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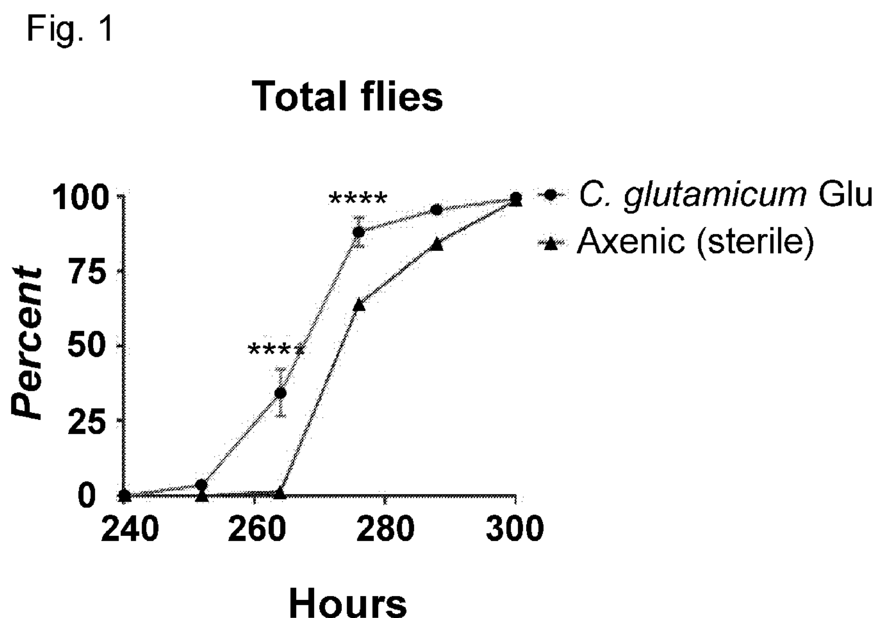
Fig. 1



(57) Abstract: Provided herein are agents, compositions, and methods for agricultural use, e.g., for altering the level, activity, or metabolism of one or more microorganisms resident in a host nematode or arthropod (e.g., honeybee or silkworm), the alteration resulting in an increase in the fitness of the host. The invention features a composition that includes an agent (e.g., phage, peptide, small molecule, antibiotic, or combinations thereof) that can alter the host's microbiota in a manner that is beneficial to the host. By promoting favorable microbial levels, microbial activity, microbial metabolism, and/or microbial diversity, the agents described herein may be used to increase the fitness of a variety of beneficial nematodes or arthropods, such as bees and silkworms, utilized in agriculture and commerce.

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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 5 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 10 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.

15 ## SUMMARY OF THE INVENTION

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.

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

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

In some embodiments, the organophosphorus insecticide may be fenitrothion.

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

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

30 In another aspect, 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 embodiments of the second aspect, the organophosphorus insecticide-metabolizing bacteria metabolize fenitrothion.

35 In some embodiments of the second aspect, the carrier is a seed coating.

In some embodiments of the second aspect, 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 40 cells/ml, or at least about 500,000 cells/ml).

In yet another aspect, 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 embodiments of any of the above compositions, the one or more microorganisms may be a bacterium or fungus resident in the host. In some embodiments, 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 embodiments, 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 embodiments, the modulating agent may decrease the viability of the one or more microorganisms resident in the host. In some embodiments, the modulating agent increases growth or viability of the one or more microorganisms resident in the host.

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

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

In some embodiments, 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 embodiments, the small molecule is a metabolite.

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

In some embodiments, the modulating agent is a naturally occurring bacteria. In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the pesticidal susceptibility is bactericidal or fungicidal susceptibility. In some embodiments, 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 embodiments, the composition includes a modulating agent and a pesticidal agent (e.g., a pesticide listed in Table 12). In some embodiments, the pesticidal agent is a bactericidal or fungicidal agent. In some embodiments, 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 embodiments, 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 embodiments, the targeting domain targets the modulating agent to a target site in the host. In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the composition may be formulated for delivery to a plant. In some embodiments, 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 embodiments, the composition is formulated as one selected from the group consisting of a liposome, polymer, bacteria secreting peptide, and synthetic nanocapsule. In some embodiments, the synthetic nanocapsule delivers the composition to a target site in the host. In some embodiments, the target site is the gut of the host. In some embodiments, the target site is a bacteriocyte in the host.

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

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

In yet a further aspect, 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 embodiments, the modulating agent is formulated as a powder. In some embodiments, the modulating agent is formulated as a solvent. In some embodiments, the modulating agent is formulated as a concentrate. In some embodiments, the modulating agent is formulated as a diluent. In some embodiments, the modulating agent is prepared for delivery by combining any of the previous compositions with a carrier.

In another aspect, 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 embodiments 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 embodiments 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 embodiments, the one or more microorganisms may be a bacterium and/or fungus. In some embodiments, the one or more microorganisms are required for host fitness. In some embodiments, the one or more microorganisms are required for host survival.

5 In some embodiments 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 embodiments, the delivering step includes topical application of any of the previous compositions to a plant. In some embodiments, the delivering step includes providing the modulating agent through a genetically engineered plant. In some embodiments, 10 the delivering step includes providing the modulating agent to the host as a comestible. In some embodiments, the delivering step includes providing a host carrying the modulating agent. In some embodiments the host carrying the modulating agent can transmit the modulating agent to one or more additional hosts.

In some embodiments of any of the above methods, the composition is effective to increase 15 health and/or survival of the host. In some embodiments, 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 embodiments, the composition is effective to decrease the host's sensitivity to a pesticidal agent (e.g., a pesticide listed in Table 12). In certain embodiments, the pesticidal agent is a neonicotinoid (e.g., imidacloprid). In certain embodiments, the 20 pesticidal agent is an organophosphorus insecticide (e.g., a phosphorothioate, e.g., fenitrothion). In some embodiments, the composition is effective to increase the host's resistance to an allelochemical agent produced by a plant. In some embodiments, the allelochemical agent is toxic to the host prior to delivery of the composition. In some embodiments, the allelochemical agent is caffeine, soyacystatin N, monoterpenes, diterpene acids, or phenolic compounds.

25 In some embodiments of any of the above methods, the host is an insect. In some embodiments, the insect aids in pest control, pollination, generation of a commercial product, waste degradation, or a combination thereof. In some embodiments, the insect is a species belonging to *Coccinellidae, Carabidae, Mantidae, Syrphidae, Lampyridae, Myrmeliontidase, Chrysopidae, Hemerobiidae, Brachonidae, Ichneumonidae,* or *Odonata.* In some embodiments, the insect is a species belonging to 30 *Andrenidae, Apidae, Colletidae, Halicitdae,* or *Megahlidae.* In some embodiments, the insect is a species belonging to *Bombycidae* or *Saturniidae*. In certain embodiments, the insect is a honey bee or silkworm.

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

In some embodiments of any of the above methods, the delivering step includes delivering any of 35 the previous compositions to a plant. In some embodiments, the plant is an agricultural crop. In some embodiments, the crop is an unharvested crop at the time of delivery. In some embodiments, the crop is a harvested crop at the time of delivery. The some embodiments, the crop comprises harvested fruits or vegetables. In some embodiments, the composition is delivered in an amount and for a duration effective to increase growth of the crop. In some embodiments, the crop includes corn, soybean, or wheat plants.

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

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

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

In any of the above embodiments 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

concentration inside a target host gut; to reach a target level (e.g., a predetermined or threshold level) of a modulating agent concentration inside a target host bacteriocyte; to modulate the level, or an activity, of one or more microorganism (e.g., endosymbiont) in the target host.

As used herein, the term “fitness” refers to the ability of a host organism to survive, grow, and/or to produce surviving offspring. Fitness of an organism may be measured by one or more parameters, including, but not limited to, life span, reproductive rate, mobility, body weight, and/or metabolic rate. Fitness may additionally be measured based on measures of activity (e.g., pollination) or product output (e.g., honey or silk).

As used herein, the term “gut” refers to any portion of a host’s gut, including, the foregut, midgut, or hindgut of the host.

As used herein, the term “host” refers to an organism (e.g., insect) carrying resident microorganisms (e.g., endogenous microorganisms, endosymbiotic microorganisms (e.g., primary or secondary endosymbionts), commensal organisms, and/or pathogenic microorganisms).

As used herein “increasing host fitness” or “promoting host fitness” refers to any favorable alteration in host physiology, or any activity carried out by said host, as a consequence of administration of a modulating agent, including, but not limited to, any one or more of the following desired effects: (1) increasing a population of a host by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 100% or more; (2) increasing the reproductive rate of a host (e.g., insect, e.g., bee or silkworm) by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 100% or more; (3) increasing the mobility of a host (e.g., insect, e.g., bee or silkworm) by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 100% or more; (4) increasing the body weight of a host (e.g., insect, e.g., bee or silkworm) by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 100% or more; (5) increasing the metabolic rate or activity of a host (e.g., insect, e.g., bee or silkworm) by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 100% or more; (6) increasing pollination (e.g., number of plants pollinated in a given amount of time) by a host (e.g., insect, e.g., bee or silkworm) by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 100% or more; (7) increasing production of host (e.g., insect, e.g., bee or silkworm) byproducts (e.g., honey from a honeybee or silk from a silkworm) by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 100% or more; (8) increasing nutrient content of the host (e.g., insect) (e.g., protein, fatty acids, or amino acids) by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 100% or more; or (9) increasing host resistance to pesticides (e.g., a neonicotinoid (e.g., imidacloprid) or an organophosphorus insecticide (e.g., a phosphorothioate, e.g., fenitrothion)) by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 100% or more. An increase in host fitness can be determined in comparison to a host organism to which the modulating agent has not been administered.

The term “insect” includes any organism belonging to the phylum *Arthropoda* and to the class *Insecta* or the class *Arachnida*, in any stage of development, i.e., immature or adult insects.

As used herein, “lysin” also known as endolysin, autolysin, murein hydrolase, peptidoglycan hydrolase, or cell wall hydrolase refers to a hydrolytic enzyme that can lyse a bacterium by cleaving peptidoglycan in the cell wall of the bacterium. Lysins contemplated herein include, but are not limited to, naturally occurring lysins, such as lysins produced by phages, lysins produced by bacteria, or derivatives

thereof, such as engineered lysins, recombinantly expressed lysins, or chemically synthesized lysins. A functionally active variant of the bacteriocin may have 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 synthetic, recombinant, or naturally derived bacteriocin, including any described herein.

As used herein, the term "microorganism" refers to bacteria or fungi. Microorganisms may refer to microorganisms resident in a host organism (e.g., endogenous microorganisms, endosymbiotic microorganisms (e.g., primary or secondary endosymbionts)) or microorganisms exogenous to the host, including those that may act as modulating agents. As used herein, the term "target microorganism" refers to a microorganism that is resident in the host and impacted by a modulating agent, either directly or indirectly.

As used herein, the term "agent" or "modulating agent" refers to an agent that is capable of altering the levels and/or functioning of microorganisms resident in a host organism (e.g., insect), and thereby modulate (e.g., increase) the fitness of the host organism (e.g., insect).

As used herein, the term "pesticide" or "pesticidal agent" refers to a substance that can be used in the control of agricultural, environmental, or domestic/household pests, such as insects, fungi, bacteria, or viruses. The term "pesticide" is understood to encompass naturally occurring or synthetic insecticides (larvicides or adulticides), insect growth regulators, acaricides (miticides), nematicides, ectoparasiticides, bactericides, fungicides, or herbicides (substance which can be used in agriculture to control or modify plant growth). Further examples of pesticides or pesticidal agents are listed in Table 12. In some instances, the pesticide is an allelochemical. As used herein, "allelochemical" or "allelochemical agent" is a substance produced by an organism that can effect a physiological function (e.g., the germination, growth, survival, or reproduction) of another organism (e.g., a host insect or nematode).

As used herein, the term "peptide," "protein," or "polypeptide" encompasses any chain of naturally or non-naturally occurring amino acids (either D- or L-amino acids), regardless of length (e.g., at least 2, 3, 4, 5, 6, 7, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, 100, or more amino acids), the presence or absence of post-translational modifications (e.g., glycosylation or phosphorylation), or the presence of, e.g., one or more non-amino acyl groups (for example, sugar, lipid, etc.) covalently linked to the peptide, and includes, for example, natural proteins, synthetic, or recombinant polypeptides and peptides, hybrid molecules, peptoids, or peptidomimetics.

As used herein, "percent identity" between two sequences is determined by the BLAST 2.0 algorithm, which is described in Altschul et al. (*J. Mol. Biol.* 215:403-410, 1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

As used herein, the term "phage" or "bacteriophage" refers to a virus that infects and replicates in bacteria. Bacteriophages replicate within bacteria following the injection of their genome into the cytoplasm and do so using either a lytic cycle, which results in bacterial cell lysis, or a lysogenic (non-lytic) cycle, which leaves the bacterial cell intact. The phage may be a naturally occurring phage isolate, or an engineered phage, including vectors, or nucleic acids that encode either a partial phage genome (e.g., including at least all essential genes necessary to carry out the life cycle of the phage inside a host bacterium) or the full phage genome.

As used herein, the term "plant" refers to whole plants, plant organs, plant tissues, seeds, plant cells, seeds, and progeny of the same. Plant cells include, without limitation, cells from seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, or microspores. Plant parts include differentiated or undifferentiated tissues including, but not limited to the following: roots, stems, shoots, leaves, pollen, seeds, tumor tissue, and various forms of cells and culture (e.g., single cells, protoplasts, embryos, or callus tissue). The plant tissue may be in a plant or in a plant organ, tissue, or cell culture. In addition, a plant may be genetically engineered to produce a heterologous protein or RNA, for example, of any of the modulating agents in the methods or compositions described herein.

The terms "obtainable by", "producible by" or the like are used to indicate that a claim or embodiment refers to compound, composition, product, etc. per se, i. e. that the compound, composition, product, etc. can be obtained or produced by a method which is described for manufacture of the compound, composition, product, etc., but that the compound, composition, product, etc. may be obtained or produced by other methods than the described one as well. The terms "obtained by," "produced by," or the like indicate that the compound, composition, product, is obtained or produced by a recited specific method. It is to be understood that the terms "obtainable by," "producible by" and the like also disclose the terms "obtained by", "produced by" and the like as a preferred embodiment of "obtainable by", "producible by" and the like.

