COMPOSITIONS AND RELATED METHODS FOR AGRICULTURE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/450,017, filed on January 24, 2017, and U.S. Provisional Application No. 62/583,736, filed on November 9, 2017, the contents of which are hereby incorporated herein by reference in their entireties.

BACKGROUND

Certain invertebrates, such as nematodes and arthropods (e.g., insects, e.g., European honey bees (*Apis melliferia*) or silkworms (*Bombyx mori*)), are utilized in agriculture for pollination efforts and pest control as well as in commerce for the production of commercial products, such as honey or silk. To cultivate beneficial nematodes and arthropods for use in agricultural or commercial industries, there is a need in the art for ways to promote the growth and fitness of beneficial invertebrates.

SUMMARY

Disclosed herein are compositions and methods for modulating the fitness of invertebrates for agriculture or commerce. The composition includes an agent that alters a level, activity, or metabolism of one or more microorganisms resident in a host, the alteration resulting in a modulation in the host's fitness.

In one aspect, the present disclosure refers to a method for increasing fitness of a honeybee, the method comprising: administering to the honeybee a composition comprising an effective amount of organophosphorous insecticide-metabolizing bacteria formulated with an insect comestible carrier.

In one aspect, the present disclosure refers to use of an organophosphorous insecticide-metabolizing bacteria in the manufacture of a composition for increasing fitness of a honeybee, wherein the composition is formulated with an insect comestible carrier.

In one example, provided herein is a method for increasing the fitness of a honeybee, the method including administering to the honeybee a composition including an effective amount of an organophosphorus insecticide-metabolizing bacteria formulated with an insect comestible carrier.

In some examples, the administration involves delivering the composition to a honeybee hive or at least one habitat where the honeybee grows, lives, reproduces, or feeds.

In some examples, the composition may be a liquid, a solid, an aerosol, a paste, a gel, or a gas.

In some examples, the organophosphorus insecticide may be fenitrothion.

In some examples, the carrier may be a seed coating.

In some examples, the honeybee may be in a honeybee colony.

In another aspect, the present disclosure refers to a composition comprising an effective amount of organophosphorous insecticide-metabolizing bacteria formulated with an insect comestible carrier as a liquid, a solid, an aerosol, a paste, a gel, or a gas.

In one example, provided herein is a composition including an effective amount of an organophosphorus insecticide-metabolizing bacteria formulated with an insect comestible carrier as a liquid, a solid, an aerosol, a paste, a gel, or a gas.

In some examples, the organophosphorus insecticide-metabolizing bacteria metabolize fenitrothion.

In some examples, the carrier is a seed coating.

In some examples, the organophosphorus insecticide-metabolizing bacteria are at a concentration of at least 100,000 cells/ml (e.g., at least about 100,000 cells/ml, at least about 150,000 cells/ml, at least about 200,000 cells/ml, at least about 250,000 cells/ml, at least about 300,000 cells/ml, at least about 350,000 cells/ml, at least about 400,000 cells/ml, at least about 450,000 cells/ml, or at least about 500,000 cells/ml).

In yet another example, the composition includes an agent that alters a level, activity, or metabolism of one or more microorganisms resident in an insect host, the alteration resulting in an increase in the insect host's fitness.

In another instance, the composition includes an agent that alters a level, activity, or metabolism of one or more microorganisms resident in a nematode host, the alteration resulting in an increase in the nematode host's fitness.

