syndrome.

[0117] In some embodiments, a subject is a mammal. In some embodiments, a mammal is a non-human primate (e.g, a higher primate), a sheep, a dog, a rodent (e.g., a mouse or rat), a guinea pig, a goat, a pig, a cat, a rabbit, or a cow. In some embodiments, a mammal is a human.

[0118] In some embodiments, a method comprises administering comprises administering a sufficient amount of the microbe to colonize the subject's microbiome.

[0119] Assessing Biological Impact

[0120] The present disclosure provides the insight that *C. elegans* can be used to identify, characterize, or assess microbial strain(s) of a mammalian microbiome for an ability to extend the life span of a subject, or reduce or delay an age-associated symptom and/or an age-associated condition by contacting the microbial strain(s) (e.g., feeding the microbial strain(s) to, administering to) *C. elegans*. To determine whether a microbial strain or combination of microbial strains extend the life span, or reduce or delay an age-associated symptom and/or an age-associated condition of *C. elegans* can be observed, measured, or assessed in different samples that have been contacted with the microbial strain or combination of microbial strains. As just a few examples, parameters can include muscle function/activity, e.g., locomotion or bending, reproduction, stress resistance, lipid metabolism, or a combination thereof. In some embodiments, parameters can include, alone or in addition to those previously listed, genetic mutations (e.g., the presence of SNPs, deletions, additions, inversions, or repeats in DNA), transcript levels, protein levels, metabolite levels, lipid levels, carbohydrate levels, protein (e.g., enzyme) activity levels can be observed, measured, or assessed to determine whether a microbial strain or combination of microbial strains affects the life span of a subject, or reduce or delay an age-associated symptom and/or an age-associated condition of a *C. elegans*.

[0121] In some embodiments, methods described herein utilize a first sample and a second sample. In some embodiments, a first sample is a reference sample. In some embodiments, a reference sample can be a culture of *C. elegans* contacted with (e.g., administered or fed), e.g., OP50. In some embodiments, a reference sample can be a culture of *C. elegans* contacted with (e.g., administered or fed) a microbial strain or combination of microbial strains from a microbiome of a healthy individual. In some embodiments, a reference

sample can be a culture of *C. elegans* contacted with (e.g., administered or fed) a microbial strain or combination of microbial strains from a microbiome of an individual obtained at a first time point.

[0122] In some embodiments, a second sample can be a test sample. In some embodiments, a test sample can be a culture of *C. elegans* contacted with (e.g., administered or fed) an individual microbial strain or a combination of microbial strains from a mammalian microbiome, e.g., a human microbiome. In some instances, a human microbiome is a microbiome of a human suffering from or at risk of a disease or condition, e.g., a disease or condition associated with premature aging or delayed aging. In some embodiments, a test sample can be a culture of *C. elegans* contacted with (e.g., administered or fed) a microbial strain or combination of microbial strains from a microbiome of an individual obtained at a second time point (e.g., an aged subject).

[0123] In some embodiments, methods described herein comprise comparing one or more parameters obtained from a test sample with one or more parameters obtained from a reference sample. In some embodiments, by comparing one or more parameters obtained from a test sample with one or more parameters obtained from a reference sample, it can be determined that an individual microbial strain or a combination of microbial strains from a microbiome affect the life span, or reduce or delay an age-associated symptom and/or an age-associated condition of a *C. elegans* culture. In some embodiments, by comparing one or more parameters obtained from a test sample with one or more parameters obtained from a reference sample, it can be determined that an individual microbial strain or a combination of microbial strains from a microbiome extend the life span, or reduce or delay an age-associated symptom and/or an age-associated condition of the cultured *C. elegans*.

[0124] *C. elegans* and methods using *C. elegans* provided herein can be useful in assessing, characterizing, or identifying microbial strains of a microbiome that affect life span, or reduce or delay an age-associated symptom and/or an age-associated condition. The present disclosure provides the recognition that *C. elegans* and methods using *C. elegans* provided herein can be used to define and/or characterize a microbial signature associated with the life span of a subject, or one or more age-associated symptoms and/or an age-associated conditions.

[0125] The present disclosure also provides the recognition that *C. elegans* and methods using *C. elegans* provided herein can be used to monitor age progression.

[0126] The present disclosure also provides the insight that *C. elegans* and methods using *C. elegans* provided herein can be used to tailor therapeutics (e.g., therapies, nutraceuticals, and/or probiotics) to an individual patient. In some cases, microbial strains within an individual can be assessed, characterized, or identified to determine if they have an effect on an age-associated symptom and/or an age-associated condition. Based on the results, the individual can be administered one or more microbial strains to adjust the microbial strains (and/or component or compound thereof) in their microbiome. In some instances, this will affect aging of the individual. For example, if an individual is determined to have a relatively low amount of one or more microbial strains that have been determined to extend life spans, administration of the one or more microbial strains can extend the life span of the individual.

[0127] Among other things, the present disclosure provides technologies for assessing one or more microbes for usefulness as described herein. In some embodiments, technologies for identifying and/or characterizing microbes as described herein may involve comparisons of observations or measurements made on *C. elegans* administered the microbes with an appropriate reference (*e.g.,* with a positive control references and/or with a negative control reference). In some embodiments, a reference may be or comprise a historical reference; in some embodiments, a reference may be or comprise a contemporaneous reference.

EXAMPLES

[0128] The following examples are provided so as to describe to the skilled artisan how to make and use methods and compositions described herein, and are not intended to limit the scope of the present disclosure.

[0129] A library of ~30 bacterial species was screened for their effect on *C. elegans* lifespan. Bacterial species were chosen based on an abundant representation in 16s RNA sequencing studies. In this screen, 3 bacterial species were identified that significantly increased the lifespan of wildtype *C. elegans* animals. Such a screen can be repeated with additional

microbial species to determine if such species affect lifespan according to technologies described herein.

[0130] Example 1: Administration of Acetobacteraceae increased the lifespan of *C. elegans*

[0131] *C. elegans* (N2) longevity assays were performed on NGM plates seeded with either *E. coli* OP50, *G. oxydans*, *A. aceti*, or *G. hansenii* (Fig. 1, panels A-D). *C. elegans* animals administered (e.g. fed) either *G. oxydans*, *A. aceti*, or *G. hansenii* exhibited a significantly (P <0.00001) increased lifespan compared to animals administered *E. coli* OP50 (Fig. 1, panel A). A cumulative hazard plot analysis generated using the OASIS 2 platform (Han et al., 2016) showed that the hazard rates were different between animals administered *E. coli* OP50 and either *G. oxydans*, *A. aceti*, or *G. hansenii* (Fig. 1, panel B). Hazard plots of animals administered *G. hansenii* compared with animals administered either *G. oxydans* or *A. aceti* was slightly different from each other (Fig. 1, panel B). Hazard plots were not different between animals administered *G. oxydans* and animals administered *A. aceti* (Fig. 1, panel B).

[0132] Using the OASIS 2 software, restricted mean life span (RMLS) of animals administered either *G. oxydans*, *A. aceti*, or *G. hansenii* were calculated. The RMLS of animals administered *E. coli* OP50, 14.86±0.27 (RMLS± s.e, n=104) days, were significantly different compared to the RMLS of animals administered *G. oxydans*, 20.52±0.39 (RMLS± s.e, n=117) days, with two-tailed p value<0.0001. Similarly, the RMLS of animals administered *E. coli* OP50, 14.86±0.27 (RMLS± s.e, n=104) days, were significantly different compared to the RMLS of animals administered *A. aceti*, 19.17±0.41 (RMLS± s.e, n=124), with two-tailed p value<0.0001. Similarly, the RMLS of animals administered *E. coli* OP50, 14.86±0.27 (RMLS± s.e, n=104) days, were significantly different compared to the RMLS of animals administered *G. hansenii*, 22.95± 0.39 (RMLS± s.e, n=103) days, with two-tailed p value<0.0001 (Fig. 1, panel C). A smaller but statistically significant difference (two-tailed p value=0.0181) were observed when the RMLS of animals administered *G. oxydans* compared to the RMLS of animals administered *A. aceti*. However, statistically significant differences (two-tailed p value<0.0001) were observed when RMLS of animals administered *G. oxydans* or *A. aceti* were compared to

RMLS of animals administered *G. hansenii*. Comparing the survival curves of animals administered *E. coli* OP50 and either *G. oxydans*, *A. aceti*, or *G. hansenii* revealed significant differences in the survival rates (Fig. 1, panel D). Smaller but statistically different hazard rates were observed between animals administered either *G. oxydans* or *A. aceti* compared to animals administered *G. hansenii* (Fig. 1, panel D). However, the survival rates were not significantly different between animals administered *G. oxydans* compared to animals administered *A. aceti* (Fig. 1, panel D). From these observations, in the context of a lifespan assay, administration of *G. hansenii* significantly improved the lifespan of animals compared to *E. coli* OP50, *G. oxydans* or *A. aceti*.

[0133] Example 2: Administration of Acetobacteraceae improved muscle function/activity

[0134] Since muscle function/activity decreases as animals grow old, pharyngeal pumping activity and locomotion rate in animals administered *E. coli* OP50, *G. oxydans*, *A. aceti*, or *G. hansenii* was monitored. Pharyngeal pumping activity was significantly decreased in 10-day old animals administered *E. coli* OP50 compared to 5-day old animals administered *E. coli* OP50 (Fig. 2, panel A). However, compared to animals administered *E. coli* OP50 animals, animals administered *G. oxydans*, *A. aceti*, or *G. hansenii* exhibited an improved pharyngeal pumping function whether it was in 5-day old animals or 10-day old animals (Fig. 2, panel A). This result suggests that the muscle function was better preserved in old animals administered *G. oxydans*, *A. aceti*, or *G. hansenii* compared to administered *E. coli* OP50. Pharyngeal pumping rates of 5-day old animals administered either *G. oxydans*, *A. aceti*, or *G. hansenii* were significantly higher than the rates in animals administered *E. coli* OP50 suggesting that the animals did not starve, and therefore, caloric restriction effect can be ruled out.