Other features and advantages of the invention will be apparent from the following Detailed Description and the Claims.

BRIEF DESCRIPTION OF THE FIGURES

The figures are meant to be illustrative of one or more features, aspects, or embodiments of the invention and are not intended to be limiting.

Fig. 1 is a graph showing the time to reach adulthood from embryos in *Drosophila melanogaster*. Embryos of *Drosophila melanogaster* were either raised on diet seeded with *Corynebacterium glutamicum* (a strain that produces glutamate – *C. glutamicum* Glu) or on axenic diet without any bacteria. The percentage of adults emerging from their pupa was measured every 12 hours from the time of the emergence of the first adult. The organisms raised on bacteria supplemented diet reach adulthood faster than their bacteria free counterparts.

Fig. 2A is a graph showing the effects of male gender on the developmental rate differences in *Drosophila melanogaster*. The adults emerging from Fig. 1 were sexed and their rate of emergence was plotted.

Fig. 2B is a graph showing the effects of female gender on the developmental rate differences in *Drosophila melanogaster*. The adults emerging from Fig. 1 were sexed and their rate of emergence was plotted. The enhancement in the rate of development in the females due to the presence of bacteria in the diet is significantly more than in their male counterparts. The benefits of the presence of bacteria in the fly diet are higher in the females compared to the males.

Fig. 3 is a graph showing *C. glutamicum* strains promoted larval biomass. Larvae raised on diet supplemented with *C. glutamicum* strains either producing glutamate or methionine are bigger than those

raised on sterile diet or diet supplemented with *Escherichia coli*. The areas of the larvae are measured as the number of pixels in the images of the larvae. The medians and the 95% confidence intervals are shown as lines on the graph.

Fig. 4 is a panel of graphs showing the results of a Seahorse flux assay for bacterial respiration. Bacteria were grown to logarithmic phase and loaded into Seahorse XFe96 plates for temporal measurements of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) as described in methods. Treatments were injected into the wells after approximately 20 minutes and bacteria were monitored to detect changes in growth. Rifampicin = 100 µg/mL; Chloramphenicol = 25 µg/mL; Phages (T7 for *E. coli* and ΦSmVL-C1 for *Serratia marcescens*) were lysates diluted either 1:2 or 1:100 in SM Buffer. The markers on each line are solely provided as indicators of the condition to which each line corresponds, and are not indicative of data points

Fig. 5 is a graph showing phage against *S. marcescens* reduced fly mortality. Flies that were pricked with *S. marcescens* were all dead within a day, whereas a sizeable portion of the flies that were pricked with both *S. marcescens* and the phage survived for five days after the treatment. Almost all of the control flies which were not treated in anyway survived till the end of the experiment. Log-rank test was used to compare the curves for statistical significance, asterisk denotes $p<0.0001$.

DETAILED DESCRIPTION

Provided herein are methods and compositions for agricultural use, e.g., for altering a level, activity, or metabolism of one or more microorganisms resident in a host nematode or arthropod (e.g., honeybee or silkworm), the alteration resulting in an increase in the fitness of the host. The invention features a composition that includes a modulating agent (e.g., phage, peptide, small molecule, antibiotic, or combinations thereof) that can alter the host's microbiota in a manner that is beneficial to the host. By promoting favorable microbial levels, microbial activity, microbial metabolism, and/or microbial diversity, the modulating agent described herein may be used to increase the fitness of a variety of beneficial nematodes or arthropods, such as bees and silkworms, utilized in agriculture and commerce.

The methods and compositions described herein are based in part on the examples which illustrate how different agents, for example imidacloprid-degrading microorganisms, fenitrothion-degrading microorganisms, and different phages can be used in insect hosts such as honeybees or *Drosophila* to indirectly improve the health of these hosts by altering the level, activity or metabolism of microorganisms within these hosts. Imidacloprid-degrading microorganisms are a representative example of neonicotinoid-degrading microorganisms and more generally are representative of insecticide- or pesticide-degrading microorganisms. Similarly, fenitrothion-degrading microorganisms are a representative example of organophosphorus insecticide-degrading microorganisms and more generally are representative of insecticide- or pesticide-degrading microorganisms. On this basis the present disclosure describes a variety of different approaches to the use of agents that alter 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.

I. Hosts

i. Hosts

The host of any of the compositions or methods described herein may be any organism belonging to the phyla Nematoda (e.g., nematodes, e.g., beneficial nematodes) or Arthropoda (e.g., insects, e.g., beneficial insects), including any arthropods described herein. In some instances, the host is a beneficial insect or nematode (e.g., a pollinator, a natural competitor of a pest, or a producer of useful substances for humans). The term “beneficial insect” or “beneficial nematode,” as used herein, refers to an insect or nematode that confers a benefit (e.g., economical and/or ecological) to humans, animals, an ecosystem, and/or the environment. For example, the host may be an insect that is involved in the production of a commercial product, including, but not limited to, insects cultivated to produce food (e.g., honey from honey bees, e.g., *Apis mellifera*), materials (such as silk from *Bombyx mori*), and/or substances (e.g., lac from *Laccifer lacca* or pigments from *Dactylopius coccus* and *Cynipidae*). Additionally, the host may include insects or nematodes that are used in agricultural applications, including insects that aid in the pollination of crops, spreading seeds, or pest control. Further, in some instances, the host may be an insect that is useful for waste disposal and/or organic recycling (e.g., earthworms, termites, or *Diptera* larvae).

In some instances, the host produces a useable product (e.g., honey, silk, beeswax, or shellac). In some instances, the host is a bee. Exemplary bee genera include, but are not limited to *Apis, Bombus, Trigona,* and *Osmia*. In some instances, the bee is a honeybee (e.g., an insect belonging to the genus *Apis*). In some instances, the honeybee is the species *Apis mellifera* (the European or Western honey bee), *Apis cerana* (the Asiatic, Eastern, or Himalayan honey bee), *Apis dorsata* (the “giant” honey bee), *Apis florea* (the “red dwarf” honey bee), *Apis andreniformis* (the “black dwarf” honey bee), or *Apis nigrocincta*. In some instances, the host is a silkworm. The silkworm may be a species in the family *Bombycidae* or *Saturniidae*. In some instances, the silkworm is *Bombyx mori*. In some instances, the host is a lac bug. The lac bug may be a species in the family *Kerriidae*. In some instances, the lac bug is *Kerria lacca*.

In some instances, the host aids in pollination of a plant (e.g., bees, beetles, wasps, flies, butterflies, or moths). In some examples, the host aiding in pollination of a plant is beetle. In some instances, the beetle is a species in the family *Buprestidae, Cantharidae, Cerambycidae, Chrysomelidae, Cleridae, Coccinellidae, Elateridae, Melandryidae, Meloidae, Melyridae, Mordellidae, Nitidulidae, Oedemeridae*, *Scarabaeidae,* or *Staphyllinidae*. In some instances, the host aiding in pollination of a plant is a butterfly or moth (e.g., *Lepidoptera*). In some instances, the butterfly or moth is a species in the family *Geometridae, Hesperiidae, Lycaenidae, Noctuidae, Nymphalidae, Papilionidae, Pieridae,* or *Sphingidae*. In some instances, the host aiding in pollination of a plant is a fly (e.g., *Diptera*). In some instances, the fly is in the family *Anthomyiidae*, *Bibionidae, Bombyliidae, Calliphoridae, Cecidomiidae, Certopogonidae, Chrionomidae, Conopidae, Culicidae, Dolichopodidae, Empididae, Ephydridae, Lonchopteridae, Muscidae, Mycetophilidae, Phoridae, Simuliidae, Stratiomyidae,* or *Syrphidae.* In some instances, the host aiding in pollination is an ant (e.g., *Formicidae*), sawfly (e.g., *Tenthredinidae*), or wasp (e.g., *Sphecidae* or *Vespidae*). In some instances, the host aiding in pollination of a plant is a bee. In some instances, the bee is in the family *Andrenidae, Apidae, Colletidae, Halictidae,* or *Megachilidae.*

In some instances, the host aids in pest control. In some instances, the host aiding in pest control is a predatory nematode. In particular examples, the nematode is a species of *Heterorhabditis* or *Steinernema*. In some instances, the host aiding in pest control is an insect. For example, the host aiding in pest control may be a species belonging to the family *Braconidae* (e.g., parasitoid wasps), *Carabidae* (e.g., ground beetles), *Chrysopidae* (e.g., green lacewings), *Coccinellidae* (e.g., ladybugs), *Hemerobiidae* (e.g., brown lacewings), *Ichneumonidae* (e.g., ichneumon wasps), *Lampyridae* (e.g., fireflies), *Mantidae* (e.g., praying mantises), *Myrmeleontidae* (e.g., antilions), *Odonata* (e.g., dragonflies and damselflies), or *Syrphidae* (e.g., hoverfly). In other instances, the host aiding in pest control is an insect that competes with an insect that is considered a pest (e.g., an agricultural pest). For example, the Mediterranean fruit fly, *Ceratitis capitata* is a common pest of fruits and vegetables worldwide. One way to control *C. captitata* is to release the sterilized male insect into the environment to compete with wild males to mate the females. In these instances, the host may be a sterilized male belonging to a species that is typically considered a pest.

In some instances, the host aids in degradation of waste or organic material. In some examples, the host aiding in degradation of waste or organic material belongs to *Coleoptera* or *Diptera*. In some instances, the host belonging to *Diptera* is in the family *Calliphoridae, Curtonotidae*, *Drosophilidae, Fanniidae, Heleomyzidae, Milichiidae, Muscidae, Phoridae, Psychodidae, Scatopsidae, Sepsidae, Sphaeroceridae, Stratiomyidae, Syrphidae, Tephritidae,* or *Ulidiidae*. In some instances, the host belonging to *Coleoptera* is in the family *Carabidae, Hydrophilidae, Phalacaridae*, *Ptiliidae*, or *Staphylinidae*.

In particular instances, the modulating agents disclosed herein may be used to increase the fitness of honeybee or silkworm hosts.

ii. *Host Fitness*

The methods and compositions provided herein may be used to increase the fitness of any of the hosts described herein. The increase in fitness may arise from any alterations in microorganisms resident in the host, wherein the alterations are a consequence of administration of a modulating agent and have beneficial or advantageous effects on the host.

In some instances, the increase in host fitness may manifest as an improvement in the physiology of the host (e.g., improved health or survival) as a consequence of administration of a modulating agent. In some instances, the fitness of an organism may be measured by one or more parameters, including, but not limited to, reproductive rate, lifespan, mobility, fecundity, body weight, metabolic rate or activity, or survival in comparison to a host organism to which the modulating agent has not been administered. For example, the methods or compositions provided herein may be effective to improve the overall health of the host or to improve the overall survival of the host in comparison to a host organism to which the modulating agent has not been administered. In some instances, the improved survival of the host is about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% greater relative to a reference level (e.g., a level found in a host that does not receive a modulating agent). In some instances, the methods and compositions are effective to increase host reproduction (e.g., reproductive rate) in comparison to a host organism to which the modulating agent has

not been administered. In some instances, the methods and compositions are effective to increase other physiological parameters, such as mobility, body weight, life span, fecundity, or metabolic rate, by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a host that does not receive a modulating agent).

5 In some instances, the increase in host fitness may manifest as an increased production of a product generated by said host in comparison to a host organism to which the modulating agent has not been administered. In some instances, the methods or compositions provided herein may be effective to increase the production of a product generated by the host, as described herein (e.g., honey, beeswax, beebread, propolis, silk, or lac), by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 10 100%, or greater than 100% relative to a reference level (e.g., a level found in a host that does not receive a modulating agent).

In some instances, the increase in host fitness may manifest as an increase in the frequency or efficacy of a desired activity carried out by the host (e.g., pollination, predation on pests, seed spreading, or breakdown of waste or organic material) in comparison to a host organism to which the modulating 15 agent has not been administered. In some instances, the methods or compositions provided herein may be effective to increase the frequency or efficacy of a desired activity carried out by the host (e.g., pollination, predation on pests, seed spreading, or breakdown of waste or organic material) by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a host that does not receive a modulating agent).

20 In some instances, the increase in host fitness may manifest as an increase in the production of one or more nutrients in the host (e.g., vitamins, carbohydrates, amino acids, or polypeptides) in comparison to a host organism to which the modulating agent has not been administered. In some instances, the methods or compositions provided herein may be effective to increase the production of nutrients in the host (e.g., vitamins, carbohydrates, amino acids, or polypeptides) by about 2%, 5%, 10%, 25 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a host that does not receive a modulating agent). In some instances, the methods or compositions provided herein may increase nutrients in the host by increasing the production of nutrients by one or more microorganisms (e.g., endosymbiont) in the host.

In some instances, the increase in host fitness may manifest as a decrease in the host's 30 sensitivity to a pesticidal agent (e.g., a pesticide listed in Table 12) and/or an increase in the host's resistance to a pesticidal agent (e.g., a pesticide listed in Table 12) in comparison to a host organism to which the modulating agent has not been administered. In some instances, the methods or compositions provided herein may be effective to decrease the host's sensitivity to a pesticidal agent (e.g., a pesticide listed in Table 12) by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater 35 than 100% relative to a reference level (e.g., a level found in a host that does not receive a modulating agent). In some instances, the host's sensitivity to the pesticidal agent is altered by administering a modulating agent that degrades a pesticidal agent (e.g., a pesticidal-degrading bacteria, e.g., a neonicotinoid-degrading bacteria or an organophosphorus insecticide-degrading bacteria). The pesticidal agent may be any pesticidal agent known in the art, including insecticidal agents. In some instances, the 40 pesticidal agent is a neonicotinoid (e.g., imidacloprid) or an organophosphorus insecticide (e.g., a

phosphorothioate, e.g., fenitrothion). In some instances, the methods or compositions provided herein may decrease the host's sensitivity to a pesticidal agent (e.g., a pesticide listed in Table 12) by increasing the host's ability to metabolize or degrade the pesticidal agent into usable substrates.

In some instances, the host's sensitivity to the pesticidal agent is altered by administering a modulating agent that detoxifies a xenobiotic.