In some examples of any of the above compositions, the one or more microorganisms may be a bacterium or fungus resident in the host. In some examples, the bacterium resident in the host is at least one selected from the group consisting of *Candidatus spp, Buchenera spp, Blattabacterium spp, Baumania spp, Wigglesworthia spp, Wolbachia spp, Rickettsia spp, Orientia spp, Sodalis spp, Burkholderia spp, Cupriavidus spp, Frankia spp, Snirhizobium spp, Streptococcus spp, Wolinella spp, Xylella spp, Erwinia spp, Agrobacterium spp, Bacillus spp, Paenibacillus spp, Streptomyces spp, Micrococcus spp, Corynebacterium spp, Acetobacter spp, Cyanobacteria spp, Salmonella spp, Rhodococcus spp, Pseudomonas spp, Lactobacillus spp, Enterococcus spp, Alcaligenes spp, Klebsiella spp, Paenibacillus spp, Arthrobacter spp, Corynebacterium spp, Brevibacterium spp, Thermus spp, Pseudomonas spp, Clostridium spp,* and *Escherichia spp.* In some examples, the fungus resident in the host is at least one selected from the group consisting of *Candida, Metschnikowia, Debaromyces, Starmerella, Pichia, Cryptococcus, Pseudozyma, Symbiotaphrina bucneri, Symbiotaphrina kochii, Scheffersomyces shehatae, Scheffersomyces stipites, Cryptococcus, Trichosporon, Amylostereum areolatum, Epichloe spp, Pichia pinus, Hansenula capsulate, Daldinia decipien, Ceratocytis spp, Ophiostoma spp,* and *Attamyces bromatificus.*

In any of the above compositions, the agent, which hereinafter may also be referred to as a modulating agent, may alter the growth, division, viability, metabolism, and/or longevity of the microorganism resident in the host. In any of the above examples, the modulating agent may decrease the viability of the one or more microorganisms resident in the host. In some examples, the modulating agent increases growth or viability of the one or more microorganisms resident in the host.

In any of the above examples, the modulating agent is a phage, a polypeptide, a small molecule, an antibiotic, a bacterium, or any combination thereof.

In some examples, the phage binds a cell surface protein on a bacterium resident in the host. In some examples, the phage is virulent to a bacterium resident in the host. In some examples, the phage is at least one selected from the group consisting of Myoviridae, Siphoviridae, Podoviridae, Lipothrixviridae, Rudiviridae, Ampullaviridae, Bicaudaviridae, Clavaviridae, Corticoviridae, Cystoviridae, Fuselloviridae, Globuloviridae, Guttaviridae, Inoviridae, Leviviridae, Microviridae, Plasmaviridae, and Tectiviridae.

In some examples, the polypeptide is at least one of a bacteriocin, R-type bacteriocin, nodule C-rich peptide, antimicrobial peptide, lysin, or bacteriocyte regulatory peptide.

In some examples, the small molecule is a metabolite.

In some examples, the antibiotic is a broad-spectrum antibiotic.

In some examples, the modulating agent is a naturally occurring bacteria. In some examples, the bacteria is at least one selected from the group consisting of *Bartonella apis,*

Parasaccharibacter apium, Frischella perrara, Snodgrassella alvi, Gilliamela apicola, Bifidobacterium spp, and *Lactobacillus spp.* In some examples, the bacterium is at least one selected from the group consisting of *Candidatus spp, Buchenera spp, Blattabacterium spp, Baumania spp, Wigglesworthia spp, Wolbachia spp, Rickettsia spp, Orientia spp, Sodalis spp, Burkholderia spp, Cupriavidus spp, Frankia spp, Snirhizobium spp, Streptococcus spp, Wolinella spp, Xylella spp, Erwinia spp, Agrobacterium spp, Bacillus spp, Paenibacillus spp, Streptomyces spp, Micrococcus spp, Corynebacterium spp, Acetobacter spp, Cyanobacteria spp, Salmonella spp, Rhodococcus spp, Pseudomonas spp, Lactobacillus spp, Enterococcus spp, Alcaligenes spp, Klebsiella spp, Paenibacillus spp, Arthrobacter spp, Corynebacterium spp, Brevibacterium spp, Thermus spp, Pseudomonas spp, Clostridium spp,* and *Escherichia spp.* In certain instances, the bacterium is a naturally occurring bacterium that is capable of degrading pesticides such as organophosphorus insecticides (e.g., phosphorothioate, e.g., fenitrothion).