[0135] To analyze the efficacy of *G. oxydans*, *A. aceti*, or *G. hansenii* in delaying aging, the rate of locomotion during the time course of aging (6^{th} and 12^{th} day post adulthood) was measured. The locomotion rate was significantly decreased in 12-day old animals administered *E. coli* OP50 animals compared to 6-day old adults (Fig. 2, panel B; p-value<0.00001). However, in animals administered *G. oxydans*, *A. aceti*, or *G. hansenii*, the locomotion rate of

12-day old animals was significantly higher than in animals of same age administered *E. coli* OP50 (p-value<0.00001). Interestingly, the locomotion rate of 6-day old animals administered *G. oxydans*, *A. aceti*, or *G. hansenii* was significantly higher than in animals of same age administered *E. coli* OP50 (Fig. 2, panel B; p-value<0.00001). These results suggested that the neuromuscular function required for locomotion was better preserved in old animals administered *G. hansenii*.

[0136] Example 3: Administration of Acetobacteraceae did not impact reproduction

[0137] Many long-lived *C. elegans* mutants exhibit a reduced reproductive capacity (Larsen et al., 1995)(Hughes et al., 2007). Therefore, the impact of *G. hansenii* on reproduction was tested. Fertility of animals administered *G. hansenii* was slightly lower than that of the animals administered *E. coli* OP50. The total number of progenies produced also decreased significantly in animals administered *G. hansenii*. Animals administered *E. coli* OP50 produced 180±31 (mean±s.d. of 15 animals) progeny, whereas 151±32 (mean±s.d. of 15 animals) progeny were produced by animals administered *G. hansenii* (two-tailed p value=0.0188). Measuring the time-course distribution of progeny production did not reveal any apparent differences in the rate of progeny production.

[0138] Example 4: Administration of Acetobacteraceae improved stress resistance

[0139] Since lifespan extension in *C. elegans* has been linked to stress resistance, the effect of *G. oxydans*, *A. aceti*, or *G. hansenii* administration on resistance to UV and thermotolerance was determined. Resistance to UV irradiation was significantly increased in animals administered either *G. oxydans*, *A. aceti*, or *G. hansenii* compared to animals administered either with *E. coli* OP50 (Fig. 3, panel A). Animals administered either with *E. coli* OP50, *G. oxydans*, *A. aceti*, or *G. hansenii* were exposed to UV irradiation (254nm) at a dose of 1,000 J/m^2. The number of dead and viable animals were scored every day until all animals died. Animals administered either *G. oxydans*, *A. aceti*, or *G. hansenii* survived longer than the animals administered *E. coli* OP50 (Fig. 3, panel A). Mean survival times of wildtype animals administered either *G. oxydans*, *A. aceti*, or *G. hansenii* were 4.69±0.15 (RMLS± s.e, n=97,

p<0.0001) days, 4.99±0.14 (RMLS± s.e, n=98, p<0.0001) days, and 5.4±0.14 (RMLS± s.e, n=98, p<0.0001) days respectively compared to 2.79±0.12 (RMLS± s.e, n=99) days in animals administered *E. coli* OP50.

[0140] To assess thermal shock stress resistance, the animals administered either with *E. coli* OP50, *G. oxydans*, *A. aceti*, or *G. hansenii* were transferred from 20°C to 37°C. The viable and the dead nematodes were scored every hour until all animals died. Animals administered *G. oxydans*, *A. aceti*, or *G. hansenii*, extended the mean survival time of the animals after they were shifted to an elevated temperature significantly compared to the animals administered *E. coli* OP50. While >60% of wildtype animals administered *E. coli* OP50 died within 2 hours of shifting to 37°C, 100% of wildtype animals administered either *G. oxydans*, *A. aceti*, or *G. hansenii* were alive even after 4 hours of shifting to 37°C (Fig. 3, panel B). Further, while 100% of wildtype animals administered *E. coli* OP50 died within 3 hours of shifting to 37°C, 100% of wildtype animals administered either *G. oxydans*, *A. aceti*, or *G. hansenii* died after 6 hours of shifting to 37°C. Thus, this data suggested that administration of *G. oxydans*, *A. aceti*, or *G. hansenii* conferred thermal stress resistance.

[0141] Example 5: Administration of Acetobacteraceae decreased fat deposition

[0142] Aging can be associated with dysregulation of lipid metabolism in some animals. Therefore, effects of administration of *G. hansenii* on lipid levels with aging were tested. While large amounts of intestinal fat were observed with Oil red O staining in *E. coli* OP50-administered animals, this accumulation was not observed in *G. hansenii*-administered animals (Fig. 4).

[0143] Example 6: *prx-5*, *tcer-1* and *aak-2* were involved in *G. hansenii*-induced lifespan extension

[0144] Because administration of *G. hansenii* had significant effect on various aspects of aging compared to *G. oxydans* or *A. aceti*, *G. hansenii* was focused on for further studies. Several conserved pathways including the insulin/IGF-1 like signaling (IIS) pathway

(Tissenbaum and Ruvkun, 1998)(Kenyon, 2011), the target of rapamycin (TOR) (Robida-Stubbs et al., 2012)(Johnson et al., 2013), Nrf2/Antioxidant stress response pathway (Blackwell et al., 2015), TGF beta signaling (Kaplan et al., 2015)(Luo et al., 2010), Sirtuins (Dang, 2014)(Guarente, 2007)(Longo and Kennedy, 2006), autophagy(Gelino et al., 2016)(Hansen et al., 2008)(Chang et al., 2017), and the AMP-activated protein kinase (AMPK) pathways (Burkewitz et al., 2014)(Curtis et al., 2006)(Onken and Driscoll, 2010) have been reported to be involved in determining the lifespan in *C. elegans*. To identify the genetic pathways through which *G. hansenii* to improve lifespan, longevity assays on *prx-5*, *tcer-1*, *aak-2* and *daf-16* mutants were performed.

[0145] *prx-5* encodes the ortholog of human PEX5, which is required for the peroxisomal import of cytosolic proteins containing peroxisomal targeting sequences (Wang et al., 2013). Peroxisome is an important organelle which plays an important role in several metabolic pathways including lipid metabolism. Age-dependent decline in peroxisomal protein import was observed previously (Narayan et al., 2016) and studies in yeast showed that reduction in peroxisomal import decreased the chronological lifespan (Lefevre et al., 2013). *tcer-1* encodes a putative transcription elongation factor that regulates aging in *C. elegans* (Amrit et al., 2016)(Ghazi et al., 2009)(McCormick et al., 2012). *aak-2* encodes an AMP-activated protein kinase that regulates lifespan in *C. elegans* (Curtis et al., 2006)(Moreno-Arriola et al., 2016)(Lee et al., 2008)(Apfeld et al., 2004). *daf-16* encodes a FOXO-family transcription factor that functions downstream of insulin signaling to regulate lifespan in many animals including *C. elegans* (Kimura et al., 1997)(Murphy et al., 2003)(Lee et al., 2001).

[0146] Based on data obtained, *prx-5*, *tcer-1*, and *aak-2* were required for the *G. hansenii*-induced lifespan extension, but *daf-16* was not required for the longevity phenotype. For the lifespan assays, a *prx-5(ku517)* strain in which PRX-5 is produced as truncated product (i.e., missing the last 26 amino acids of the protein (Wang et al., 2013)) was used. This strain is referred to herein as *prx-5(0)*. Comparing the survival curves revealed that the RMLS of *G. hansenii*-administered wildtype animals was significantly higher than the RMLS of *prx-5(0)* animals administered *G. hansenii* [21.94±0.34(n=109) days vs 14.86±0.27(n=104) days, $p<0.0001$] (Fig. 5, panels A-B). The RMLS of *prx-5(0)* animals administered *G. hansenii* were more similar to that of wildtype animals administered *E. coli* OP50 [13.52±0.34(n=103) days vs

13.11±0.32(n=113) days, p=0.3805] (Fig. 5, panels A-B), which suggested that *prx-5* was necessary for the *G. hansenii*-induced lifespan extension. Further, *prx-5* appeared necessary for normal lifespan because *prx-5(0)* animals administered *E. coli* OP50 had decreased RMLS compared to that of wildtype animals administered *E. coli* OP50 [9.59±0.29 (n=108) days vs 13.11±0.32(n=113) days, p<0.0001] (Fig. 5, panels A-B). *prx-5(0)* animals administered *G. hansenii* had increased RMLS compared to *prx-5(0)* animals administered *E. coli* OP50 [13.52±0.34(n=103) days vs 9.59±0.29 (n=108) days, p<0.0001] (Fig. 5, panels A-B). This result suggested that although the lifespan extension in animals administered *G. hansenii* was dependent on *prx-5*, *G. hansenii* also improved the lifespan of *prx-5(0)* mutants. Similar results were obtained by comparing the cumulative hazard rate of wildtype and *prx-5(0)* animals administered either *E. coli* OP50 or *G. hansenii* (Fig. 5, panel C). Lifespan assays on *tcer-1(0)* animals revealed that while administration of *G. hansenii* extended the RMLS of wildtype animals compared to that of wildtype animals administered *E. coli* OP50 [22.15±0.37(n=110) days vs 14.43±0.30(n=98) days, p<0.0001], *G. hansenii*-administration did not extend the RMLS of *tcer-1(0)* animals compared to that of *tcer-1(0)* animals administered *E. coli* OP50 [15.15±0.29(n=97) days vs 15.24±0.31(n=92) days, p<0.0001] (Fig. 6, panel A-B). RMLS of *tcer-1(0)* animals administered *G. hansenii* was not significantly different from the RMLS of wildtype animals administered *E. coli* OP50 [15.15±0.29(n=97) days vs 14.43±0.3(n=98) days p=0.0861] or RMLS of of *tcer-1(0)* animals administered *E. coli* OP50 [15.15±0.29(n=97) days vs 15.24±0.31(n=92) days p=0.8322] (Fig. 6, panel A-B). This result suggested that *tcer-1* was required for *G. hansenii*-induced lifespan extension. Lifespan assays on *aak-2(0)* animals revealed that while administration of *G. hansenii* extended the RMLS of wildtype animals compared to that of wildtype animals administered *E. coli* OP50 [22.10±0.36(n=105) days vs 13.29±0.31(n=89) days, p<0.0001], *G. hansenii*-administration did not extend the RMLS of *aak-2(0)* animals compared to that of *aak-2(0)* animals administered *E. coli* OP50 [15.13±0.31(n=105) days vs 14.73±0.30 (n=110) days, p<0.3548] (Fig. 6, panel C-D).