In some instances, the increase in host fitness may manifest as a decrease in the host's sensitivity to an allelochemical agent and/or an increase in the host's resistance to an allelochemical agent in comparison to a host organism to which the modulating agent has not been administered. In some instances, the methods or compositions provided herein may be effective to increase the host's resistance to an allelochemical agent by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a host that does not receive a modulating agent). In some instances, the allelochemical agent is caffeine, soyacystatin N, monoterpenes, diterpene acids, or phenolic compounds. In some instances, the methods or compositions provided herein may decrease the host's sensitivity to an allelochemical agent by increasing the host's ability to metabolize or degrade the allelochemical agent into usable substrates.

In some instances, the methods or compositions provided herein may be effective to increase the host's resistance to parasites or pathogens (e.g., fungal, bacterial, or viral pathogens; or parasitic mites (e.g., *Varroa destructor* mite in honeybees)) in comparison to a host organism to which the modulating agent has not been administered. In some instances, the methods or compositions provided herein may be effective to increase the host's resistance to a pathogen or parasite (e.g., fungal, bacterial, or viral pathogens; or parasitic mites (e.g., *Varroa destructor* mite in honeybees)) by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or greater than 100% relative to a reference level (e.g., a level found in a host that does not receive a modulating agent).

In some instances, the increase in host fitness may manifest as other fitness advantages, such as improved tolerance to certain environmental factors (e.g., a high or low temperature tolerance), improved ability to survive in certain habitats, or an improved ability to sustain a certain diet (e.g., an improved ability to metabolize soy vs corn) in comparison to a host organism to which the modulating agent has not been administered. In some instances, the methods or compositions provided herein may be effective to increase host fitness in any plurality of ways described herein. Further, the modulating agent may increase host fitness in any number of host classes, orders, families, genera, or species (e.g., 1 host species, 2, 3, 4, 5, 6, 7, 8, 9 ,10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 200, 250, 500, or more host species). In some instances, the modulating agent acts on a single host class, order, family, genus, or species.

Host fitness may be evaluated using any standard methods in the art. In some instances, host fitness may be evaluated by assessing an individual host. Alternatively, host fitness may be evaluated by assessing a host population. For example, an increase in host fitness may manifest as an increase in successful competition against other insects, thereby leading to an increase in the size of the host population.

iii. Host invertebrates in agriculture

By increasing the fitness of beneficial nematodes or insects, the modulating agents provided herein may be effective to promote the growth of plants that benefit from said hosts. The modulating agent may be delivered using any formulations and delivery methods described herein, in an amount and for a duration effective to increase the fitness of the hosts of interest and thereby benefit the plant (e.g., increase crop growth, increase crop yield, decrease pest infestation, and/or decrease damage to plants). This may or may not involve direct application of the modulating agent to the plant. For example, in instances where the primary host habitat is different than the region of plant growth, the modulating agent may be applied to either the primary host habitat, the plants of interest, or a combination of both.

In some instances, the plant may be an agricultural crop, such as a cereal, grain, legume, fruit, or vegetable crop. The compositions described herein may be delivered to the crop any time prior to or after harvesting the cereal, grain, legume, fruit, or vegetable. Crop yield is a measurement often used for, e.g., a cereal, grain, or legume and is normally measured in metric tons per hectare (or kilograms per hectare). Crop yield can also refer to the actual seed generation from the plant. In some instances, the modulating agent may be effective to increase crop yield (e.g., increase metric tons of cereal, grain, legume, fruit, or vegetable per hectare and/or increase seed generation) by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more in comparison to a reference level (e.g., a crop to which the modulating agent has not been administered.

In some instances, the plant (e.g., crop) may be at risk of developing a pest infestation (e.g., by an insect) or may have already developed a pest infestation. The methods and compositions described herein may be used to reduce or prevent pest infestation in such crops by promoting the fitness of beneficial insects that prey on agricultural pests. In some instances, the modulating agent may be effective to reduce crop infestation (e.g., reduce the number of plants infested, reduce the pest population size, or reduce damage to plants) by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more in comparison to a reference level (e.g., a crop to which the modulating agent has not been administered). In other instances, the modulating agent may be effective to prevent or reduce the likelihood of crop infestation by about 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more in comparison to a reference level (e.g., a crop to which the modulating agent has not been administered).

Any suitable plant tissues may benefit from the compositions and methods described herein, including, but not limited to, somatic embryos, pollen, leaves, stems, calli, stolons, microtubers, and shoots. The methods described herein may include treatment of angiosperm and gymnosperm plants such as acacia, alfalfa, apple, apricot, artichoke, ash tree, asparagus, avocado, banana, barley, beans, beet, birch, beech, blackberry, blueberry, broccoli, brussels sprouts, cabbage, canola, cantaloupe, carrot, cassaya, cauliflower, cedar, a cereal, celery, chestnut, cherry, Chinese cabbage, citrus, clementine, clover, coffee, corn, cotton, cowpea, cucumber, cypress, eggplant, elm, endive, eucalyptus, fava beans, fennel, figs, fir, geranium, grape, grapefruit, groundnuts, ground cherry, gum hemlock, hickory, kale, kiwifruit, kohlrabi, larch, lettuce, leek, lemon, lime, locust, pine, maidenhair, maize, mango, maple, melon, millet, mushroom, mustard, nuts, oak, oats, okra, onion, orange, an ornamental plant or flower or tree, papaya, palm, parsley, parsnip, pea, peach, peanut, pear, peat, pepper, persimmon, pigeon pea, pine,

pineapple, plantain, plum, pomegranate, potato, pumpkin, radicchio, radish, rapeseed, raspberry, rice, rye, sorghum, sallow, soybean, spinach, spruce, squash, strawberry, sugarbeet, sugarcane, sunflower, sweet potato, sweet corn, tangerine, tea, tobacco, tomato, trees, triticale, turf grasses, turnips, a vine, walnut, watercress, watermelon, wheat, yams, yew, and zucchini.

5

II. Target Microorganisms

The microorganisms targeted by the modulating agent described herein may include any microorganism resident in or on the host, including, but not limited to, any bacteria and/or fungi described herein. Microorganisms resident in the host may include, for example, symbiotic (e.g., endosymbiotic
10 microorganisms that provide beneficial nutrients or enzymes to the host), commensal, pathogenic, or parasitic microorganisms. A symbiotic microorganism (e.g., bacteria or fungi) may be an obligate symbiont of the host or a facultative symbiont of the host. Microorganisms resident in the host may be acquired by any mode of transmission, including vertical, horizontal, or multiple origins of transmission.

15 *i. Bacteria*

Exemplary bacteria that may be targeted in accordance with the methods and compositions provided herein, include, but are not limited to, *Xenorhabdus spp, Photorhabdus spp, Candidatus spp, Buchnera spp, Blattabacterium spp, Baumania spp, Wigglesworthia spp, Wolbachia spp, Rickettsia spp, Orientia spp, Sodalis spp, Burkholderia spp, Cupriavidus spp, Frankia spp, Snirhizobium spp,*
20 *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.* Non-
25 limiting examples of bacteria that may be targeted by the methods and compositions provided herein are shown in Table 1.

Table 1: Examples of Target Bacteria and Host Insects

Endosymbiont	Host Insect	Location	16S rRNA
Snodgrassella alvi	honeybee (*Apis mellifera*) and *Bombus spp.*	Ileum	GAGAGTTTGATCCTGGCTCAGATTGAACGC TGGCGGCATGCCTTACACATGCAAGTCGAA CGGCAGCACGGAGAGCTTGCTCTCTGGTG GCGAGTGGCGAACGGGTGAGTAATGCATC GGAACGTACCGAGTAATGGGGGATAACTG TCCGAAAGGATGGCTAATACCGCATACGCC CTGAGGGGGAAAGCGGGGGATCGAAAGAC CTCGCGTTATTTGAGCGGCCGATGTTGGAT TAGCTAGTTGGTGGGGTAAAGGCCTACCAA GGCGACGATCCATAGCGGGTCTGAGAGGA TGATCCGCCACATTGGGACTGAGACACGG

			CCCAAACTCCTACGGGAGGCAGCAGTGGG GAATTTTGGACAATGGGGGGAACCCTGATC CAGCCATGCCGCGTGTCTGAAGAAGGCCT TCGGGTTGTAAAGGACTTTTGTTAGGGAAG AAAAGCCGGGTGTTAATACCATCTGGTGCT GACGGTACCTAAAGAATAAGCACCGGCTAA CTACGTGCCAGCAGCCGCGGTAATACGTA GGGTGCGAGCGTTAATCGGAATTACTGGG CGTAAAGCGAGCGCAGACGGTTAATTAAGT CAGATGTGAAATCCCCGAGCTCAACTTGGG ACGTGCATTTGAAACTGGTTAACTAGAGTG TGTCAGAGGGAGGTAGAATTCCACGTGTAG CAGTGAAATGCGTAGAGATGTGGAGGAATA CCGATGGCGAAGGCAGCCTCCTGGGATAA CACTGACGTTCATGCTCGAAAGCGTGGGTA GCAAACAGGATTAGATACCCTGGTAGTCCA CGCCCTAAACGATGACAATTAGCTGTTGGG ACACTAGATGTCTTAGTAGCGAAGCTAACG CGTGAAATTGTCCGCCTGGGGAGTACGGT CGCAAGATTAAAACTCAAAGGAATTGACGG GGACCCGCACAAGCGGTGGATGATGTGGA TTAATTCGATGCAACGCGAAGAACCTTACC TGGTCTTGACATGTACGGAATCTCTTAGAG ATAGGAGAGTGCCTTCGGGAACCGTAACA CAGGTGCTGCATGGCTGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAAC GAGCGCAACCCTTGTCATTAGTTGCCATCA TTAAGTTGGGCACTCTAATGAGACTGCCGG TGACAAACCGGAGGAAGGTGGGGATGACG TCAAGTCCTCATGGCCCTTATGACCAGGGC TTCACACGTCATACAATGGTCGGTACAGAG GGTAGCGAAGCCGCGAGGTGAAGCCAATC TCAGAAAGCCGATCGTAGTCCGGATTGCAC TCTGCAACTCGAGTGCATGAAGTCGGAATC GCTAGTAATCGCAGGTCAGCATACTGCGGT GAATACGTTCCCGGGTCTTGTACACACCGC CCGTCACACCATGGGAGTGGGGGATACCA GAATTGGGTAGACTAACCGCAAGGAGGTC GCTTAACACGGTATGCTTCATGACTGGGGT

			GAAGTCGTAACAAGGTAGCCGTAG (SEQ ID NO: 1)
Gilliamella apicola	honeybee (*Apis mellifera*) and *Bombus* spp.	Ileum	TTAAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCTTAACACATGCAAGTCGAACGGTAACATGAGTGCTTGCACTTGATGACGAGTGGCGGACGGGTGAGTAAAGTATGGGGATCTGCCGAATGGAGGGGGACAACAGTTGGAAACGACTGCTAATACCGCATAAAGTTGAGAGACCAAAGCATGGGACCTTCGGGCCATGCGCCATTTGATGAACCCATATGGGATTAGCTAGTTGGTAGGGTAATGGCTTACCAAGGCGACGATCTCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAAACCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCGGTGATGAGGAAGGTGGTGTATCTAATAGGTGCATCAATTGACGTTAATTACAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCGAGCGTTAATCGGAATGACTGGGCGTAAAGGGCATGTAGGCGGATAATTAAGTTAGGTGTGAAAGCCCTGGGCTCAACCTAGGAATTGCACTTAAAACTGGTTAACTAGAGTATTGTAGAGGAAGGTAGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAATACCGGTGGCGAAGGCGGCCTTCTGGACAGATACTGACGCTGAGATGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAGTTTGTTGCCTAGAGTGATGGGCTCCGAAGCTAACGCGATAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAATCTTGCAGAGATGCGGGAGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGT

			TGCCATCGGTTAGGCCGGGAACTCAAAGG AGACTGCCGTTGATAAAGCGGAGGAAGGT GGGGACGACGTCAAGTCATCATGGCCCTT ACGACCAGGGCTACACACGTGCTACAATG GCGTATACAAAGGGAGGCGACCTCGCGAG AGCAAGCGGACCTCATAAAGTACGTCTAAG TCCGGATTGGAGTCTGCAACTCGACTCCAT GAAGTCGGAATCGCTAGTAATCGTGAATCA GAATGTCACGGTGAATACGTTCCCGGGCCT TGTACACACCGCCCGTCACACCATGGGAG TGGGTTGCACCAGAAGTAGATAGCTTAACC TTCGGGAGGGCGTTTACCACGGTGTGGTC CATGACTGGGGTGAAGTCGTAACAAGGTAA CCGTAGGGGAACCTGCGGTTGGATCACCT CCTTAC (SEQ ID NO: 2)
Bartonella apis	honeybee (*Apis mellifera*)	Gut	AAGCCAAAATCAAATTTTCAACTTGAGAGTT TGATCCTGGCTCAGAACGAACGCTGGCGG CAGGCTTAACACATGCAAGTCGAACGCACT TTTCGGAGTGAGTGGCAGACGGGTGAGTA ACGCGTGGGAATCTACCTATTTCTACGGAA TAACGCAGAGAAATTTGTGCTAATACCGTA TACGTCCTTCGGGAGAAAGATTTATCGGAG ATAGATGAGCCCGCGTTGGATTAGCTAGTT GGTGAGGTAATGGCCCACCAAGGCGACGA TCCATAGCTGGTCTGAGAGGATGACCAGC CACATTGGGACTGAGACACGGCCCAGACT CCTACGGGAGGCAGCAGTGGGGAATATTG GACAATGGGCGCAAGCCTGATCCAGCCAT GCCGCGTGAGTGATGAAGGCCCTAGGGTT GTAAAGCTCTTTCACCGGTGAAGATAATGA CGGTAACCGGAGAAGAAGCCCCGGCTAAC TTCGTGCCAGCAGCCGCGGTAATACGAAG GGGGCTAGCGTTGTTCGGATTTACTGGGC GTAAAGCGCACGTAGGCGGATATTTAAGTC AGGGGTGAAATCCCGGGGCTCAACCCCGG AACTGCCTTTGATACTGGATATCTTGAGTAT GGAAGAGGTAAGTGGAATTCCGAGTGTAG AGGTGAAATTCGTAGATATTCGGAGGAACA CCAGTGGCGAAGGCGGCTTACTGGTCCAT TACTGACGCTGAGGTGCGAAAGCGTGGGG