In any of the above compositions, host fitness may be measured by survival, reproduction, or metabolism of the host. In some examples, the modulating agent modulates the host's fitness by decreasing pesticidal susceptibility of the host (e.g., susceptibility to a pesticide listed in Table 12). In some examples, the pesticidal susceptibility is bactericidal or fungicidal susceptibility. In some examples, the pesticidal susceptibility is insecticidal or nematicidal susceptibility.

In any of the above compositions, the composition may include a plurality of different modulating agents. In some examples, the composition includes a modulating agent and a pesticidal agent (e.g., a pesticide listed in Table 12). In some examples, the pesticidal agent is a bactericidal or fungicidal agent. In some examples, the pesticidal agent is an insecticidal or nematicidal agent.

In any of the above compositions, the composition may include a modulating agent and an agent that increases crop growth.

In any of the above compositions, modulating agent may be linked to a second moiety. In some examples, the second moiety is a modulating agent.

In any of the above compositions, the modulating agent may be linked to a targeting domain. In some examples, the targeting domain targets the modulating agent to a target site in the host. In some examples, the targeting domain targets the modulating agent to the one or more microorganisms resident in the host.

In any of the above compositions, the modulating agent may include an inactivating pre- or pro-sequence, thereby forming a precursor modulating agent. In some examples, the precursor modulating agent is converted to an active form in the host.

In any of the above compositions, the modulating agent may include a linker. In some examples, the linker is a cleavable linker.

In any of the above compositions, the composition may further include a carrier. In some instances, the carrier may be an agriculturally acceptable carrier.

In any of the above compositions, the composition may further include a host bait, a sticky agent, or a combination thereof. In some examples, the host bait is a comestible agent and/or a chemoattractant.

In any of the above compositions, the composition may be at a dose effective to modulate host fitness.

In any of the above compositions, the composition may be formulated for delivery to a microorganism inhabiting the gut of the host.

In any of the above compositions, the composition may be formulated for delivery to a microorganism inhabiting a bacteriocyte of the host and/or the gut of the host. In some examples, the composition may be formulated for delivery to a plant. In some examples, the composition may be formulated for use in a host feeding station.

In any of the above compositions, the composition may be formulated as a liquid, a powder, granules, or nanoparticles. In some examples, the composition is formulated as one selected from the group consisting of a liposome, polymer, bacteria secreting peptide, and synthetic nanocapsule. In some examples, the synthetic nanocapsule delivers the composition to a target site in the host. In some examples, the target site is the gut of the host. In some examples, the target site is a bacteriocyte in the host.

In a further example, also provided herein are hosts that include any of the above compositions. In some examples, the host is an insect. In some examples, the insect aids in pest control, pollination, generation of a commercial product, or a combination thereof. In some examples, the insect is a species belonging to *Coccinellidae, Carabidae, Mantidae, Syrphidae, Lampyridae, Myrmeliontidase, Chrysopidae, Hemerobiidae, Brachonidae, Ichneumonidae,* or *Odonata.* In some examples, the insect is a species belonging to *Andrenidae, Apidae, Colletidae, Halicitdae,* or *Megahlidae*. In some examples, the insect is a species belonging to *Bombycidae* or *Saturniidae*. In certain examples, the insect is a honey bee or silkworm.

In some examples, the host is a nematode. In some examples, the nematode is a species belonging to Heterorhabditis or Steinernema.

In yet a further example, also provided herein is a system for modulating a host's fitness comprising a modulating agent that targets a microorganism that is required for a host's fitness, wherein the system is effective to modulate the host's fitness, and wherein the host is an insect or nematode. The modulating agent may include any of the compositions described herein. In some examples, the modulating agent is formulated as a powder. In some examples, the modulating agent is formulated as a solvent. In some examples, the modulating agent is formulated as a concentrate. In some examples, the modulating agent is formulated as a diluent. In some examples, the modulating agent is prepared for delivery by combining any of the previous compositions with a carrier.