[0147] Example 7: *daf-16* was not required for the *G. hansenii*-induced lifespan extension

[0148] While *prx-5*, *tcer-1* or *aak-2* animals appeared to be necessary for *G. hansenii*-induced lifespan extension phenotype, results suggested that *daf-16* was not required for the longevity phenotype. Lifespan assays revealed that the RMLS of *daf-16(0)* animals administered *G. hansenii* was significantly increased compared to that of *daf-16(0)* animals administered *E. coli* OP50 [22.61±0.26(n=97) days vs 11.51±0.30(n=98) days, p<0.0001] (Fig. 7, panel A-B).

[0149] **Example 8: *hsf-1* was involved in *G. hansenii*-induced thermotolerance phenotype**

[0150] Thermotolerance in *C. elegans* has been associated with expression of heat shock proteins under the control of heat shock factor-1 (HSF-1) transcription factor (Hajdu-Cronin et al., 2004)(Link et al., 1999); therefore, *G. oxydans*, *A. aceti*, and *G. hansenii*-administration were examined to determine if such administration would induce *hsp-16.2::gfp* expression. *hsp-16.2* is a heat shock protein that is induced in heat stress in a heat shock factor-1 (HSF-1) transcription factor. While 2.5±1.1% (n=225) animals administered *E. coli* OP50 and grown at 20°C showed *hsp-16.2::gfp* GFP induction, 86.6±8.3% (n=252) of animals administered *E. coli* OP50 and shifted to 35°C for 1 hour had *hsp-16.2::gfp* expression. Animals administered either *G. oxydans*, *A. aceti*, or *G. hansenii* and grown at 20°C did not show induction of *hsp-16.2::gfp* expression suggesting that induction of heat shock protein expression is not required for the thermotolerance phenotype. When the animals administered either *G. oxydans*, *A. aceti*, or *G. hansenii* were shifted to 35°C for 1 hour, *hsp-16.2::gfp* expression was observed in 94.1±4.3% (n=243), 88.9±2.4% (n=220), 90.2±1.3% (n=216) of animals respectively (Fig. 8). This result suggested that administration with either *G. oxydans*, *A. aceti*, or *G. hansenii* did not affect the induction of heat shock response genes.

[0151] Although, induction of heat response genes in animals administered *G. hansenii* and grown at 20°C was not observed, the thermotolerance phenotype was found to be dependent on HSF-1. While 100% of wildtype animals administered *G. hansenii* were alive even after 3 hours shifting to 37°C, 100% of *hsf-1(0)* animals administered *G. hansenii* were dead (Fig. 8, panel B). Further, while 25.6±4.7% (n=300) of wildtype animals administered *E. coli* OP50 were alive after 2 hours of shifting to 37°C, 100±0% (n=300) of *hsf-1(0)* animals administered *E.*

coli OP50 were dead within 2 hours of shifting to 37°C, which suggested HSF-1 was required for thermotolerance (Fig. 8, panel B). Compared to the *hsf-1(0)* animals administered *E. coli* OP50 and shifted to 37°C, *hsf-1(0)* animals administered *G. hansenii* and shifted to 37°C survived better (Fig. 8, panel B), which suggested that there might be HSF-1 independent pathways as well.

[0152] The results suggested that even though administration of *G. hansenii* did not induce *hsp-16.2::gfp*, the thermo-tolerance phenotype was dependent on HSF-1, as well as HSF-1 independent pathways. To test whether the thermotolerance phenotype of animals administered *G. hansenii* is dependent on PRX-5, TCER-1 or AAK-2, thermotolerance assays in *tcer-1(0)*, *prx-5(0)* or *aak-2(0)* mutants were conducted. The survival curves of *tcer-1(0)* administered *G. hansenii* were similar to that of wildtype animals administered *G. hansenii*, which suggested that TCER-1 was not required for the thermotolerance phenotype (Fig. 9, panel A). *prx-5(0)* animals were found to be hypersensitive to heat stress compared to the wildtype; while 22±1% (n=300) of wildtype animals administered *E. coli* OP50 were alive after 2 hours of shifting to 37°C, 100±0% (n=300) of *prx-5(0)* animals administered *E. coli* OP50 were dead within 2 hours of shifting to 37°C suggesting as PRX-5 is required for thermotolerance (Fig. 9, panel B). Further, while 100% of wildtype animals administered *G. hansenii* were alive even after 3 hours of shifting to 37°C, 100% of *prx-5(0)* animals administered *G. hansenii* were dead (Fig. 8, panel B), which suggested that PRX-5 was required for thermotolerance phenotype of animals administered *G. hansenii*. AAK-2 was found to be required for the thermotolerance phenotype of animals administered *G. hansenii*. Compared to the survival rate of wildtype animals administered *G. hansenii*, the survival rates of *aak-2(0)* animals administered *G. hansenii* was significantly reduced (Fig. 9, panel C). The survival curves of *aak-2(0)* animals administered *E. coli* OP50 was similar to that of wildtype animals administered *E. coli* OP50 suggesting that AAK-2 is not required for normal thermotolerance (Fig. 9, panel C).

OTHER EMBODIMENTS

[0153] It is to be appreciated by those skilled in the art that various alterations, modifications, and improvements to the present disclosure will readily occur to those skilled in

the art. Such alterations, modifications, and improvements are intended to be part of the present disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawing are by way of example only and any invention described in the present disclosure if further described in detail by the claims that follow.

[0154] Those skilled in the art will appreciate typical standards of deviation or error attributable to values obtained in assays or other processes as described herein. The publications, websites and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference in their entireties.

[0155] It is to be understood that while embodiments of the invention have 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.

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[0156] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

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WHAT IS CLAIMED IS:

1. A composition comprising:

(a) at least one bacterial strain or extracts or components thereof, wherein the at least one bacterial strain comprises *Gluconobacter spp.*, *Acetobacter spp.*, *Gluconoacaetobacter spp.*, *Acidomonas spp.*, *Ameyamaea spp.*, *Asaia spp.*, *Granulibacter spp.*, *Kozakia spp.*, *Neoasaia spp.*, *Neokomagataea spp.*, *Saccharibacter spp.*, *Swaminathania spp.*, *Tanticharoenia spp.*, or a combination thereof; and

(b) an excipient.

2. The composition of claim 1, wherein the at least one bacterial strain comprises *Gluconacetobacter hansenii*, *Gluconobacter oxydans*, *Acetobacter aceti*, or a combination thereof.

3. The composition of claim 1 or 2, wherein the at least one bacterial strain comprises *Gluconacetobacter hansenii*.

4. The composition of any one of claims 1-3, wherein the at least one bacterial strain or extracts or components thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, an average life span of the *C. elegans* animals in the *C. elegans* culture is extended by at least 20%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extracts or components thereof.

5. The composition of any one of claims 1-4, wherein the at least one bacterial strain or extracts or components thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, average pharyngeal pumping activity of the *C. elegans* animals in the *C. elegans* culture is increased by at least 20%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extracts or components thereof.

6. The composition of any one of claims 1-5, wherein the at least one bacterial strain or extracts or components thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, average locomotion rate of the *C. elegans* animals in the *C. elegans* culture is increased by at least 20%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extracts or components thereof.

7. The composition of any one of claims 1-6, wherein the at least one bacterial strain or extracts or components thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, fertility of the *C. elegans* animals in the *C. elegans* culture is decreased by at least 20%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extracts or components thereof.

8. The composition of any one of claims 1-7, wherein the at least one bacterial strain or extracts or components thereof is characterized in that, when a *C. elegans* culture comprising *C. elegans* animals is exposed to Ultra Violet irradiation, average survival time of the *C. elegans* animals in the *C. elegans* culture to which the at least one bacterial strain or extracts or components thereof has been administered is increased by at least 20%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extracts or components thereof.

9. The composition of any one of claims 1-8, wherein the at least one bacterial strain or extracts or components thereof is characterized in that, when a *C. elegans* culture comprising *C. elegans* animals is exposed to an elevated temperature, average survival time of *C. elegans* animals in the *C. elegans* culture to which the at least one bacterial strain or extracts or components thereof has been administered is increased by at least 20%, as compared to that *C. elegans* animals in of a comparable *C. elegans* culture without administration of the at least one bacterial strain or extracts or components thereof.

10. The composition of claim 9, wherein the elevated temperature is at least 37°C.

11. The composition of any one of claims 1-10, wherein the at least one bacterial strain or extracts or components thereof is characterized in that, when administered to a *C. elegans* culture comprising *C. elegans* animals, average amount of intestinal fat observed in the *C. elegan* animals in the *C. elegans* culture is decreased by at least 20%, as compared to that of *C. elegans* animals in a comparable *C. elegans* culture without administration of the at least one bacterial strain or extracts or components thereof.

12. The composition of any one of claims 4-11, wherein the *C. elegans* animals are adult *C. elegans* animals.

13. The composition of any one of claims 4-12, wherein the *C. elegans* animals are at least 5 days old.

14. The composition of any one of claims 1-13, wherein the composition is formulated for oral administration.

15. The composition of any one of claims 1-14, wherein the composition is a food, a beverage, a feed composition, or a nutritional supplement.