			AGCAAACAGGATTAGATACCCTGGTAGTCC ACGCTGTAAACGATGAATGTTAGCCGTTGG ACAGTTTACTGTTCGGTGGCGCAGCTAACG CATTAAACATTCCGCCTGGGGAGTACGGTC GCAAGATTAAAACTCAAAGGAATTGACGGG GGCCCGCACAAGCGGTGGAGCATGTGGTT TAATTCGAAGCAACGCGCAGAACCTTACCA GCCCTTGACATCCCGATCGCGGATGGTGG AGACACCGTCTTTCAGTTCGGCTGGATCGG TGACAGGTGCTGCATGGCTGTCGTCAGCT CGTGTCGTGAGATGTTGGGTTAAGTCCCGC AACGAGCGCAACCCTCGCCCTTAGTTGCCA TCATTTAGTTGGGCACTCTAAGGGGACTGC CGGTGATAAGCCGAGAGGAAGGTGGGGAT GACGTCAAGTCCTCATGGCCCTTACGGGCT GGGCTACACACGTGCTACAATGGTGGTGA CAGTGGGCAGCGAGACCGCGAGGTCGAG CTAATCTCCAAAAGCCATCTCAGTTCGGAT TGCACTCTGCAACTCGAGTGCATGAAGTTG GAATCGCTAGTAATCGTGGATCAGCATGCC ACGGTGAATACGTTCCCGGGCCTTGTACAC ACCGCCCGTCACACCATGGGAGTTGGTTTT ACCCGAAGGTGCTGTGCTAACCGCAAGGA GGCAGGCAACCACGGTAGGGTCAGCGACT GGGGTGAAGTCGTAACAAGGTAGCCGTAG GGGAACCTGCGGCTGGATCACCTCCTTTCT AAGGAAGATGAAGAATTGGAA (SEQ ID NO: 3)
Parasaccharibacter apium	honeybee (*Apis mellifera*)	Gut	CTACCATGCAAGTCGCACGAAACCTTTCGG GGTTAGTGGCGGACGGGTGAGTAACGCGT TAGGAACCTATCTGGAGGTGGGGGATAAC ATCGGGAAACTGGTGCTAATACCGCATGAT GCCTGAGGGCCAAAGGAGAGATCCGCCAT TGGAGGGGCCTGCGTTCGATTAGCTAGTT GGTTGGGTAAAGGCTGACCAAGGCGATGA TCGATAGCTGGTTTGAGAGGATGATCAGCC ACACTGGGACTGAGACACGGCCCAGACTC CTACGGGAGGCAGCAGTGGGGAATATTGG ACAATGGGGGCAACCCTGATCCAGCAATG CCGCGTGTGTGAAGAAGGTCTTCGGATTGT

			AAAGCACTTTCACTAGGGAAGATGATGACG GTACCTAGAGAAGAAGCCCCGGCTAACTTC GTGCCAGCAGCCGCGGTAATACGAAGGGG GCTAGCGTTGCTCGGAATGACTGGGCGTA AAGGGCGCGTAGGCTGTTTGTACAGTCAG ATGTGAAATCCCCGGGCTTAACCTGGGAAC TGCATTTGATACGTGCAGACTAGAGTCCGA GAGAGGGTTGTGGAATTCCCAGTGTAGAG GTGAAATTCGTAGATATTGGGAAGAACACC GGTTGCGAAGGCGGCAACCTGGCTNNNNN NNNNNNNNNNNNNNNNNNNNNNNNNNNNN NNNNNNNNNNNNNNNNNNNNNNNNNNNNN NNNNNNNNNNNNNNNNNNNNNNNNNNNNN NNNNNNNNNNNNNNNNNNNNNNNGAGCTAA CGCGTTAAGCACACCGCCTGGGGAGTACG GCCGCAAGGTTGAAACTCAAAGGAATTGAC GGGGGCCCGCACAAGCGGTGGAGCATGT GGTTTAATTCGAAGCAACGCGCAGAACCTT ACCAGGGCTTGCATGGGGAGGCTGTATTC AGAGATGGATATTTCTTCGGACCTCCCGCA CAGGTGCTGCATGGCTGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAAC GAGCGCAACCCTTGTCTTTAGTTGCCATCA CGTCTGGGTGGGCACTCTAGAGAGACTGC CGGTGACAAGCCGGAGGAAGGTGGGGATG ACGTCAAGTCCTCATGGCCCTTATGTCCTG GGCTACACACGTGCTACAATGGCGGTGAC AGAGGGATGCTACATGGTGACATGGTGCT GATCTCAAAAAACCGTCTCAGTTCGGATTG TACTCTGCAACTCGAGTGCATGAAGGTGGA ATCGCTAGTAATCGCGGATCAGCATGCCGC GGTGAATACGTTCCCGGGCCTTGTACACAC CGCCCGTCACACCATGGGAGTTGGTTTGA CCTTAAGCCGGTGAGCGAACCGCAAGGAA CGCAGCCGACCACCGGTTCGGGTTCAGCG ACTGGGGGA (SEQ ID NO: 4)
Lactobacillus kunkeei	honeybee (*Apis mellifera*)	Gut	TTCCTTAGAAAGGAGGTGATCCAGCCGCAG GTTCTCCTACGGCTACCTTGTTACGACTTC ACCCTAATCATCTGTCCCACCTTAGACGAC

			TAGCTCCTAAAAGGTTACCCCATCGTCTTT GGGTGTTACAAACTCTCATGGTGTGACGGG CGGTGTGTACAAGGCCCGGGAACGTATTC ACCGTGGCATGCTGATCCACGATTACTAGT GATTCCAACTTCATGCAGGCGAGTTGCAGC CTGCAATCCGAACTGAGAATGGCTTTAAGA GATTAGCTTGACCTCGCGGTTTCGCGACTC GTTGTACCATCCATTGTAGCACGTGTGTAG CCCAGCTCATAAGGGGCATGATGATTTGAC GTCGTCCCCACCTTCCTCCGGTTTATCACC GGCAGTCTCACTAGAGTGCCCAACTAAATG CTGGCAACTAATAATAAGGGTTGCGCTCGT TGCGGGACTTAACCCAACATCTCACGACAC GAGCTGACGACAACCATGCACCACCTGTCA TTCTGTCCCCGAAGGGAACGCCCAATCTCT TGGGTTGGCAGAAGATGTCAAGAGCTGGT AAGGTTCTTCGCGTAGCATCGAATTAAACC ACATGCTCCACCACTTGTGCGGGCCCCCG TCAATTCCTTTGAGTTTCAACCTTGCGGTC GTACTCCCCAGGCGGAATACTTAATGCGTT AGCTGCGGCACTGAAGGGCGGAAACCCTC CAACACCTAGTATTCATCGTTTACGGCATG GACTACCAGGGTATCTAATCCTGTTCGCTA CCCATGCTTTCGAGCCTCAGCGTCAGTAAC AGACCAGAAAGCCGCCTTCGCCACTGGTG TTCTTCCATATATCTACGCATTTCACCGCTA CACATGGAGTTCCACTTTCCTCTTCTGTACT CAAGTTTTGTAGTTTCCACTGCACTTCCTCA GTTGAGCTGAGGGCTTTCACAGCAGACTTA CAAAACCGCCTGCGCTCGCTTTACGCCCAA TAAATCCGGACAACGCTTGCCACCTACGTA TTACCGCGGCTGCTGGCACGTAGTTAGCC GTGGCTTTCTGGTTAAATACCGTCAAAGTG TTAACAGTTACTCTAACACTTGTTCTTCTTT AACAACAGAGTTTTACGATCCGAAAACCTT CATCACTCACGCGGCGTTGCTCCATCAGAC TTTCGTCCATTGTGGAAGATTCCCTACTGC TGCCTCCCGTAGGAGTCTGGGCCGTGTCT CAGTCCCAATGTGGCCGATTACCCTCTCAG GTCGGCTACGTATCATCGTCTTGGTGGGCT

			TTTATCTCACCAACTAACTAATACGGCGCG GGTCCATCCCAAAGTGATAGCAAAGCCATC TTTCAAGTTGGAACCATGCGGTTCCAACTA ATTATGCGGTATTAGCACTTGTTTCCAAATG TTATCCCCCGCTTCGGGGCAGGTTACCCAC GTGTTACTCACCAGTTCGCCACTCGCTCCG AATCCAAAAATCATTTATGCAAGCATAAAAT CAATTTGGGAGAACTCGTTCGACTTGCATG TATTAGGCACGCCGCCAGCGTTCGTCCTGA GCCAGGATCAAACTCTCATCTTAA (SEQ ID NO: 190)
Lactobacillus Firm-4	honeybee (*Apis mellifera*)	Gut	ACGAACGCTGGCGGCGTGCCTAATACATG CAAGTCGAGCGCGGGAAGTCAGGGAAGCC TTCGGGTGGAACTGGTGGAACGAGCGGCG GATGGGTGAGTAACACGTAGGTAACCTGC CCTAAAGCGGGGGATACCATCTGGAAACA GGTGCTAATACCGCATAAACCCAGCAGTCA CATGAGTGCTGGTTGAAAGACGGCTTCGG CTGTCACTTTAGGATGGACCTGCGGCGTAT TAGCTAGTTGGTGGAGTAACGGTTCACCAA GGCAATGATACGTAGCCGACCTGAGAGGG TAATCGGCCACATTGGGACTGAGACACGG CCCAAACTCCTACGGGAGGCAGCAGTAGG GAATCTTCCACAATGGACGCAAGTCTGATG GAGCAACGCCGCGTGGATGAAGAAGGTCT TCGGATCGTAAAATCCTGTTGTTGAAGAAG AACGGTTGTGAGAGTAACTGCTCATAACGT GACGGTAATCAACCAGAAAGTCACGGCTAA CTACGTGCCAGCAGCCGCGGTAATACGTA GGTGGCAAGCGTTGTCCGGATTTATTGGG CGTAAAGGGAGCGCAGGCGGTCTTTTAAG TCTGAATGTGAAAGCCCTCAGCTTAACTGA GGAAGAGCATCGGAAACTGAGAGACTTGA GTGCAGAAGAGGAGAGTGGAACTCCATGT GTAGCGGTGAAATGCGTAGATATATGGAAG AACACCAGTGGCGAAGGCGGCTCTCTGGT CTGTTACTGACGCTGAGGCTCGAAAGCATG GGTAGCGAACAGGATTAGATACCCTGGTAG TCCATGCCGTAAACGATGAGTGCTAAGTGT TGGGAGGTTTCCGCCTCTCAGTGCTGCAG

			CTAACGCATTAAGCACTCCGCCTGGGGAGT ACGACCGCAAGGTTGAAACTCAAAGGAATT GACGGGGGCCCGCACAAGCGGTGGAGCA TGTGGTTTAATTCGAAGCAACGCGAAGAAC CTTACCAGGTCTTGACATCTCCTGCAAGCC TAAGAGATTAGGGGTTCCCTTCGGGGACA GGAAGACAGGTGGTGCATGGTTGTCGTCA GCTCGTGTCGTGAGATGTTGGGTTAAGTCC CGCAACGAGCGCAACCCTTGTTACTAGTTG CCAGCATTAAGTTGGGCACTCTAGTGAGAC TGCCGGTGACAAACCGGAGGAAGGTGGGG ACGACGTCAAATCATCATGCCCCTTATGAC CTGGGCTACACACGTGCTACAATGGATGGT ACAATGAGAAGCGAACTCGCGAGGGGAAG CTGATCTCTGAAAACCATTCTCAGTTCGGA TTGCAGGCTGCAACTCGCCTGCATGAAGCT GGAATCGCTAGTAATCGCGGATCAGCATGC CGCGGTGAATACGTTCCCGGGCCTTGTAC ACACCGCCC (SEQ ID NO: 191)
Enterococcus	*Bombyx mori*	Gut	AGGTGATCCAGCCGCACCTTCCGATACGG CTACCTTGTTACGACTTCACCCCAATCATCT ATCCCACCTTAGGCGGCTGGCTCCAAAAAG GTTACCTCACCGACTTCGGGTGTTACAAAC TCTCGTGGTGTGACGGGCGGTGTGTACAA GGCCCGGGAACGTATTCACCGCGGCGTGC TGATCCGCGATTACTAGCGATTCCGGCTTC ATGCAGGCGAGTTGCAGCCTGCAATCCGA ACTGAGAGAAGCTTTAAGAGATTTGCATGA CCTCGCGGTCTAGCGACTCGTTGTACTTCC CATTGTAGCACGTGTGTAGCCCAGGTCATA AGGGGCATGATGATTTGACGTCATCCCCAC CTTCCTCCGGTTTGTCACCGGCAGTCTCGC TAGAGTGCCCAACTAAATGATGGCAACTAA CAATAAGGGTTGCGCTCGTTGCGGGACTTA ACCCAACATCTCACGACACGAGCTGACGAC AACCATGCACCACCTGTCACTTTGTCCCCG AAGGGAAAGCTCTATCTCTAGAGTGGTCAA AGGATGTCAAGACCTGGTAAGGTTCTTCGC GTTGCTTCGAATTAAACCACATGCTCCACC

			GCTTGTGCGGGCCCCCGTCAATTCCTTTGA GTTTCAACCTTGCGGTCGTACTCCCCAGGC GGAGTGCTTAATGCGTTTGCTGCAGCACTG AAGGGCGGAAACCCTCCAACACTTAGCACT CATCGTTTACGGCGTGGACTACCAGGGTAT CTAATCCTGTTTGCTCCCCACGCTTTCGAG CCTCAGCGTCAGTTACAGACCAGAGAGCC GCCTTCGCCACTGGTGTTCCTCCATATATC TACGCATTTCACCGCTACACATGGAATTCC ACTCTCCTCTTCTGCACTCAAGTCTCCCAG TTTCCAATGACCCTCCCCGGTTGAGCCGG GGGCTTTCACATCAGACTTAAGAAACCGCC TGCGCTCGCTTTACGCCCAATAAATCCGGA CAACGCTTGCCACCTACGTATTACCGCGGC TGCTGGCACGTAGTTAGCCGTGGCTTTCTG GTTAGATACCGTCAGGGGACGTTCAGTTAC TAACGTCCTTGTTCTTCTCTAACAACAGAGT TTTACGATCCGAAAACCTTCTTCACTCACG CGGCGTTGCTCGGTCAGACTTTCGTCCATT GCCGAAGATTCCCTACTGCTGCCTCCCGTA GGAGTCTGGGCCGTGTCTCAGTCCCAGTG TGGCCGATCACCCTCTCAGGTCGGCTATG CATCGTGGCCTTGGTGAGCCGTTACCTCAC CAACTAGCTAATGCACCGCGGGTCCATCCA TCAGCGACACCCGAAAGCGCCTTTCACTCT TATGCCATGCGGCATAAACTGTTATGCGGT ATTAGCACCTGTTTCCAAGTGTTATCCCCCT CTGATGGGTAGGTTACCCACGTGTTACTCA CCCGTCCGCCACTCCTCTTTCCAATTGAGT GCAAGCACTCGGGAGGAAAGAAGCGTTCG ACTTGCATGTATTAGGCACGCCGCCAGCGT TCGTCCTGAGCCAGGATCAAACTCT (SEQ ID NO: 5)
Delftia	*Bombyx mori*	Gut	CAGAAAGGAGGTGATCCAGCCGCACCTTC CGATACGGCTACCTTGTTACGACTTCACCC CAGTCACGAACCCCGCCGTGGTAAGCGCC CTCCTTGCGGTTAGGCTACCTACTTCTGGC GAGACCCGCTCCCATGGTGTGACGGGCGG TGTGTACAAGACCCGGGAACGTATTCACCG CGGCATGCTGATCCGCGATTACTAGCGATT