In another example, also provided herein are methods for modulating the fitness of an insect or nematode using any of the compositions described herein. In one instance, the method of modulating the fitness of an insect or nematode host includes delivering the composition of any one of the previous claims to the host, wherein the modulating agent targets the one or more microorganisms resident in the host, and thereby modulates the host's fitness. In another instance, the method of modulating microbial diversity in an insect or nematode host includes delivering the composition of any one of the previous claims to the host, wherein the modulating agent targets the one or more microorganisms resident in the host, and thereby modulates microbial diversity in the host.

In some examples of any of the above methods, the modulating agent may alter the levels of the one or more microorganisms resident in the host. In some examples of any of the above

methods, the modulating agent may alter the function of the one or more microorganisms resident in the host. In some examples, the one or more microorganisms may be a bacterium and/or fungus. In some examples, the one or more microorganisms are required for host fitness. In some examples, the one or more microorganisms are required for host survival.

In some examples of any of the above methods, the delivering step may include providing the modulating agent at a dose and time sufficient to effect the one or more microorganisms, thereby modulating microbial diversity in the host. In some examples, the delivering step includes topical application of any of the previous compositions to a plant. In some examples, the delivering step includes providing the modulating agent through a genetically engineered plant. In some examples, the delivering step includes providing the modulating agent to the host as a comestible. In some examples, the delivering step includes providing a host carrying the modulating agent. In some examples the host carrying the modulating agent can transmit the modulating agent to one or more additional hosts.

In some examples of any of the above methods, the composition is effective to increase health and/or survival of the host. In some examples, the composition is effective to increase host fitness, increase host lifespan, increase effective pollination, increase generation of a host product, increase host reproduction, or a combination thereof. In some examples, the composition is effective to decrease the host's sensitivity to a pesticidal agent (e.g., a pesticide listed in Table 12). In certain examples, the pesticidal agent is a neonicotinoid (e.g., imidacloprid). In certain examples, the pesticidal agent is an organophosphorus insecticide (e.g., a phosphorothioate, e.g., fenitrothion). In some examples, the composition is effective to increase the host's resistance to an allelochemical agent produced by a plant. In some examples, the allelochemical agent is toxic to the host prior to delivery of the composition. In some examples, the allelochemical agent is caffeine, soyacystatin N, monoterpenes, diterpene acids, or phenolic compounds.

In some examples of any of the above methods, the host is an insect. In some examples, the insect aids in pest control, pollination, generation of a commercial product, waste degradation, or a combination thereof. In some examples, the insect is a species belonging to *Coccinellidae, Carabidae, Mantidae, Syrphidae, Lampyridae, Myrmeliontidase, Chrysopidae, Hemerobiidae, Brachonidae, Ichneumonidae,* or *Odonata.* In some examples, the insect is a species belonging to *Andrenidae, Apidae, Colletidae, Halicitdae,* or *Megahlidae.* In some examples, the insect is a species belonging to *Bombycidae* or *Saturniidae*. In certain examples, the insect is a honey bee or silkworm.

In some examples, the host is a nematode. In some examples, the nematode is a species belonging to *Heterorhabditis* or *Steinernema*.

In some examples of any of the above methods, the delivering step includes delivering any of the previous compositions to a plant. In some examples, the plant is an agricultural crop. In some examples, the crop is an unharvested crop at the time of delivery. In some examples, the crop is a harvested crop at the time of delivery. The some examples, the crop comprises harvested fruits or vegetables. In some examples, the composition is delivered in an amount and for a duration effective to increase growth of the crop. In some examples, the crop includes corn, soybean, or wheat plants.

In another example, also provided herein are screening assays to identify modulating agent that modulate the fitness of a host. In one instance, the screening assay to identify a modulating agent that modulates the fitness of a host, includes the steps of (a) exposing a microorganism that can be resident in the host to one or more candidate modulating agents and (b) identifying a modulating agent that increases the fitness of the host.

In some examples of the screening assay, the modulating agent is a microorganism resident in the host. In some examples, the microorganism is a bacterium. In some examples, the bacterium, when resident in the host, increases host fitness. In some examples, the bacterium degrades a pesticide (e.g., a pesticide listed in Table 12). In certain examples, the pesticide is a neonicotinoid (e.g., imidacloprid) or an organophosphorus insecticide (e.g., a phosphorothioate, e.g., fenitrothion). In some examples, the bacterium secretes an amino acid. In certain examples, wherein the amino acid is methionine.