16. The composition of any one of claims 1-15, wherein the composition is a liquid, syrup, tablet, troche, gummy, capsule, powder, gel, or film.

17. The composition of any one of claims 1-16, wherein the composition is a pharmaceutical composition.

18. The composition of any one of claims 1-17, wherein the composition is an enteric-coated formulation.

19. A method comprising administering to a subject a composition of any one of claims 1-18.

20. The method of claim 19, wherein the method is a method of extending lifespan of a subject.

21. The method of claim 20, wherein the life span of the subject is extended by at least 20%, as compared to that of a comparable subject without administration of the composition.

22. The method of claim 19, wherein the method is a method of reducing or delaying the onset of at least one age-associated symptom or condition in a subject.

23. The method of claim 22, wherein the at least one age-associated symptom or condition is reduced or delayed in the subject by at least 20%, as compared to that of a comparable subject without administration of the composition.

24. The method of claim 22 or 23, wherein the at least one age-associated symptom or condition is or comprises a decline in muscle and/or neuromuscular function of the subject.

25. The method of any one of claims 22-24, wherein the at least one age-associated symptom or condition is or comprises dysregulation of lipid metabolism.

26. The method of any one of claims 19-25, wherein the subject is at least 30 years old.

27. The method of any one of claims 19-26, wherein the subject is an elderly subject.

28. The method of any one of claims 19-27, wherein the subject is a mammal.

29. The method of any one of claims 19-28, wherein the subject is a human.

30. The method of any one claims 19-29, wherein administering comprises administering a sufficient amount of the microbe to colonize the subject's microbiome.

31. Use of at least one bacterial strain or extracts or components thereof for extending the life span of a subject, wherein at least one bacterial strain comprises *Gluconobacter spp.*, *Acetobacter spp.*, *Gluconoacaetobacter spp.*, *Acidomonas spp.*, *Ameyamaea spp.*, *Asaia spp.*, *Granulibacter spp.*, *Kozakia spp.*, *Neoasaia spp.*, *Neokomagataea spp.*, *Saccharibacter spp.*, *Swaminathania spp.*, *Tanticharoenia spp.*, or a combination thereof.

32. The use of claim 31, wherein the at least one bacterial strain comprises *Gluconacetobacter hansenii*, *Gluconobacter oxydans*, *Acetobacter aceti*, or a combination thereof.

33. The use of claim 31 or 32, wherein the at least one bacterial strain comprises *Gluconacetobacter hansenii*.

34. Use of at least one bacterial strain or extracts or components thereof reducing or delaying the onset of at least one age-associated symptom or condition in a subject, wherein the at least one bacterial strain comprises *Gluconobacter spp.*, *Acetobacter spp.*, *Gluconoacaetobacter spp.*, *Acidomonas spp.*, *Ameyamaea spp.*, *Asaia spp.*, *Granulibacter spp.*, *Kozakia spp.*, *Neoasaia spp.*, *Neokomagataea spp.*, *Saccharibacter spp.*, *Swaminathania spp.*, *Tanticharoenia spp.*, or a combination thereof.

35. The use of claim 34, wherein the at least one bacterial strain comprises *Gluconacetobacter hansenii*, *Gluconobacter oxydans*, *Acetobacter aceti*, or a combination thereof.

36. The use of claim 34 or 35, wherein the at least one bacterial strain comprises *Gluconacetobacter hansenii*.

37. The use of claim 34, wherein the at least one age-associated symptom or condition is reduced or delayed in the subject by at least 20%, as compared to that of a comparable subject without administration of the composition.

38. The use of any one of claims 34-37, wherein the at least one age-associated symptom or condition is or comprises a decline in muscle and/or neuromuscular function of the subject.

39. The use of any one of claims 34-38, wherein the at least one age-associated symptom or condition is or comprises dysregulation of lipid metabolism.

40. The use of any one of claims 34-39, wherein the subject is at least 30 years old.

41. The use of any one of claims 34-40, wherein the subject is an elderly subject.

42. The use of any one of claims 34-41, wherein the subject is a mammal.

43. The use of any one of claims 34-42, wherein the subject is a human.

44. A method of characterizing the ability of one or more microbial strains to modify the life span of a subject, an age-associated symptom, and/or an age-associated condition, comprising:

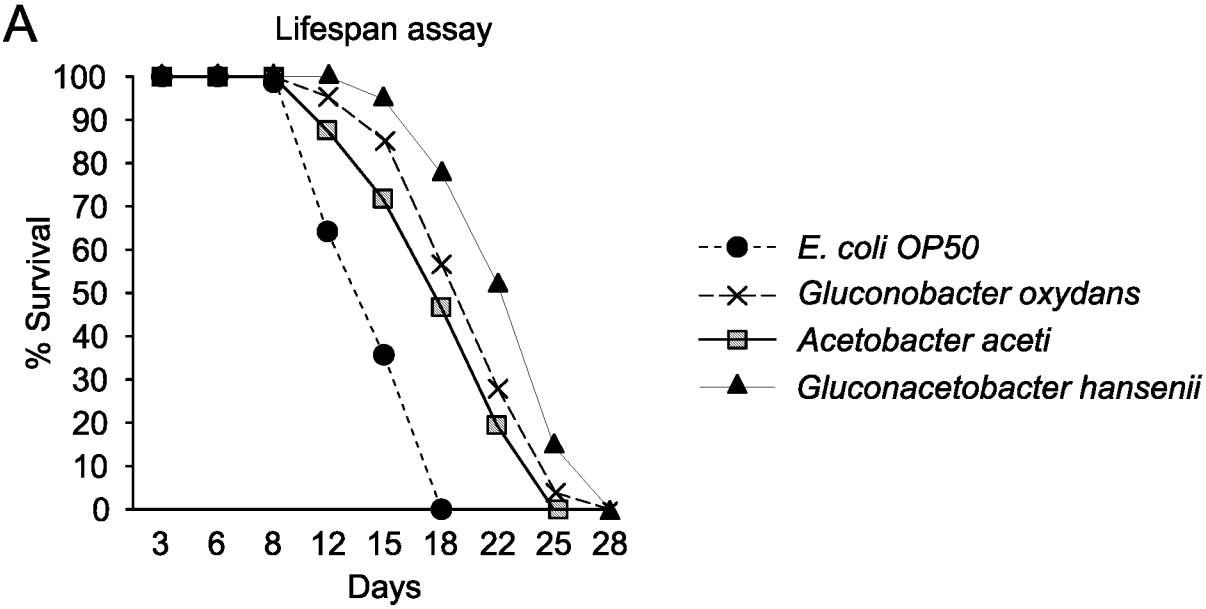
(a) adding a plurality of microbial strains of a mammalian microbiome to a plurality of *C. elegans* cultures, wherein a different microbial strain is added to each *C. elegans* culture, and wherein each culture comprises *C. elegans* animals of the same *C. elegans* strain, and

(b) determining whether each microbial strain of the plurality affects one or more parameters of the *C. elegans* animals of each culture, wherein the one or more parameters are associated with aging, an age-associated symptom, and/or an age-associated condition.

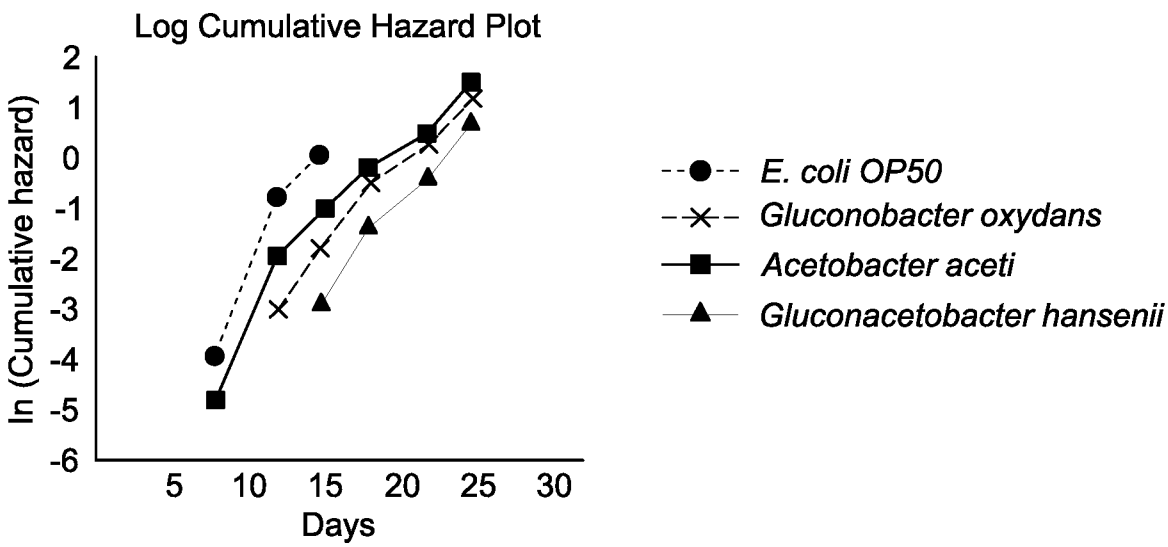
45. Use of *C. elegans* animals for characterizing the ability of one or more one or more microbial strains to modify the life span of a subject, an age-associated symptom, and/or an age-associated condition.

46. A method of making a composition according to any one of claims 1-18 comprising combining at least one bacterial strain or extracts or components thereof and the excipient.