			CCGACTTCACGCAGTCGAGTTGCAGACTG CGATCCGGACTACGACTGGTTTTATGGGAT TAGCTCCCCCTCGCGGGTTGGCAACCCTC TGTACCAGCCATTGTATGACGTGTGTAGCC CCACCTATAAGGGCCATGAGGACTTGACGT CATCCCCACCTTCCTCCGGTTTGTCACCGG CAGTCTCATTAGAGTGCTCAACTGAATGTA GCAACTAATGACAAGGGTTGCGCTCGTTGC GGGACTTAACCCAACATCTCACGACACGAG CTGACGACAGCCATGCAGCACCTGTGTGC AGGTTCTCTTTCGAGCACGAATCCATCTCT GGAAACTTCCTGCCATGTCAAAGGTGGGTA AGGTTTTTCGCGTTGCATCGAATTAAACCA CATCATCCACCGCTTGTGCGGGTCCCCGT CAATTCCTTTGAGTTTCAACCTTGCGGCCG TACTCCCCAGGCGGTCAACTTCACGCGTTA GCTTCGTTACTGAGAAAACTAATTCCCAAC AACCAGTTGACATCGTTTAGGGCGTGGACT ACCAGGGTATCTAATCCTGTTTGCTCCCCA CGCTTTCGTGCATGAGCGTCAGTACAGGTC CAGGGGATTGCCTTCGCCATCGGTGTTCCT CCGCATATCTACGCATTTCACTGCTACACG CGGAATTCCATCCCCCTCTACCGTACTCTA GCCATGCAGTCACAAATGCAGTTCCCAGGT TGAGCCCGGGGATTTCACATCTGTCTTACA TAACCGCCTGCGCACGCTTTACGCCCAGTA ATTCCGATTAACGCTCGCACCCTACGTATT ACCGCGGCTGCTGGCACGTAGTTAGCCGG TGCTTATTCTTACGGTACCGTCATGGGCCC CCTGTATTAGAAGGAGCTTTTTCGTTCCGT ACAAAAGCAGTTTACAACCCGAAGGCCTTC ATCCTGCACGCGGCATTGCTGGATCAGGC TTTCGCCCATTGTCCAAAATTCCCCACTGC TGCCTCCCGTAGGAGTCTGGGCCGTGTCT CAGTCCCAGTGTGGCTGGTCGTCCTCTCA GACCAGCTACAGATCGTCGGCTTGGTAAG CTTTTATCCCACCAACTACCTAATCTGCCAT CGGCCGCTCCAATCGCGCGAGGCCCGAAG GGCCCCCGCTTTCATCCTCAGATCGTATGC GGTATTAGCTACTCTTTCGAGTAGTTATCCC

			CCACGACTGGGCACGTTCCGATGTATTACT CACCCGTTCGCCACTCGTCAGCGTCCGAA GACCTGTTACCGTTCGACTTGCATGTGTAA GGCATGCCGCCAGCGTTCAATCTGAGCCA GGATCAAACTCTACAGTTCGATCT (SEQ ID NO: 6)
Pelomonas	*Bombyx mori*	Gut	ATCCTGGCTCAGATTGAACGCTGGCGGCAT GCCTTACACATGCAAGTCGAACGGTAACAG GTTAAGCTGACGAGTGGCGAACGGGTGAG TAATATATCGGAACGTGCCCAGTCGTGGGG GATAACTGCTCGAAAGAGCAGCTAATACCG CATACGACCTGAGGGTGAAAGCGGGGGAT CGCAAGACCTCGCNNGATTGGAGCGGCCG ATATCAGATTAGGTAGTTGGTGGGGTAAAG GCCCACCAAGCCAACGATCTGTAGCTGGT CTGAGAGGACGACCAGCCACACTGGGACT GAGACACGGCCCAGACTCCTACGGGAGGC AGCAGTGGGGAATTTTGGACAATGGGCGC AAGCCTGATCCAGCCATGCCGCGTGCGGG AAGAAGGCCTTCGGGTTGTAAACCGCTTTT GTCAGGGAAGAAAAGGTTCTGGTTAATACC TGGGACTCATGACGGTACCTGAAGAATAAG CACCGGCTAACTACGTGCCAGCAGCCGCG GTAATACGTAGGGTGCAAGCGTTAATCGGA ATTACTGGGCGTAAAGCGTGCGCAGGCGG TTATGCAAGACAGAGGTGAAATCCCCGGG CTCAACCTGGGAACTGCCTTTGTGACTGCA TAGCTAGAGTACGGTAGAGGGGGATGGAA TTCCGCGTGTAGCAGTGAAATGCGTAGATA TGCGGAGGAACACCGATGGCGAAGGCAAT CCCCTGGACCTGTACTGACGCTCATGCAC GAAAGCGTGGGGAGCAAACAGGATTAGAT ACCCTGGTAGTCCACGCCCTAAACGATGTC AACTGGTTGTTGGGAGGGTTTCTTCTCAGT AACGTANNTAACGCGTGAAGTTGACCGCCT GGGGAGTACGGCCGCAAGGTTGAAACTCA AAGGAATTGACGGGGACCCGCACAAGCGG TGGATGATGTGGTTTAATTCGATGCAACGC GAAAAACCTTACCTACCCTTGACATGCCAG GAATCCTGAAGAGATTTGGGAGTGCTCGAA

			AGAGAACCTGGACACAGGTGCTGCATGGC CGTCGTCAGCTCGTGTCGTGAGATGTTGG GTTAAGTCCCGCAACGAGCGCAACCCTTGT CATTAGTTGCTACGAAAGGGCACTCTAATG AGACTGCCGGTGACAAACCGGAGGAAGGT GGGGATGACGTCAGGTCATCATGGCCCTT ATGGGTAGGGCTACACACGTCATACAATGG CCGGGACAGAGGGCTGCCAACCCGCGAG GGGGAGCTAATCCCAGAAACCCGGTCGTA GTCCGGATCGTAGTCTGCAACTCGACTGC GTGAAGTCGGAATCGCTAGTAATCGCGGAT CAGCTTGCCGCGGTGAATACGTTCCCGGG TCTTGTACACACCGCCCGTCACACCATGGG AGCGGGTTCTGCCAGAAGTAGTTAGCCTAA CCGCAAGGAGGGCGATTACCACGGCAGGG TTCGTGACTGGGGTGAAGTCGTAACAAGGT AGCCGTATCGGAAGGTGCGGCTGGATCAC (SEQ ID NO: 7)

Any number of bacterial species may be targeted by the compositions or methods described herein. For example, in some instances, the modulating agent may target a single bacterial species. In some instances, the modulating agent may target at least about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, or more distinct bacterial species. In some instances, the modulating agent may target any one of about 1 to about 5, about 5 to about 10, about 10 to about 20, about 20 to about 50, about 50 to about 100, about 100 to about 200, about 200 to about 500, about 10 to about 50, about 5 to about 20, or about 10 to about 100 distinct bacterial species. In some instances, the modulating agent may target at least about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more phyla, classes, orders, families, or genera of bacteria.

In some instances, the modulating agent may increase a population of one or more bacteria (e.g., symbiotic bacteria) by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in the host in comparison to a host organism to which the modulating agent has not been administered. In some instances, the modulating agent may reduce the population of one or more bacteria (e.g., pathogenic or parasitic bacteria) by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in the host in comparison to a host organism to which the modulating agent has not been administered. In some instances, the modulating agent may eradicate the population of a bacterium (e.g., a pathogenic or parasitic bacteria) in the host. In some instances, the modulating agent may increase the level of one or more symbiotic bacteria by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in the host and/or decreases the level of one or more pathogenic bacteria

by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in the host in comparison to a host organism to which the modulating agent has not been administered.

In some instances, the modulating agent may alter the bacterial diversity and/or bacterial composition of the host. In some instances, the modulating agent may increase the bacterial diversity in the host relative to a starting diversity by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in comparison to a host organism to which the modulating agent has not been administered. In some instances, the modulating agent may decrease the bacterial diversity in the host relative to a starting diversity by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in comparison to a host organism to which the modulating agent has not been administered.

In some instances, the modulating agent may alter the function, activity, growth, and/or division of one or more bacterial cells. For example, the modulating agent may alter the expression of one or genes in the bacteria. In some instances, the modulating agent may alter the function of one or more proteins in the bacteria. In some instances, the modulating agent may alter the function of one or more cellular structures (e.g., the cell wall, the outer or inner membrane) in the bacteria. In some instances, the modulating agent may kill (e.g., lyse) the bacteria.

The target bacterium may reside in one or more parts of the insect. Further, the target bacteria may be intracellular or extracellular. In some instances, the bacteria reside in or on one or more parts of the host gut, including, e.g., the foregut, midgut, and/or hindgut. For example, in honey bees (e.g., *Apis mellifera*), bacterial symbionts confined to the hindguts of adults are acquired in the first few days following emergence of adults from the pupal stage, through social interactions with other adult worker bees in the colony. Honey bee gut inhabitants belong to a small number of distinctive lineages found only in honey bees and also in other *Apis* species and in *Bombus* species (bumble bees). In some instances, the target bacteria are resident in a honeybee. In some instances, one or more bacteria targeted in the honeybee is a *Snodgrassella* spp. (e.g., *Snodgrasella alvi*), a *Gilliamella* spp. (e.g., *Gilliamella apicola*), a *Bartonella* spp. (e.g., *Bartonella apis*), a *Parasaccharibacter* spp. (e.g., *Parasaccharibacter apium*), or a *Lactobacillus* spp. (e.g., *Lactobacillus kunkeei*, *Lactobacillus Firm-4*).

In some instances, the bacteria reside as an intracellular bacteria within a cell of the host insect. In some instances, the bacteria reside in a bacteriocyte of the host insect.

Changes to the populations of bacteria in the host may be determined by any methods known in the art, such as microarray, polymerase chain reaction (PCR), real-time PCR, flow cytometry, fluorescence microscopy, transmission electron microscopy, fluorescence *in situ* hybridization (e.g., FISH), spectrophotometry, matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS), and DNA sequencing. In some instances, a sample of the host treated with a modulating agent is sequenced (e.g., by metagenomics sequencing of 16S rRNA or rDNA) to determine the microbiome of the host after delivery or administration of the modulating agent. In some instances, a sample of a host that did not receive the modulating agent is also sequenced to provide a reference.

ii. Fungi

Exemplary fungi that may be targeted in accordance with the methods and compositions provided herein, include, but are not limited to *Amylostereum areolatum, Epichloe spp, Pichia pinus, Hansenula*

capsulate, Daldinia decipien, Ceratocytis spp, Ophiostoma spp, and Attamyces bromatificus. Non-limiting examples of yeast and yeast-like symbionts found in insects include *Candida, Metschnikowia, Debaromyces, Scheffersomyces shehatae and Scheffersomyces stipites, Starmerella, Pichia, Trichosporon, Cryptococcus, Pseudozyma,* and yeast-like symbionts from the subphylum *Pezizomycotina* (e.g., *Symbiotaphrina bucneri* and *Symbiotaphrina kochii*). Non-limiting examples of yeast that may be targeted by the methods and compositions herein are listed in Table 2.