In some examples of the screening assay, the modulating agent affects an allelochemical-degrading microorganism. In some examples, the modulating agent is a phage, an antibiotic, or a test compound. In certain examples, the antibiotic is timentin or azithromycin.

In some examples of the screening assay, the host may be an invertebrate. In some examples, the invertebrate is an insect or a nematode. In certain examples, the insect is a honey bee. In other particular examples, the insect is a silkworm.

In any of the above examples of the screening assay, host fitness may be modulated by altering the host microbiota.

Definitions

As used herein, the term “bacteriocin” refers to a peptide or polypeptide that possesses anti-microbial properties. Naturally occurring bacteriocins are produced by certain prokaryotes and act against organisms related to the producer strain, but not against the producer strain itself. Bacteriocins contemplated herein include, but are not limited to, naturally occurring bacteriocins, such as bacteriocins produced by bacteria, or derivatives thereof, such as engineered bacteriocins, recombinantly expressed bacteriocins, or chemically synthesized bacteriocins. In some instances, the bacteriocin is a functionally active variant of the bacteriocins described herein. In some instances, the variant of the bacteriocin has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity, e.g., over a specified region or over the entire sequence, to a sequence of a bacteriocin described herein or a naturally occurring bacteriocin.

As used herein, the term “bacteriocyte” refers to a specialized cell found in certain insects where intracellular bacteria reside with symbiotic bacterial properties.

As used herein, the term “effective amount” refers to an amount of a modulating agent (e.g., a phage, lysin, bacteriocin, small molecule, or antibiotic) or composition including said agent sufficient to effect the recited result, e.g., to increase or promote the fitness of a host organism (e.g., insect); to reach a target level (e.g., a predetermined or threshold level) of a modulating agent concentration inside a target host; to reach a target level (e.g., a predetermined or threshold level) of a modulating agent

CLAIMS

1. A method for increasing fitness of a honeybee, the method comprising:
administering to the honeybee a composition comprising an effective amount of organophosphorous insecticide-metabolizing bacteria formulated with an insect comestible carrier.

2. The method of claim 1, wherein the administration comprises delivering the composition to a honeybee hive or at least one habitat where the honeybee grows, lives, reproduces, or feeds.

3. The method of any one of claims 1-2, wherein the composition is a liquid, a solid, an aerosol, a paste, a gel, or a gas.

4. The method of any one of claims 1-3, wherein the organophosphorous insecticide is fenitrothion.

5. The method of any one of claims 1-4, wherein the carrier is a seed coating.

6. The method of any one of claims 1-5, wherein the honeybee is in a honeybee colony.

7. A composition comprising an effective amount of organophosphorous insecticide-metabolizing bacteria formulated with an insect comestible carrier as a liquid, a solid, an aerosol, a paste, a gel, or a gas.

8. The composition of claim 7, wherein the organophosphorous insecticide-metabolizing bacteria metabolize fenitrothion.

9. The composition of any one of claims 7-8, wherein the carrier is a seed coating.

10. The composition of any one of claims 7-9, wherein the organophosphorous insecticide-metabolizing bacteria are at a concentration of at least 100,000 cells/ml.

11. Use of an organophosphorous insecticide-metabolizing bacteria in the manufacture of a composition for increasing fitness of a honeybee, wherein the composition is formulated with an insect comestible carrier.

12. The use of claim 11, wherein the composition is to be delivered to a honeybee hive or at least one habitat where the honeybee grows, lives, reproduces, or feeds.

13. The use of any one of claims 11-12, wherein the composition is a liquid, a solid, an aerosol, a paste, a gel, or a gas.

14. The use of any one of claims 11-13, wherein the organophosphorous insecticide is fenitrothion.

15. The use of any one of claims 11-14, wherein the carrier is a seed coating.

16. The use of any one of claims 11-15, wherein the honeybee is in a honeybee colony.