A



B



C

C. elegans administered	Mean ± SE	N	95% C.I.
E. coli OP50	14.86 ± 0.27	104	14.33 ~ 15.40
Gluconobacter oxydans	20.52 ± 0.39	117	19.76 ~ 21.29
Acetobacter aceti	19.17 ± 0.41	124	18.37 ~ 19.98
Gluconacetobacter hansenii	22.95 ± 0.39	103	22.19 ~ 23.70

D

Condition	X^2	pvalue
E. coli OP50 v.s. *Gluconobacter oxydans*	92.48	0.00001
E. coli OP50 v.s. *Acetobacter aceti*	60.58	0.00001
E. coli OP50 v.s. *Gluconacetobacter hansenii*	137.42	0.00001
Gluconobacter oxydans v.s. *Acetobacter aceti*	4.8	0.0285
Gluconobacter oxydans v.s. *Gluconacetobacter hansenii*	14.46	0.0001
Acetobacter aceti v.s. *Gluconacetobacter hansenii*	33.8	0.0001

FIG. 1

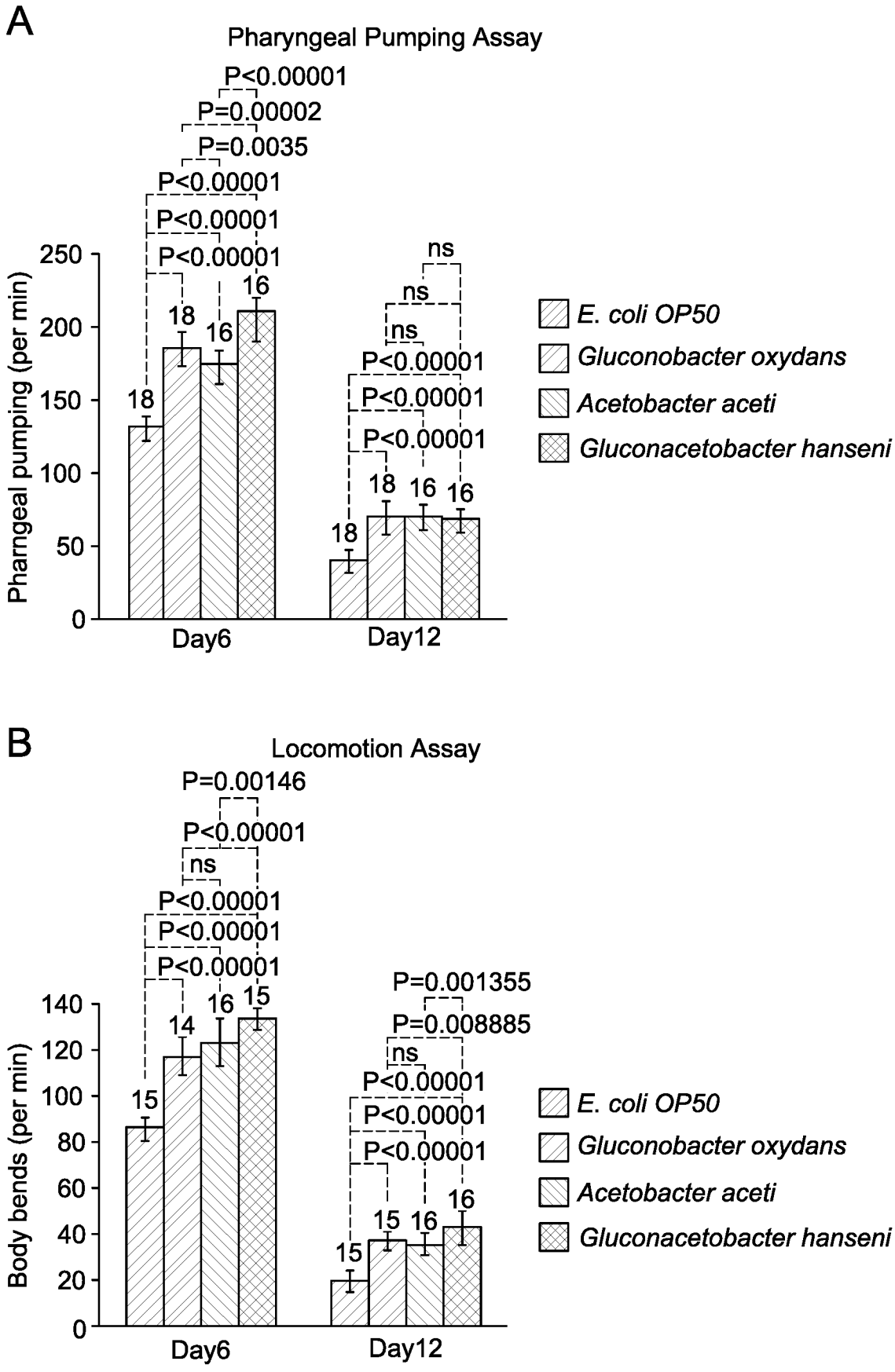

A
Pharyngeal Pumping Assay
P<0.00001
P=0.00002
P=0.0035
P<0.00001
P<0.00001
P<0.00001
ns
ns
ns
P<0.00001
P<0.00001
P<0.00001
Pharngeal pumping (per min)
250
200
150
100
50
0
18
18
16
16
18
18
16
16
Day6
Day12
E. coli OP50
Gluconobacter oxydans
Acetobacter aceti
Gluconacetobacter hanseni
B
Locomotion Assay
P=0.00146
P<0.00001
ns
P<0.00001
P<0.00001
P<0.00001
P=0.001355
P=0.008885
ns
P<0.00001
P<0.00001
P<0.00001
Body bends (per min)
140
120
100
80
60
40
20
0
15
14
16
15
15
15
16
16
Day6
Day12
E. coli OP50
Gluconobacter oxydans
Acetobacter aceti
Gluconacetobacter hanseni

FIG. 2

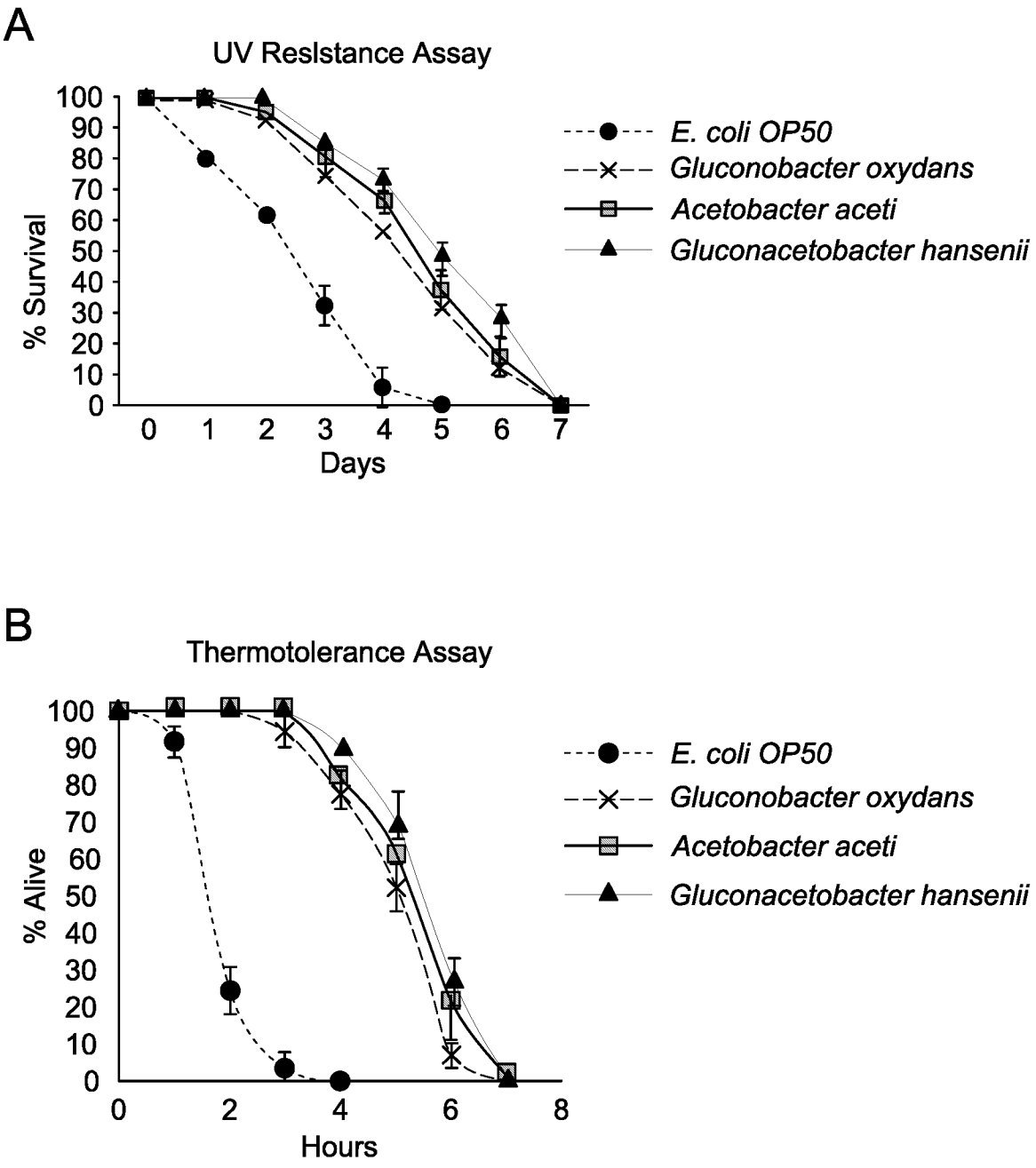

A
UV Reslstance Assay
% Survival
Days
E. coli OP50
Gluconobacter oxydans
Acetobacter aceti
Gluconacetobacter hansenii
B
Thermotolerance Assay
% Alive
Hours
E. coli OP50
Gluconobacter oxydans
Acetobacter aceti
Gluconacetobacter hansenii