Table 2: Examples of Yeast in Insects

Insect Species	**Order: Family**	**Yeast Location (Species)**
Stegobium paniceum	Coleoptera: Anobiidae	Mycetomes
(= Sitodrepa panicea)		(*Saccharomyces*)
		Cecae (*Torulopsis buchnerii*)
		Mycetome between foregut and midgut
		Mycetomes (*Symbiotaphrina buchnerii*)
		Mycetomes and digestive tube (*Torulopsis buchnerii*)
		Gut cecae (*Symbiotaphrina buchnerii*)
Lasioderma serricorne	Coleoptera: Anobiidae	Mycetome between foregut and midgut
		(*Symbiotaphrina kochii*)
Ernobius abietis	Coleoptera: Anobiidae	Mycetomes (*Torulopsis karawaiewii*)
		(*Candida karawaiewii*)
Ernobius mollis	Coleoptera: Anobiidae	Mycetomes (*Torulopsis ernobii*)
		(*Candida ernobii*)
Hemicoelus gibbicollis	Coleoptera: Anobiidae	Larval mycetomes
Xestobium plumbeum	Coleoptera: Anobiidae	Mycetomes (*Torulopsis xestobii*)
		(*Candida xestobii*)
Criocephalus rusticus	Coleoptera: Cerambycidae	Mycetomes
Phoracantha semipunctata	Coleoptera: Cerambycidae	Alimentary canal (*Candida guilliermondii*, *C. tenuis*)
		Cecae around midgut (*Candida guilliermondii*)
Harpium inquisitor	Coleoptera: Cerambycidae	Mycetomes (*Candida rhagii*)
Harpium mordax *H. sycophanta*	Coleoptera: Cerambycidae	Cecae around midgut (*Candida tenuis*)
Gaurotes virginea	Coleoptera: Cerambycidae	Cecae around midgut (*Candida rhagii*)

Leptura rubra	Coleoptera: Cerambycidae	Cecae around midgut (*Candida tenuis*)
		Cecae around midgut (*Candida parapsilosis*)
Leptura maculicornis	Coleoptera: Cerambycidae	Cecae around midgut (*Candida parapsilosis*)
L. cerambyciformis		
Leptura sanguinolenta	Coleoptera: Cerambycidae	Cecae around midgut (*Candida sp.*)
Rhagium bifasciatum	Coleoptera: Cerambycidae	Cecae around midgut (*Candida tenuis*)
Rhagium inquisitor	Coleoptera: Cerambycidae	Cecae around midgut (*Candida guilliermondii*)
Rhagium mordax	Coleoptera: Cerambycidae	Cecae around midgut (*Candida*)
Carpophilus hemipterus	Coleoptera: Nitidulidae	Intestinal tract (10 yeast species)
Odontotaenius disjunctus	Coleoptera: Passalidae	Hindgut (*Enteroramus dimorphus*)
Odontotaenius disjunctus	Coleoptera: Passalidae	Gut (*Pichia stipitis*, *P. segobiensis*, *Candida shehatae*)
Verres sternbergianus		(*C. ergatensis*)
Scarabaeus semipunctatus	Coleoptera: Scarabaeidae	Digestive tract (10 yeast species)
Chironitis furcifer		
Unknown species	Coleoptera: Scarabaeidae	Guts (*Trichosporon cutaneum*)
Dendroctonus and Ips spp.	Coleoptera: Scolytidae	Alimentary canal (13 yeast species)
Dendroctonus frontalis	Coleoptera: Scolytidae	Midgut (*Candida sp.*)
Ips sexdentatus	Coleoptera: Scolytidae	Digestive tract (*Pichia bovis*, *P. rhodanensis*)
		Hansenula holstii (*Candida rhagii*)
		Digestive tract
		(*Candida pulcherina*)
Ips typographus	Coleoptera: Scolytidae	Alimentary canal
		Alimentary tracts (*Hansenula capsulata*, *Candida parapsilosis*)

		Guts and beetle homogenates (*Hansenula holstii*, *H. capsulata*, *Candida diddensii*, *C. mohschtana*, *C. nitratophila*, *Cryptococcus albidus*, *C. laurentii*)
Trypodendron lineatum	Coleoptera: Scolytidae	Not specified
Xyloterinus politus	Coleoptera: Scolytidae	Head, thorax, abdomen (*Candida*, *Pichia*, *Saccharomycopsis*)
Periplaneta americana	Dictyoptera: Blattidae	Hemocoel (*Candida sp. nov.*)
Blatta orientalis	Dictyoptera: Blattidae	Intestinal tract (*Kluyveromyces blattae*)
Blatella germanica	Dictyoptera: Blattellidae	Hemocoel
Cryptocercus punctulatus	Dictyoptera: Cryptocercidae	Hindgut (1 yeast species)
Philophylla heraclei	Diptera: Tephritidae	Hemocoel
Aedes (4 species)	Diptera: Culicidae	Internal microflora (9 yeast genera)
Drosophila pseudoobscura	Diptera: Drosophilidae	Alimentary canal (24 yeast species)
Drosophila (5 spp.)	Diptera: Drosophilidae	Crop (42 yeast species)
Drosophila melanogaster	Diptera: Drosophilidae	Crop (8 yeast species)
Drosophila (4 spp.)	Diptera: Drosophilidae	Crop (7 yeast species)
Drosophila (6 spp.)	Diptera: Drosophilidae	Larval gut (17 yeast species)
Drosophila (20 spp.)	Diptera: Drosophilidae	Crop (20 yeast species)
Drosophila (8 species groups)	Diptera: Drosophilidae	Crop (*Kloeckera, Candida, Kluyveromyces*)
Drosophila serido	Diptera: Drosophilidae	Crop (18 yeast species)
Drosophila (6 spp.)	Diptera: Drosophilidae	Intestinal epithelium (*Coccidiascus legeri*)
Protaxymia melanoptera	Diptera	Unknown (*Candida*, *Cryptococcus*, *Sporoblomyces*)
Astegopteryx styraci	Homoptera: Aphididae	Hemocoel and fat body
Tuberaphis sp.	Homoptera: Aphididae	Tissue sections
Hamiltonaphis styraci		

Glyphinaphis bambusae		
Cerataphis sp.		
Hamiltonaphis styraci	Homoptera: Aphididae	Abdominal hemocoel
Cofana unimaculata	Homoptera: Cicadellidae	Fat body
Leofa unicolor	Homoptera: Cicadellidae	Fat body
Lecaniines, etc.	Homoptera:Coccoidea d	Hemolymph, fatty tissue, etc.
Lecanium sp.	Homoptera: Coccidae	Hemolymph, adipose tissue
Ceroplastes (4 sp.)	Homoptera: Coccidae	Blood smears
Laodelphax striatellus	Homoptera: Delphacidae	Fat body
		Eggs
		Eggs (*Candida*)
Nilaparvata lugens	Homoptera: Delphacidae	Fat body
		Eggs (2 unidentified yeast species)
		Eggs, nymphs (*Candida*)
		Eggs (7 unidentified yeast species)
		Eggs (*Candida*)
Nisia nervosa	Homoptera: Delphacidae	Fat body
Nisia grandiceps		
Perkinsiella spp.		
Sardia rostrata		
Sogatella furcifera		
Sogatodes orizicola	Homoptera: Delphacidae	Fat body
Amrasca devastans	Homoptera: Jassidae	Eggs, mycetomes, hemolymph
Tachardina lobata	Homoptera: Kerriidae	Blood smears (*Torulopsis*)
Laccifer (=Lakshadia) sp.	Homoptera: Kerriidae	Blood smears (*Torula variabilis*)
Comperia merceti	Hymenoptera: Encyrtidae	Hemolymph, gut, poison gland
Solenopsis invicta	Hymenoptera: Formicidae	Hemolymph (*Myrmecomyces annellisae*)
S. quinquecuspis		

Solenopsis invicta	Hymenoptera: Formicidae	Fourth instar larvae (*Candida parapsilosis*, *Yarrowia lipolytica*)
		Gut and hemolymph (*Candida parapsilosis*, *C. lipolytica*, *C. guillermondii*, *C. rugosa*, *Debaryomyces hansenii*)
Apis mellifera	Hymenoptera: Apidae	Digestive tracts (*Torulopsis sp.*)
		Intestinal tract (*Torulopsis apicola*)
		Digestive tracts (8 yeast species)
		Intestinal contents (12 yeast species)
		Intestinal contents (7 yeast species)
		Intestines (14 yeast species)
		Intestinal tract (*Pichia melissophila*)
		Intestinal tracts (7 yeast species)
		Alimentary canal (*Hansenula silvicola*)
		Crop and gut (13 yeast species)
Apis mellifera	Hymenoptera: Apidae	Midguts (9 yeast genera)
Anthophora occidentalis	Hymenoptera:Anthophoridae	
Nomia melanderi	Hymenoptera:Halictidae	
Halictus rubicundus	Hymenoptera:Halictidae	
Megachile rotundata	Hymenoptera:Megachilidae	

Any number of fungal species may be targeted by the compositions or methods described herein. For example, in some instances, the modulating agent may target a single fungal species. In some instances, the modulating agent may target at least about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, or more distinct fungal species. In some instances, the modulating agent may target any one of about 1 to about 5, about 5 to about 10, about 10 to about 20, about 20 to about 50, about 50 to about 100, about 100 to about 200, about 200 to about 500, about 10 to about 50, about 5 to about 20, or about 10 to about 100 distinct fungal species. In some instances, the modulating agent may target at least about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more phyla, classes, orders, families, or genera of fungi.

In some instances, the modulating agent may increase a population of one or more fungi (e.g., symbiotic fungi) by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in the host in comparison to a host organism to which the modulating agent has not been administered. In

some instances, the modulating agent may reduce the population of one or more fungi (e.g., pathogenic or parasitic fungi) by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in the host in comparison to a host organism to which the modulating agent has not been administered. In some instances, the modulating agent may eradicate the population of a fungi (e.g., a pathogenic or parasitic fungi) in the host. In some instances, the modulating agent may increase the level of one or more symbiotic fungi by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in the host and/or may decrease the level of one or more pathogenic fungi by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in the host in comparison to a host organism to which the modulating agent has not been administered.

In some instances, the modulating agent may alter the fungal diversity and/or fungal composition of the host. In some instances, the modulating agent may increase the fungal diversity in the host relative to a starting diversity by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in comparison to a host organism to which the modulating agent has not been administered. In some instances, the modulating agent may decrease the fungal diversity in the host relative to a starting diversity by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more in comparison to a host organism to which the modulating agent has not been administered.

In some instances, the modulating agent may alter the function, activity, growth, and/or division of one or more fungi. For example, the modulating agent may alter the expression of one or more genes in the fungus. In some instances, the modulating agent may alter the function of one or more proteins in the fungus. In some instances, the modulating agent may alter the function of one or more cellular components in the fungus. In some instances, the modulating agent may kill the fungus.

Further, the target fungus may reside in one or more parts of the insect. In some instances, the fungus resides in or on one or more parts of the insect gut, including, e.g., the foregut, midgut, and/or hindgut. In some instances, the fungus lives extracellularly in the hemolymph, fat bodies or in specialized structures in the host.

Changes to the population of fungi in the host may be determined by any methods known in the art, such as microarray, polymerase chain reaction (PCR), real-time PCR, flow cytometry, fluorescence microscopy, transmission electron microscopy, fluorescence *in situ* hybridization (e.g., FISH), spectrophotometry, matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS), and DNA sequencing. In some instances, a sample of the host treated with modulating agent is sequenced (e.g., by metagenomics sequencing) to determine the microbiome of the host after delivery or administration of the modulating agent. In some instances, a sample of a host that did not receive the modulating agent is also sequenced to provide a reference.

III. Modulating Agents

The modulating agent of the methods and compositions provided herein may include a phage, a polypeptide, a small molecule, an antibiotic, a secondary metabolite, a bacterium, or any combination thereof.

i. Phage

The modulating agent described herein may include a phage (e.g., a lytic phage or a non-lytic phage). In some instances, an effective concentration of any phage described herein may altering a level, activity, or metabolism of one or more microorganisms (as described herein) resident in a host
5 described herein, the alteration resulting in an increase in the host's fitness. In some instances, the modulating agent includes at least one phage selected from the order *Tectiviridae, Myoviridae, Siphoviridae, Podoviridae, Caudovirales, Lipothrixviridae, Rudiviridae,* or *Ligamenvirales.* In some instances, the composition includes at least one phage selected from the family *Myoviridae, Siphoviridae, Podoviridae, Lipothrixviridae, Rudiviridae, Ampullaviridae, Bicaudaviridae, Clavaviridae, Corticoviridae,*
10 *Cystoviridae, Fuselloviridae, Gluboloviridae, Guttaviridae, Inoviridae, Leviviridae, Microviridae, Plasmaviridae,* and *Tectiviridae.* Further non-limiting examples of phages useful in the methods and compositions are listed in Table 3.

Table 3: Examples of Phage and Targeted Bacteria

Phage and Accession #	**Target bacteria**	**Target host**
SA1(NC_027991), phiP68 (NC_004679)	*Staphylococcus sp.*	*Apidae* family
WO (AB036666.1)	*Wolbachia sp.*	*Aedes aegypt*; *Drosophila melanogaster*; *Plasmodium sp*; *Teleogryllus taiwanemma*; *Bombyx mori*
KL1 (NC_018278), BcepNazgul (NC_005091) PhiE125 (NC_003309)	*Burkholderia sp.*	*Riptortus sp.*; *Pyrrhocoris apterus.*
Fern (NC_028851), Xenia (NC_028837), HB10c2 (NC_028758)	*Paenibacillus larvae*	bumble bees: *Bombus sp.*; honey bees: *A. mellifera*
CP2 (NC_020205), XP10 (NC_004902), XP15 (NC_007024), phiL7 (NC_012742)	*Xanthomonas sp.*	*Plebeina denoiti*; *Apidae* family; *Apis mellifera*; *Drosphilidae* family; and *Chloropidae* family
PP1 (NC_019542), PM1 (NC_023865)	*Pectobacterium carotovorum* subsp. *carotovorum*	*Apidae* family
ΦRSA1 (NC_009382), ΦRSB1 (NC_011201), ΦRSL1 (NC_010811), RSM1 (NC_008574)	*Ralstonia solanacearum*	*Bombyx mori*

SF1(NC_028807)	*Streptomyces scabies*	*Philantus sp.*; *Trachypus sp*
ECML-4 (NC_025446), ECML-117 (NC_025441), ECML-134 (NC_025449)	*Escherichia coli*	*Apidae* family; *Varroa destructor*
SSP5(JX274646.1), SSP6 (NC_004831), SFP10 (NC_016073), F18SE (NC_028698)	*Salmonella* sp.	*Drosphilidae* family
γ (NC_001416), Bcp1 (NC_024137)	*Bacillus sp.*	Gypsy moth; *Lymantria dispar*; *Varroa destructor*
Phi1 (NC_009821)	*Enterococcus sp.*	*Schistocerca gragaria*
ΦKMV (NC_005045), ΦEL(AJ697969.1), ΦKZ (NC_004629)	*Pseudomonas sp.*	*Lymantria dispar*; *Apidae* family
A2 (NC_004112), phig1e (NC_004305)	*Lactobacilli sp.*	*Apidae family*; *Drosophila* family; *Varroa destructor*
KLPN1 (NC_028760)	*Klebsiella sp*	*C. capitata*
vB_AbaM_Acibel004 (NC_025462), vB_AbaP_Acibel007 (NC_025457)	*Acinetobacter sp.*	*Schistocerca gragaria*

In some instances, a modulating agent includes a lytic phage. Thus, after delivery of the lytic phage to a bacterial cell resident in the host, the phage causes lysis in the target bacterial cell. In some instances, the lytic phage targets and kills a bacterium resident in a host insect to increase the fitness of the host. Alternatively or additionally, the phage of the modulating agent may be a non-lytic phage (also referred to as lysogenic or temperate phage). Thus, after delivery of the non-lytic phage to a bacterial cell resident in the host, the bacterial cell may remain viable and able to stably maintain expression of genes encoded in the phage genome. In some instances, a non-lytic phage is used to alter gene expression in a bacterium resident in a host insect to increase the fitness of the host. In some instances, the modulating agent includes a mixture of lytic and non-lytic phage.

The modulating agent described herein may include phage with either a narrow or broad bacterial target range. In some instances, the phage has a narrow bacterial target range. In some instances, the phage is a promiscuous phage with a large bacterial target range. For example, the promiscuous phage may target at least about any of 5, 10, 20, 30, 40, 50, or more bacterium resident in the host. A phage with a narrow bacterial target range may target a specific bacterial strain in the host without affecting another, e.g., non-targeted, bacterium in the host. For example, the phage may target no more than about any of 50, 40, 30, 20, 10, 8, 6, 4, 2, or 1 bacterium resident in the host. For example, the compositions described herein may target the bacterial pathogen *Paenibacillus larvae* in honeybees but may not target other bacteria (e.g., symbiotic bacteria) including *Lactobacillus Firm4*, *Lactobacillus Firm5*, *Bifidobacterium* sp, *Snodgrassella alvi*, *Gilliamella apicola*, *Bartonella apis*, *Parasaccharibacter apium*, or *Lactobacillus kunkeei*. In some instances, the phage infects one or more specific bacteria by binding to a

cell surface protein (e.g., OmpF, LamB, BtuB, TolC, etc) or to a different recognition molecule (e.g., glycolipids, LPS, lipoteichoic acid, etc.).

The compositions described herein may include any number of phage, such as at least about any one of 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 100, or more phage. In some instances, the composition includes phage from one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phage) families, one or more orders (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phage), or one or more species (e.g., 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 100, or more phage). Compositions including one or more phage are also referred herein as “phage cocktails.” Phage cocktails are useful because they allow for targeting of a wider host range of bacteria. Furthermore, they allow for each bacterial strain (i.e. subspecies) to be targeted by multiple orthogonal phages, thereby preventing or significantly delaying the onset of resistance. In some instances, a cocktail includes multiple phages targeting one bacterial species. In some instances, a cocktail includes multiple phages targeting multiple bacterial species. In some instances, a one-phage “cocktail” includes a single promiscuous phage (i.e. a phage with a large host range) targeting many strains within a species.

Suitable concentrations of the phage in the modulating agent described herein depends on factors such as efficacy, survival rate, transmissibility of the phage, number of distinct phage, and/or lysin types in the compositions, formulation, and methods of application of the composition. In some instances, the phage is in a liquid or a solid formulation. In some instances, the concentration of each phage in any of the compositions described herein is at least about any of 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10} or more pfu/ml. In some instances, the concentration of each phage in any of the compositions described herein is no more than about any of 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9 pfu/ml. In some instances, the concentration of each phage in the composition is any of about 10^2 to about 10^3, about 10^3 to about 10^4, about 10^4 to about 10^5, about 10^5 to about 10^6, about 10^7 to about 10^8, about 10^8 to about 10^9, about 10^2 to about 10^4, about 10^4 to about 10^6, about 10^6 to about 10^9, or about 10^3 to about 10^8 pfu/ml. In some instances, wherein the composition includes at least two types of phages, the concentration of each type of the phages may be the same or different. For example, in some instances, the concentration of one phage in the cocktail is about 10^8 pfu/ml and the concentration of a second phage in the cocktail is about 10^6 pfu/ml.

A modulating agent including a phage as described herein can be contacted with the target host in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of phage concentration inside a target host; (b) reach a target level (e.g., a predetermined or threshold level) of phage concentration inside a target host gut; (c) reach a target level (e.g., a predetermined or threshold level) of phage concentration inside a target host bacteriocyte; (d) modulate the level, or an activity, of one or more microorganism (e.g., endosymbiont) in the target host; or/and (e) modulate fitness of the target host.

As illustrated by Example 7 and 11, phages can be used as a modulating agents by eliminating bacterial pathogens such as *Serratia marcescens, Erwinia catotovora,* and *Pseudomonas enzomophila* from insect hosts, such as honeybees or silkworms.

ii. Polypeptides

Numerous polypeptides (e.g., a bacteriocin, R-type bacteriocin, nodule C-rich peptide, antimicrobial peptide, lysin, or bacteriocyte regulatory peptide) may be used in the compositions and methods described herein. In some instances, an effective concentration of any peptide or polypeptide described herein may alter a level, activity, or metabolism of one or more microorganisms (as described herein) resident in a host, the alteration resulting in an increase in the host's fitness. Polypeptides included herein may include naturally occurring polypeptides or recombinantly produced variants. For example, the polypeptide may be a functionally active variant of any of the polypeptides described herein with 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 polypeptide described herein or a naturally occurring polypeptide.

A modulating agent comprising a polypeptide as described herein can be contacted with the target host in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of concentration inside a target host; (b) reach a target level (e.g., a predetermined or threshold level) of concentration inside a target host gut; (c) reach a target level (e.g., a predetermined or threshold level) of concentration inside a target host bacteriocyte; (d) modulate the level, or an activity, of one or more microorganism (e.g., endosymbiont) in the target host; or/and (e) modulate fitness of the target host.

The polypeptide modulating agents discussed hereinafter, namely bacteriocins, lysins, antimicrobial peptides, nodule C-rich peptides, and bacteriocyte regulatory peptides, can be used to alter the level, activity, or metabolism of target microorganisms as indicated in the section for increasing the fitness of insects, such as honeybees and silkworms.

(a) Bacteriocins

The modulating agent described herein may include a bacteriocin. In some instances, the bacteriocin is naturally produced by Gram-positive bacteria, such as *Pseudomonas, Streptomyces, Bacillus, Staphylococcus*, or lactic acid bacteria (LAB, such as *Lactococcus lactis*). In some instances, the bacteriocin is naturally produced by Gram-negative bacteria, such as *Hafnia alvei*, *Citrobacter freundii*, *Klebsiella oxytoca, Klebsiella pneumonia, Enterobacter cloacae*, *Serratia plymithicum, Xanthomonas campestris, Erwinia carotovora, Ralstonia solanacearum,* or *Escherichia coli*. Exemplary bacteriocins include, but are not limited to, Class I-IV LAB antibiotics (such as lantibiotics), colicins, microcins, and pyocins. Non-limiting examples of bacteriocins are listed in Table 4.

Table 4: Examples of Bacteriocins

Class	**Name**	**Producer**	**Targets**	**Sequence**
Class I	Nisin	*Lactococcus lactis*	Active on Gram-positive bacteria: *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Listeria, Clostridium*	ITSISLCTPGCKT GALMGCNMKTA TCHCSIHVSK (SEQ ID NO: 8)
	Epidermin	*Staphylococcus epidermis*	Gram-positive bacteria	IASKFICTPGCA KTGSFNSYCC (SEQ ID NO: 9)
Class II				
Class II a	Pediocin PA-1	*Pediococcus acidilactici*	*Pediococci, Lactobacilli, Leuconostoc, Brochothrix thermosphacta, Propionibacteria, Bacilli, Enterococci, Staphylococci, Listeria clostridia, Listeria monocytogenes, Listeria innocua*	KYYGNGVTCG KHSCSVDWGK ATTCIINNGAMA WATGGHQGNH KC (SEQ ID NO: 10)
Class II b	Enterocin P	*Enterococcus faecium*	*Lactobacillus sakei, Enterococcus faecium*	ATRSYGNGVYC NNSKCWVNWG EAKENIAGIVISG WASGLAGMGH (SEQ ID NO: 11)
Class II c	Lactococcin G	*Streptococcus lactis*	Gram-positive bacteria	GTWDDIGQGIG RVAYWVGKAM GNMSDVNQAS RINRKKKH (SEQ ID NO: 12)

Class II d	Lactacin-F	*Lactobacillus johnsonii*	*Lactobacilli, Enterococcus faecalis*	NRWGDTVLSAA SGAGTGIKACK SFGPWGMAICG VGGAAIGGYFG YTHN (SEQ ID NO: 13)
Class III				
Class III a	Enterocin AS-48	*Enterococcus faecalis*	Broad spectrum: Gram positive and Gram negative bacteria.	MAKEFGIPAAVA GTVLNVVEAGG WVTTIVSILTAV GSGGLSLLAAA GRESIKAYLKKE IKKKGKRAVIAW (SEQ ID NO: 14)
Class III b	Aureocin A70	*Staphylococcus aureus*	Broad spectrum: Gram positive and Gram negative bacteria.	MSWLNFLKYIAK YGKKAVSAAWK YKGKVLEWLNV GPTLEWVWQKL KKIAGL (SEQ ID NO: 15)
Class IV	Garvicin A	*Lactococcus garvieae*	Broad spectrum: Gram positive and Gram negative bacteria.	IGGALGNALNGL GTWANMMNGG GFVNQWQVYA NKGKINQYRPY (SEQ ID NO: 16)
Unclassified	Colicin V	*Escherichia coli*	Active against *Escherichia coli* (also closely related bacteria), Enterobacteriaceae	MRTLTLNELDS VSGGASGRDIA MAIGTLSGQFV AGGIGAAAGGV AGGAIYDYAST HKPNPAMSPSG LGGTIKQKPEGI PSEAWNYAAGR LCNWSPNNLSD VCL (SEQ ID NO: 17)

In some instances, the bacteriocin is a colicin, a pyocin, or a microcin produced by Gram-negative bacteria. In some instances, the bacteriocin is a colicin. The colicin may be a group A colicin (e.g., uses the Tol system to penetrate the outer membrane of a target bacterium) or a group B colicin (e.g., uses the Ton system to penetrate the outer membrane of a target bacterium). In some instances, the bacteriocin is a microcin. The microcin may be a class I microcin (e.g., < 5 kDa, has post-translational modifications) or a class II microcin (e.g., 5-10 kDa, with or without post-translational modifications). In some instances, the class II microcin is a class IIa microcin (e.g., requires more than one genes to synthesize and assemble functional peptides) or a class IIb microcin (e.g., linear peptides with or without post-translational modifications at C-terminus). In some instances, the bacteriocin is a pyocin. In some instances, the pyocin is an R-pyocin, F-pyocin, or S-pyocin.

In some instances, the bacteriocin is a class I, class II, class III, or class IV bacteriocin produced by Gram-positive bacteria. In some instances, the modulating agent includes a Class I bacteriocin (e.g., lanthionine-containing antibiotics (lantibiotics) produced by a Gram-positive bacteria). The class I bacteriocins or lantibiotic may be a low molecular weight peptide (e.g., less than about 5 kDa) and may possess post-translationally modified amino acid residues (e.g., lanthionine, β-methyllanthionine, or dehydrated amino acids).

In some instances, the bacteriocin is a Class II bacteriocin (e.g., non-lantibiotics produced by Gram-positive bacteria). Many are positively charged, non-lanthionine-containing peptides, which unlike lantibiotics, do not undergo extensive post-translational modification. The Class II bacteriocin may belong to one of the following subclasses: “pediocin-like” bacteriocins (e.g., pediocin PA-1 and carnobacteriocin X (Class IIa)); two-peptide bacteriocins (e.g., lactacin F and ABP-118 (Class IIb)); circular bacteriocins (e.g., carnocyclin A and enterocin AS-48 (Class IIc)); or unmodified, linear, non-pediocin-like bacteriocins (e.g., epidermicin NI01 and lactococcin A (Class IId)).

In some instances, the bacteriocin is a Class III bacteriocin (e.g., produced by Gram-positive bacteria). Class III bacteriocins may have a molecular weight greater than10 kDa and may be heat unstable proteins. The Class III bacteriocins can be further subdivided into Group IIIA and Group IIIB bacteriocins. The Group IIIA bacteriocins include bacteriolytic enzymes which kill sensitive strains by lysis of the cell well, such as Enterolisin A. Group IIIB bacteriocins include non-lytic proteins, such as Caseicin 80, Helveticin J, and lactacin B.

In some instances, the bacteriocin is a Class IV bacteriocin (e.g., produced by Gram-positive bacteria). Class IV bacteriocins are a group of complex proteins, associated with other lipid or carbohydrate moieties, which appear to be required for activity. They are relatively hydrophobic and heat stable. Examples of Class IV bacteriocins leuconocin S, lactocin 27, and lactocin S.

In some instances, the bacteriocin is an R-type bacteriocin. R-type bacteriocins are contractile bacteriocidal protein complexes. Some R-type bacteriocins have a contractile phage-tail-like structure. The C-terminal region of the phage tail fiber protein determines target-binding specificity. They may attach to target cells through a receptor-binding protein, e.g., a tail fiber. Attachment is followed by sheath contraction and insertion of the core through the envelope of the target bacterium. The core penetration results in a rapid depolarization of the cell membrane potential and prompt cell death. Contact with a single R-type bacteriocin particle can result in cell death. An R-type bacteriocin, for

example, may be thermolabile, mild acid resistant, trypsin resistant, sedimentable by centrifugation, resolvable by electron microscopy, or a combination thereof. Other R-type bacteriocins may be complex molecules including multiple proteins, polypeptides, or subunits, and may resemble a tail structure of bacteriophages of the myoviridae family. In naturally occurring R-type bacteriocins, the subunit structures may be encoded by a bacterial genome, such as that of *C. difficile* or *P. aeruginosa* and form R-type bacteriocins to serve as natural defenses against other bacteria. In some instances, the R-type bacteriocin is a pyocin. In some instances, the pyocin is an R-pyocin, F-pyocin, or S-pyocin.

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.

In some instances, the bacteriocin may be bioengineered, according to standard methods, to modulate their bioactivity, e.g., increase or decrease or regulate, or to specify their target microorganisms. In other instances, the bacteriocin is produced by the translational machinery (e.g. a ribosome, etc.) of a microbial cell. In some instances, the bacteriocin is chemically synthesized. Some bacteriocins can be derived from a polypeptide precursor. The polypeptide precursor can undergo cleavage (e.g., processing by a protease) to yield the polypeptide of the bacteriocin itself. As such, in some instances, the bacteriocin is produced from a precursor polypeptide. In some other instances, the bacteriocin includes a polypeptide that has undergone post-translational modifications, for example, cleavage, or the addition of one or more functional groups.