FIG. 3

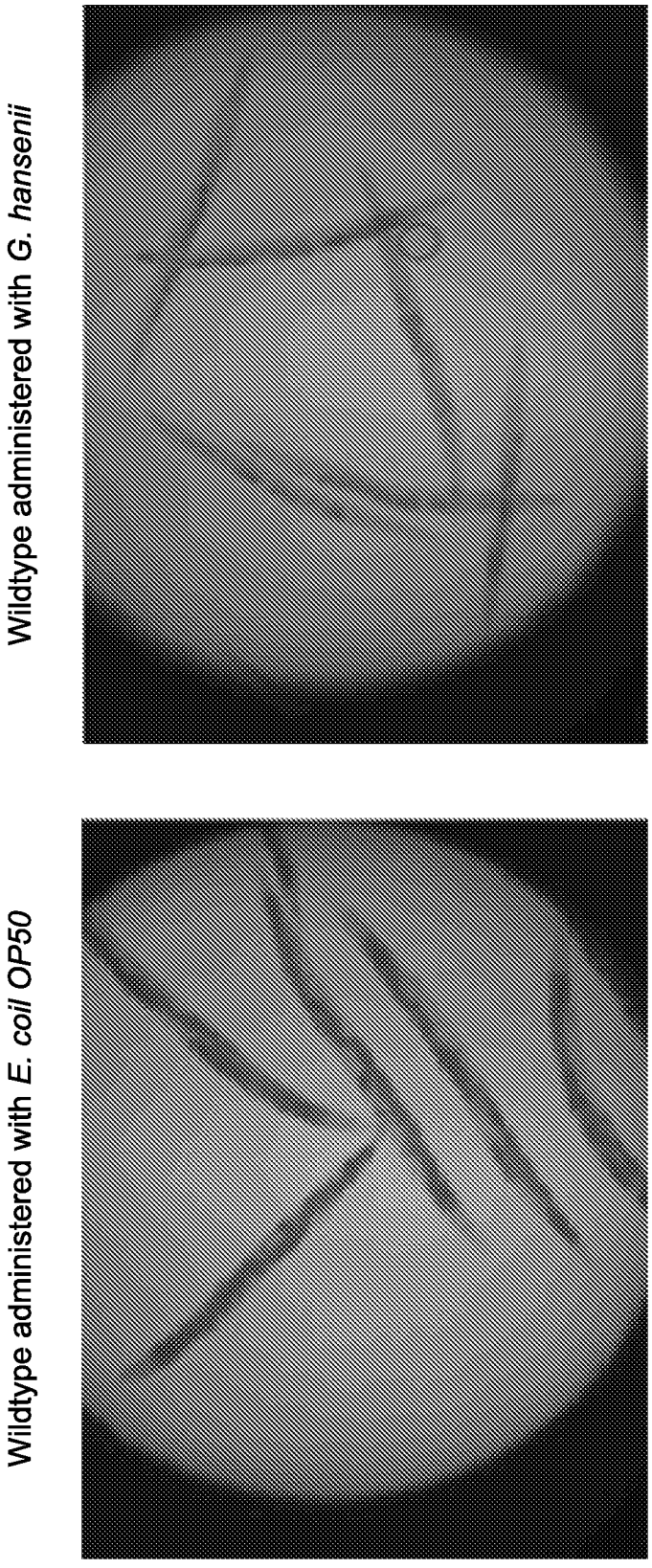
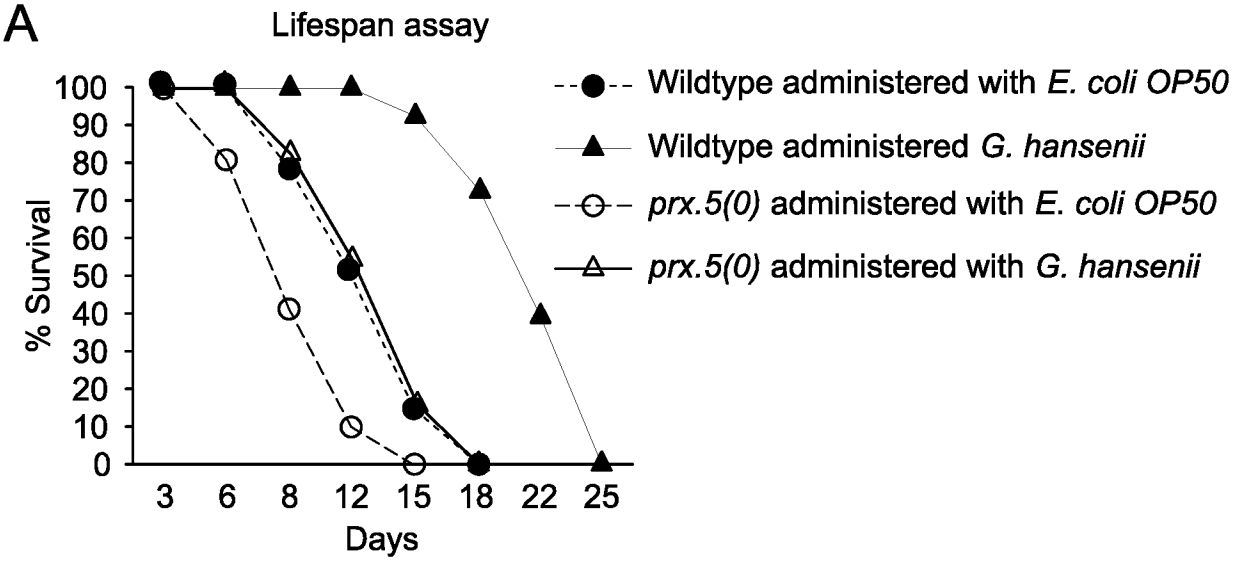

Wildtype administered with *G. hansenii*
Wildtype administered with *E. coil OP50*

FIG. 4

A



B

C. elegans administered	Mean ± SE	N	95% C.I.
Wildtype administered with *E. coli OP50*	13.11 ± 0.32	113	12.48 ~ 13.74
Wildtype administered with *G. hansenii*	21.94 ± 0.34	109	21.28 ~ 22.60
prx.5(0) administered with *E. coli OP50*	9.59 ± 0.29	108	9.03 ~ 10.15
prx.5(0) administered with *G. hansenii*	13.52 ± 0.34	103	12.85 ~ 14.18

C

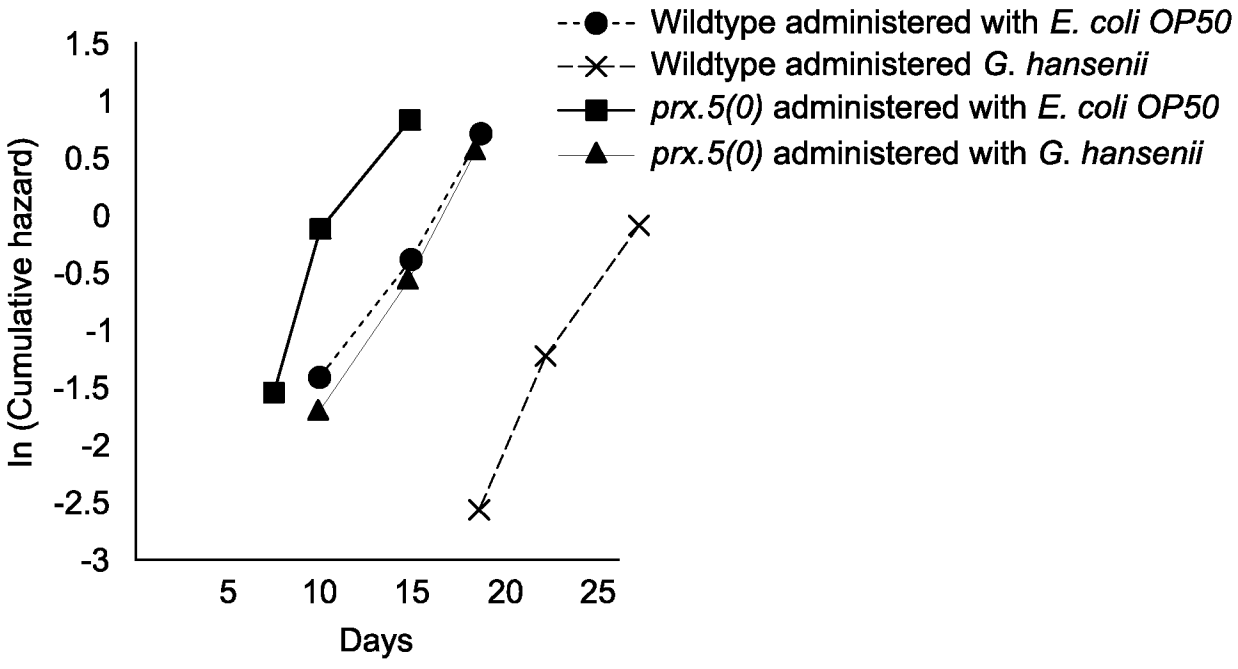
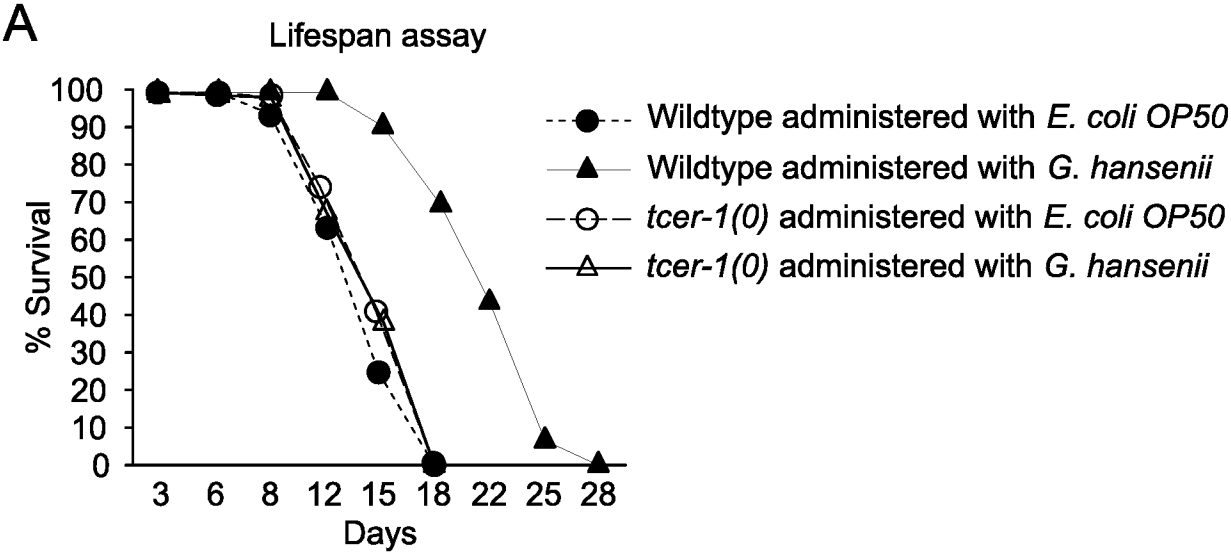


FIG. 5

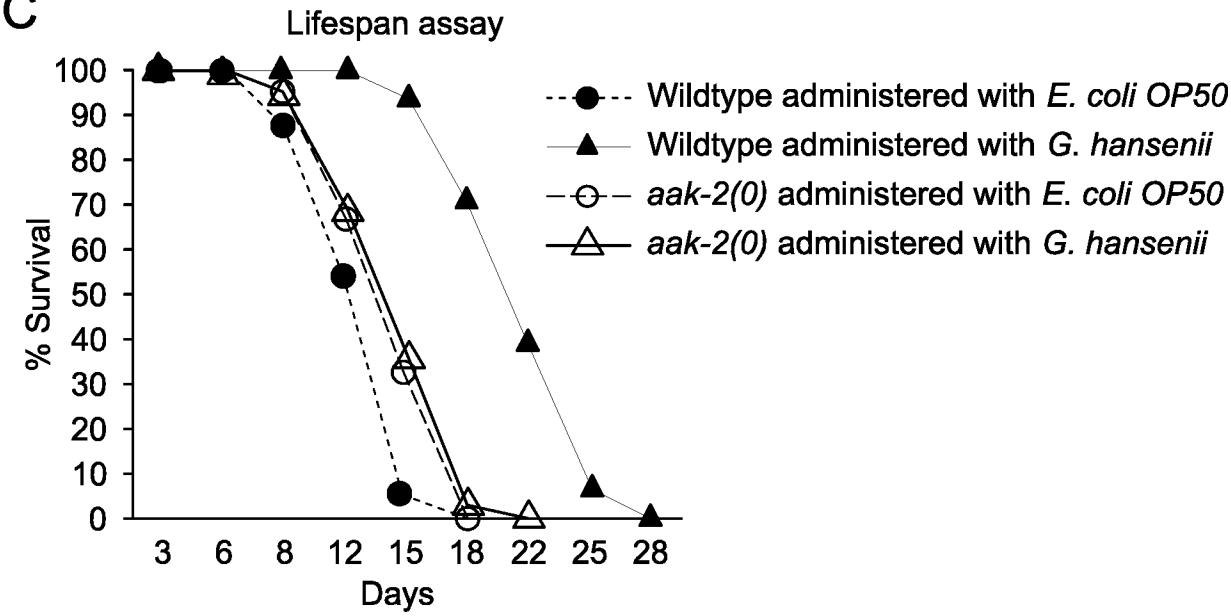
A



B

C. elegans administered	Mean ± SE	N	95% C.I.
Wildtype administered with *E. coli OP50*	14.43 ± 0.3	98	13.84 ~ 15.01
Wildtype administered with *G. hansenii*	22.15 ± 0.37	110	21.42 ~ 22.89
tcer-1(0) administered with *E. coli OP50*	15.24 ± 0.31	92	14.63 ~ 15.85
tcer-1(0) administered with *G. hansenii*	15.15 ± 0.29	97	14.58 ~ 15.71

C

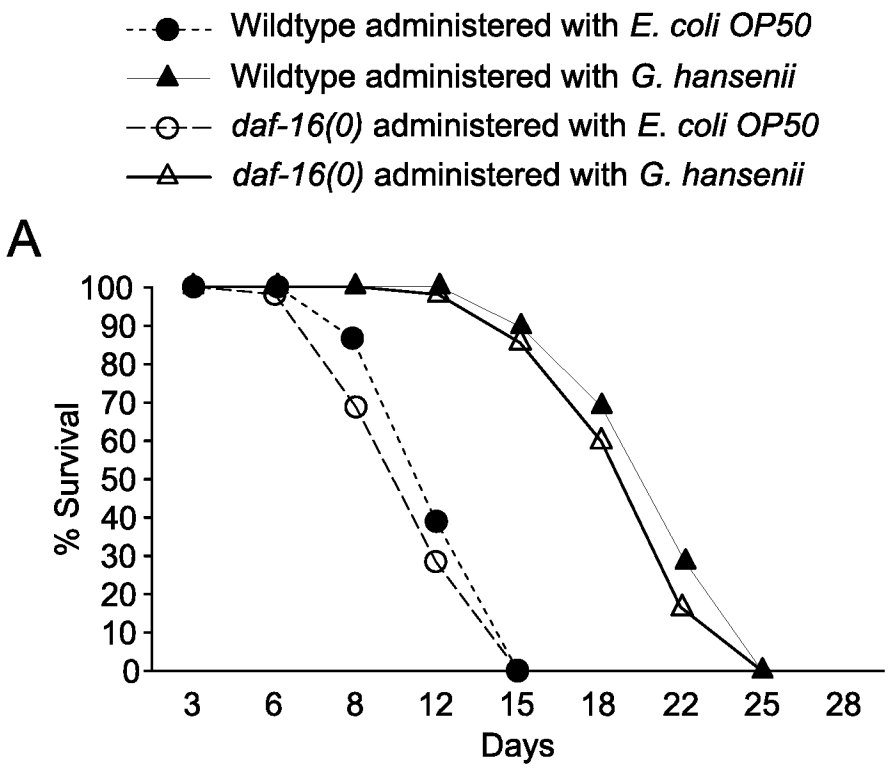


D

C. elegans administered	Mean ± SE	N	95% C.I.
Wildtype administered with *E. coli OP50*	13.29 ± 0.31	89	12.69 ~ 13.89
Wildtype administered with *G. hanseni*	22.10 ± 0.36	105	21.39 ~ 22.81
aak-2(0) administered with *E. coli OP50*	14.73 ± 0.30	110	14.15 ~ 15.32
aak-2(0) administered with *G. hansenii*	15.13 ± 0.31	105	14.52 ~ 15.74

FIG. 6

A



B

C. elegans administered	Mean ± SE	N	95% C.I.
Wildtype administered with *E. coli OP50*	12.61 ± 0.26	97	12.09 ~ 13.12
Wildtype administered with *G. hanseni*	21.35 ± 0.34	102	20.67 ~ 22.02
daf-16(0) administered with *E. coli OP50*	11.51 ± 0.30	98	10.92 ~ 12.10
daf-16(0) administered with *G. hansenii*	20.40 ± 0.35	106	19.71 ~ 21.09

FIG. 7

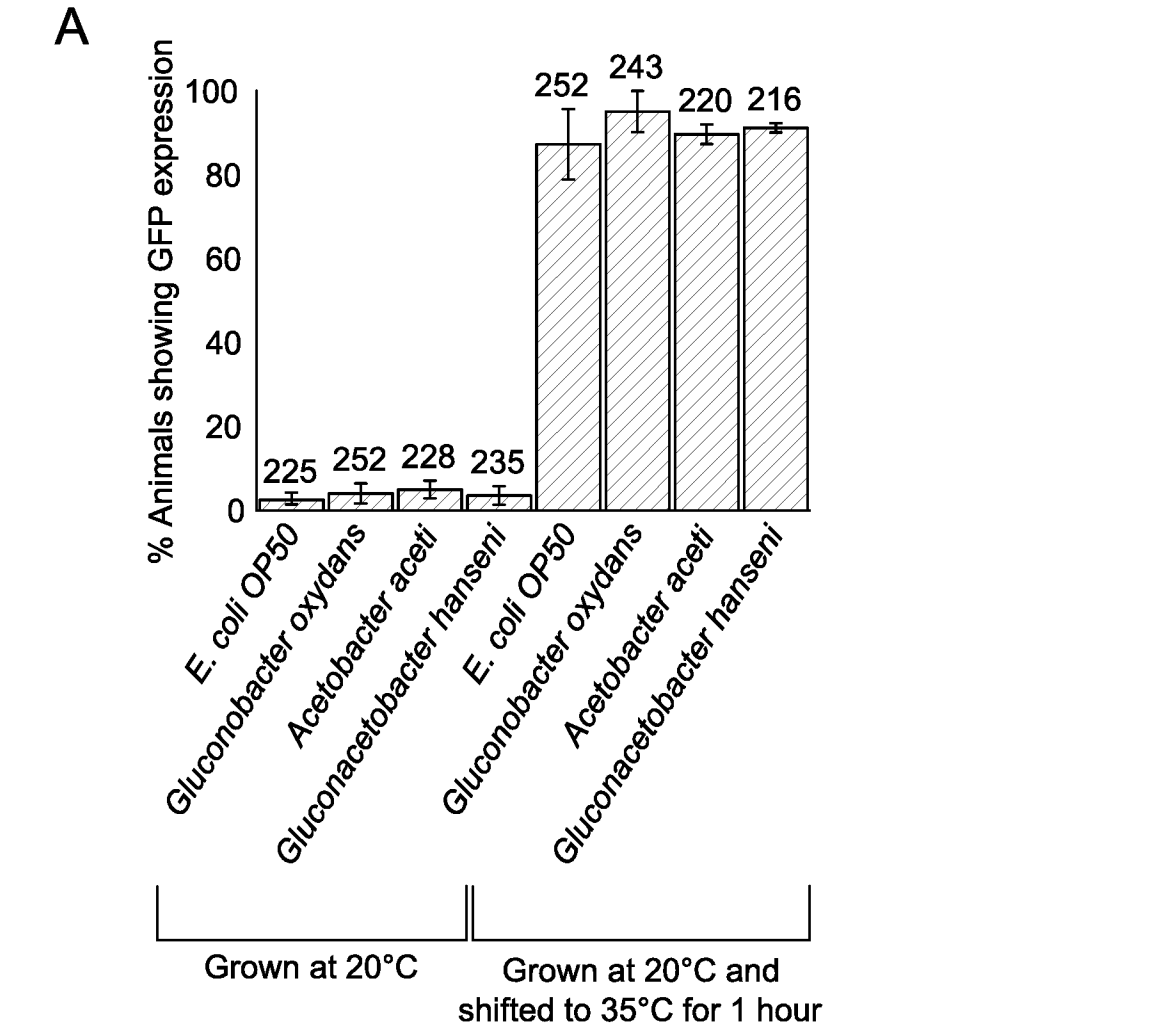

A
% Animals showing GFP expression
100
80
60
40
20
0
225
252
228
235
252
243
220
216
E. coli OP50
Gluconobacter oxydans
Acetobacter aceti
Gluconacetobacter hanseni
E. coli OP50
Gluconobacter oxydans
Acetobacter aceti
Gluconacetobacter hanseni
Grown at 20°C
Grown at 20°C and shifted to 35°C for 1 hour
B
Thermotolerance assay in hsf-1(0)
% Alive
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8
hours
Wildtype administered with E. coli OP50
Wildtype administered with G. hansenii
hsf-1(0) administered with E. coli OP50
hsf-1(0) administered with G. hansenii

FIG. 8

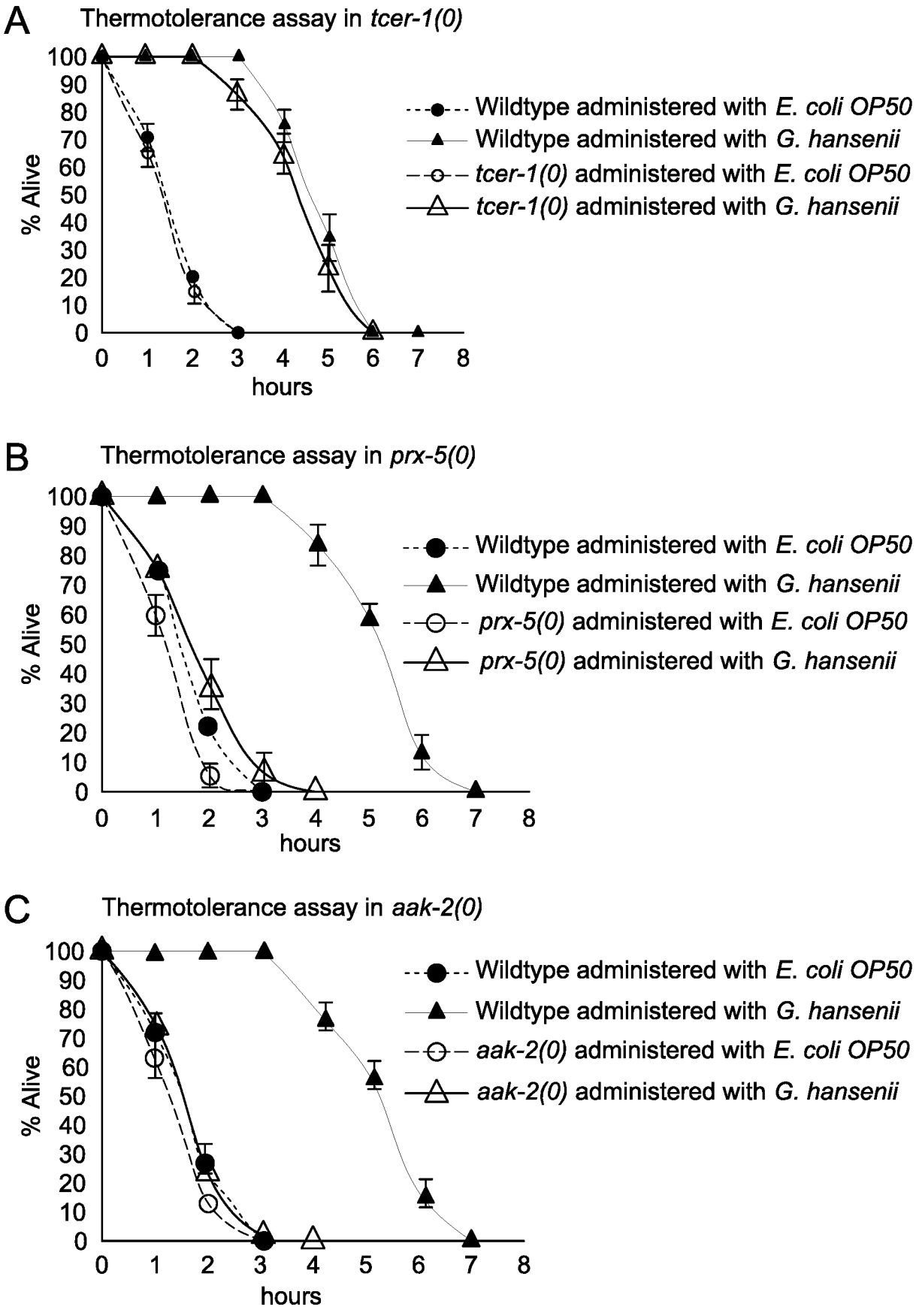

A
Thermotolerance assay in tcer-1(0)
% Alive
hours
Wildtype administered with E. coli OP50
Wildtype administered with G. hansenii
tcer-1(0) administered with E. coli OP50
tcer-1(0) administered with G. hansenii
B
Thermotolerance assay in prx-5(0)
% Alive
hours
Wildtype administered with E. coli OP50
Wildtype administered with G. hansenii
prx-5(0) administered with E. coli OP50
prx-5(0) administered with G. hansenii
C
Thermotolerance assay in aak-2(0)
% Alive
hours
Wildtype administered with E. coli OP50
Wildtype administered with G. hansenii
aak-2(0) administered with E. coli OP50
aak-2(0) administered with G. hansenii

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20/52251

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 35/74; A61K 35/66; A23L 33/135; C12N 1/20; A61K 35/00 (2020.01)

CPC - A61K 35/74; A61K 35/66; A23L 33/135; C12N 1/20; A61K 35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2016/0051596 A1 (FOOD INDUSTRY RESEARCH & DEVELOPMENT INSTITUTEE) 25 February 2016; abstract; paragraphs [0020], [0032]; claims 19, 23	1-3
X	US 2019/0255124 A1 (GENERAL HOSPITAL CORP) 22 August 2019; abstract; paragraphs [0006], [0010], [0037], [0053], [0162]-[0163], [0089], [0110],[0197], [0326], [0411], [0460]	1-3, 31-37
A	WO 2019/043951 A1 (KEWPIE CORP) 07 March 2019; see machine translation; entire document; especially page 5, third-fourth paragraphs; page 7, fourth paragraph	1-3, 31-37

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 January 2021 (11.01.2021)	09 FEB 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20/52251

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-30, 38-43, 46
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-3, 31-37;

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20/52251

-***-Continued From Box No. III: Observations where unity of invention is lacking-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-3, 31-37, Gluconobacter hansenii (bacterial strain) are directed toward compositions comprising at least one bacterial strain, and uses thereof.

The compositions and uses will be searched to the extent they encompass Gluconobacter hansenii (first exemplary bacterial strain). Applicant is invited to elect additional bacterial strain(s) to be searched. Additional bacterial strain(s) will be searched upon the payment of additional fees. It is believed that claims 1-3 (each in-part), and 31-37 (each in-part), , encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass Gluconobacter hansenii (bacterial strain). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be Gluconobacter oxydans (bacterial strain).

Group II, Claims 44 and 45 are directed toward a method of characterizing the ability of one or more microbial strains to modify the life span of a subject, and use of C. elegans for said method.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Groups I+ include Gluconobacter spp., not present in Group II; the special technical features of Group II include C. elegans, not present in any of Groups I+.

Groups I+ and II share the technical features including: one or more microbial strains modifying the life span of a subject, an age-associated symptom, and/or an age-associated condition.

However, these shared technical features are previously disclosed by the article 'Longevity in Mice Is Promoted by Probiotic-Induced Suppression of Colonic Senescence Dependent on Upregulation of Gut Bacterial Polyamine Production' by Matsumoto, et al. (hereinafter 'Matsumoto').

Matsumoto discloses one or more microbial strains modifying the life span of a subject (one or more microbial strains modifying the longevity (life span) of a subject; abstract).

No technical features are shared between the bacterial strains of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a composition comprising: (a) at least one bacterial strain or extracts or components thereof; and (b) an excipient; use of at least one bacterial strain or extracts or components thereof for extending the life span of a subject; and use of at least one bacterial strain or extracts or components thereof reducing or delaying the onset of at least one age-associated symptom or condition in a subject.

However, these shared technical features are previously disclosed by Matsumoto, as above.

Matsumoto discloses a composition comprising: (a) at least one bacterial strain or extracts or components thereof; and (b) an excipient (gavage comprising B. animalis in 10mM PBS (a composition comprising: at least one bacterial strain or extracts or components thereof and an excipient); page 9, first column, third paragraph); use of at least one bacterial strain or extracts or components thereof for extending the life span of a subject (use of at least one bacterial strain or extracts or components thereof for increasing longevity (extending the life span) of a subject; abstract); and use of at least one bacterial strain or extracts or components thereof reducing or delaying the onset of at least one age-associated symptom or condition in a subject (reducing age-associated low-grade inflammation in the colon of a subject by administration of B. animalis to a subject (use of at least one bacterial strain or extracts or components thereof reducing or delaying the onset of at least one age-associated symptom or condition in a subject); abstract).

Since none of the special technical features of the Groups I+ and II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Matsumoto reference, unity of invention is lacking.