The bacteriocins described herein may be formulated in a composition for any of the uses described herein. The compositions disclosed herein may include any number or type (e.g., classes) of bacteriocins, such as at least about any one of 1 bacteriocin, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 100, or more bacteriocins. Suitable concentrations of each bacteriocin in the compositions described herein depends on factors such as efficacy, stability of the bacteriocin, number of distinct bacteriocin types in the compositions, formulation, and methods of application of the composition. In some instances, each bacteriocin in a liquid composition is from about 0.01 ng/ml to about 100 mg/mL. In some instances, each bacteriocin in a solid composition is from about 0.01 ng/g to about 100 mg/g. In some instances, wherein the composition includes at least two types of bacteriocins, the concentration of each type of the bacteriocins may be the same or different. In some instances, the bacteriocin is provided in a composition including a bacterial cell that secretes the bacteriocin. In some instances, the bacteriocin is provided in a composition including a polypeptide (e.g., a polypeptide isolated from a bacterial cell).

Bacteriocins may neutralize (e.g., kill) at least one microorganism other than the individual bacterial cell in which the polypeptide is made, including cells clonally related to the bacterial cell and other microbial cells. As such, a bacterial cell may exert cytotoxic or growth-inhibiting effects on a plurality of microbial organisms by secreting bacteriocins. In some instances, the bacteriocin targets and kills one or more species of bacteria resident in the host via cytoplasmic membrane pore formation, cell wall interference (e.g., peptidoglycanase activity), or nuclease activity (e.g., DNase activity, 16S rRNase activity, or tRNase activity).

In some instances, the bacteriocin has a neutralizing activity. Neutralizing activity of bacteriocins may include, but is not limited to, arrest of microbial reproduction, or cytotoxicity. Some bacteriocins have cytotoxic activity, and thus can kill microbial organisms, for example bacteria, yeast, algae, and the like. Some bacteriocins can inhibit the reproduction of microbial organisms, for example bacteria, yeast, algae, and the like, for example by arresting the cell cycle.

In some instances, the bacteriocin has killing activity. The killing mechanism of bacteriocins is specific to each group of bacteriocins. In some instances, the bacteriocin has narrow-spectrum bioactivity. Bacteriocins are known for their very high potency against their target strains. Some bacteriocin activity is limited to strains that are closely related to the bacteriocin producer strain (narrow-spectrum bioactivity). In some instances, the bacteriocin has broad-spectrum bioactivity against a wide range of genera.

In some instances, bacteriocins interact with a receptor molecule or a docking molecule on the target bacterial cell membrane. For example, nisin is extremely potent against its target bacterial strains, showing antimicrobial activity even at a single-digit nanomolar concentration. The nisin molecule has been shown to bind to lipid II, which is the main transporter of peptidoglycan subunits from the cytoplasm to the cell wall

In some instances, the bacteriocin has anti-fungal activity. A number of bacteriocins with anti-yeast or anti-fungal activity have been identified. For example, bacteriocins from *Bacillus* have been shown to have neutralizing activity against some yeast strains (see, for example, Adetunji and Olaoye, *Malaysian Journal of Microbiology* 9:130-13, 2013). In another example, an *Enterococcus faecalis* peptide has been shown to have neutralizing activity against *Candida* species (see, for example, Shekh and Roy, *BMC Microbiology* 12:132, 2012). In another example, bacteriocins from *Pseudomonas* have been shown to have neutralizing activity against fungi, such as *Curvularia lunata*, *Fusarium species*, *Helminthosporium* species, and *Biopolaris* species (see, for example, Shalani and Srivastava, *The Internet Journal of Microbiology* Volume 5 Number 2, 2008). In another example, botrycidin AJ1316 and alirin B1 from *B. subtilis* have been shown to have antifungal activities.

A modulating agent including a bacteriocin as described herein can be contacted with the target host in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of bacteriocin concentration inside a target host; (b) reach a target level (e.g., a predetermined or threshold level) of bacteriocin concentration inside a target host gut; (c) reach a target level (e.g., a predetermined or threshold level) of bacteriocin concentration inside a target host bacteriocyte; (d) modulate the level, or an activity, of one or more microorganism (e.g., endoymbiont) in the target host; or/and (e) modulate fitness of the target host.

(b) Lysins

The modulating agent described herein may include a lysin (e.g., also known as a murein hydrolase or peptidoglycan autolysin). Any lysin suitable for inhibiting a bacterium resident in the host may be used. In some instances, the lysin is one that can be naturally produced by a bacterial cell. In some instances, the lysin is one that can be naturally produced by a bacteriophage. In some instances, the lysin is obtained from a phage that inhibits a bacterium resident in the host. In some instances, the

lysin is engineered based on a naturally occurring lysin. In some instances, the lysin is engineered to be secreted by a host bacterium, for example, by introducing a signal peptide to the lysin. In some instances, the lysin is used in combination with a phage holin. In some instances, a lysin is expressed by a recombinant bacterium host that is not sensitive to the lysin. In some instances, the lysin is used to
5 inhibit a Gram-positive or Gram-negative bacterium resident in the host.

The lysin may be any class of lysin and may have one or more substrate specificities. For example, the lysin may be a glycosidase, an endopeptidase, a carboxypeptidase, or a combination thereof. In some instances, the lysin cleaves the β-1-4 glycosidic bond in the sugar moiety of the cell wall, the amide bond connecting the sugar and peptide moieties of the bacterial cell wall, and/or the
10 peptide bonds between the peptide moieties of the cell wall. The lysin may belong to one or more specific lysin groups, depending on the cleavage site within the peptidoglycan. In some instances, the lysin is a *N*-acetyl- β-D-muramidase (e.g., lysozyme), lytic transglycosylase, *N*-acetyl-β-D-glucosaminidase, *N*-acetylmuramyl-L-alanine amidase, L,D-endopeptidase, D,D-endopeptidase, D,D-carboxypeptidase, L,D-carboxypeptidase, or L,D-transpeptidase. Non-limiting examples of lysins and
15 their activities are listed in Table 5.

Table 5: Examples of Lysins

Target Bacteria	**Producer**	**Lysins**	**Activity**	**Sequence**
S. pneumoniae	Cp1	Cpl-1	Muramidase	MVKKNDLFVDVSSHNGY DITGILEQMGTTNTIIKISES TTYLNPCLSAQVEQSNPI GFYHFARFGGDVAEAERE AQFFLDNVPMQVKYLVLD YEDDPSGDAQANTNACL RFMQMIADAGYKPIYYSY KPFTHDNVDYQQILAQFP NSLWIAGYGLNDGTANFE YFPSMDGIRWWQYSSNP FDKNIVLLDDEEDDKPKTA GTWKQDSKGWWFRRNN GSFPYNKWEKIGGVWYY FDSKGYCLTSEWLKDNEK WYYLKDNGAMATGWVLV GSEWYYMDDSGAMVTG WVKYKNNWYYMTNERGN MVSNEFIKSGKGWYFMNT NGELADNPSFTKEPDGLIT VA (SEQ ID NO: 18)

S. pneumoniae	Dp-1	Pal	Amidase	MGVDIEKGVAWMQARKG RVSYSMDFRDGPDSYDC SSSMYYALRSAGASSAG WAVNTEYMHAWLIENGY ELISENAPWDAKRGDIFIW GRKGASAGAGGHTGMFI DSDNIIHCNYAYDGISVND HDERWYYAGQPYYYVYR LTNANAQPAEKKLGWQK DATGFWYARANGTYPKD EFEYIEENKSWFYFDDQG YMLAEKWLKHTDGNWYW FDRDGYMATSWKRIGES WYYFNRDGSMVTGWIKY YDNWYYCDATNGDMKSN AFIRYNDGWYLLLPDGRL ADKPQFTVEPDGLITAKV (SEQ ID NO: 19)
S. pyogenes	C1	C1	Amidase	N/A
B. anthracis	γ	PlyG	Amidase	MEIQKKLVDPSKYGTKCP YTMKPKYITVHNTYNDAP AENEVSYMISNNNEVSFHI AVDDKKAIQGIPLERNAW ACGDGNGSGNRQSISVEI CYSKSGGDRYYKAEDNA VDVVRQLMSMYNIPIENV RTHQSWSGKYCPHRMLA EGRWGAFIQKVKNGNVAT TSPTKQNIIQSGAFSPYET PDVMGALTSLKMTADFIL QSDGLTYFISKPTSDAQLK AMKEYLDRKGWWYEVK (SEQ ID NO: 20)
B. anthracis	Ames prophage	PlyPH	Amidase	N/A
E. faecalis and E. faecium	Phi1	PlyV12	Amidase	N/A
S. aureus	MR11	MV-L	Endopeptidase and amidase	MQAKLTKKEFIEWLKTSE GKQFNVDLWYGFQCFDY

				ANAGWKVLFGLLLKGLGA KDIPFANNFDGLATVYQN TPDFLAQPGDMVVFGSNY GAGYGHVAWVIEATLDYII VYEQNWLGGGWTDRIEQ PGWGWEKVTRRQHAYDF PMWFIRPNFKSETAPRSI QSPTQASKKETAKPQPKA VELKIIKDVVKGYDLPKRG GNPKGIVIHNDAGSKGAT AEAYRNGLVNAPLSRLEA GIAHSYVSGNTVWQALDE SQVGWHTANQLGNKYYY GIEVCQSMGADNATFLKN EQATFQECARLLKKWGLP ANRNTIRLHNEFTSTSCPH RSSVLHTGFDPVTRGLLP EDKQLQLKDYFIKQIRVYM DGKIPVATVSNESSASSN TVKPVASAWKRNKYGTYY MEENARFTNGNQPITVRKI GPFLSCPVAYQFQPGGY CDYTEVMLQDGHVWVGY TWEGQRYYLPIRTWNGS APPNQILGDLWGEIS (SEQ ID NO: 21)
S. pyogenes	C1	PlyC	Amidase	N/A
S. agalactiae	B30	GBS lysin	Muramidase and endopeptidase	MVINIEQAIAWMASRKGK VTYSMDYRNGPSSYDCS SSVYFALRSAGASDNGW AVNTEYEHDWLIKNGYVLI AENTNWNAQRGDIFIWGK RGASAGAFGHTGMFVDP DNIIHCNYGYNSITVNNHD EIWGYNGQPYVYAYRYS GKQSNAKVDNKSVVSKFE KELDVNTPLSNSNMPYYE ATISEDYYVESKPDVNSTD KELLVAGTRVRVYEKVKG

				WARIGAPQSNQWVEDAY LIDATDM (SEQ ID NO: 22)
S. aureus	P68	Lys16	Endopeptidase	N/A
S. aureus	K	LysK	Amidase and endopeptidase	MAKTQAEINKRLDAYAKG TVDSPYRVKKATSYDPSF GVMEAGAIDADGYYHAQ CQDLITDYVLWLTDNKVR TWGNAKDQIKQSYGTGFK IHENKPSTVPKKGWIAVFT SGSYEQWGHIGIVYDGGN TSTFTILEQNWNGYANKK PTKRVDNYYGLTHFIEIPV KAGTTVKKETAKKSASKT PAPKKKATLKVSKNHINYT MDKRGKKPEGMVIHNDA GRSSGQQYENSLANAGY ARYANGIAHYYGSEGYVW EAIDAKNQIAWHTGDGTG ANSGNFRFAGIEVCQSMS ASDAQFLKNEQAVFQFTA EKFKEWGLTPNRKTVRLH MEFVPTACPHRSMVLHTG FNPVTQGRPSQAIMNKLK DYFIKQIKNYMDKGTSSST VVKDGKTSSASTPATRPV TGSWKKNQYGTWYKPEN ATFVNGNQPIVTRIGSPFL NAPVGGNLPAGATIVYDE VCIQAGHIWIGYNAYNGN RVYCPVRTCQGVPPNQIP GVAWGVFK (SEQ ID NO: 23)
L. monocytogenes	A118	Ply118	Amidase	MTSYYYSRSLANVNKLAD NTKAAARKLLDWSESNGI EVLIYETIRTKEQQAANVN SGASQTMRSYHLVGQAL DFVMAKGKTVDWGAYRS DKGKKFVAKAKSLGFEW

				GGDWSGFVDNPHLQFNY KGYGTDTFGKGASTSNSS KPSADTNTNSLGLVDYMN LNKLDSSFANRKKLATSY GIKNYSGTATQNTTLLAKL KAGKPHTPASKNTYYTEN PRKVKTLVQCDLYKSVDF TTKNQTGGTFPPGTVFTIS GMGKTKGGTPRLKTKSG YYLTANTKFVKKI (SEQ ID NO: 24)
L. monocytogenes	A511	Ply511	Amidase	MVKYTVENKIIAGLPKGKL KGANFVIAHETANSKSTID NEVSYMTRNWKNAFVTH FVGGGGRVVQVANVNYV SWGAGQYANSYSYAQVE LCRTSNATTFKKDYEVYC QLLVDLAKKAGIPITLDSG SKTSDKGIKSHKWVADKL GGTTHQDPYAYLSSWGIS KAQFASDLAKVSGGGNT GTAPAKPSTPAPKPSTPS TNLDKLGLVDYMNAKKMD SSYSNRDKLAKQYGIANY SGTASQNTTLLSKIKGGAP KPSTPAPKPSTSTAKKIYF PPNKGNWSVYPTNKAPV KANAIGAINPTKFGGLTYTI QKDRGNGVYEIQTDQFG RVQVYGAPSTGAVIKK (SEQ ID NO: 25)
L. monocytogenes	A500	Ply500	Endopeptidase	MALTEAWLIEKANRKLNA GGMYKITSDKTRNVIKKM AKEGIYLCVAQGYRSTAE QNALYAQGRTKPGAIVTN AKGGQSNHNYGVAVDLC LYTNDGKDVIWESTTSRW KKVVAAMKAEGFKWGGD WKSFKDYPHFELCDAVSG

				EKIPAATQNTNTNSNRYE GKVIDSAPLLPKMDFKSSP FRMYKVGTEFLVYDHNQY WYKTYIDDKLYYMYKSFC DVVAKKDAKGRIKVRIKSA KDLRIPVWNNIKLNSGKIK WYAPNVKLAWYNYRRGY LELWYPNDGWYYTAEYFL K (SEQ ID NO: 26)
S. pneumoniae	ΦDp-1	Pal, S	Endopeptidase and amidase	N/A
S. agalactiae	LambdaSa1 prophage	LambdaSa1	Glycosidase	MVINIEQAIAWMASRKGK VTYSMDYRNGPSSYDCS SSVYFALRSAGASDNGW AVNTEYEHDWLIKNGYVLI AENTNWNAQRGDIFIWGK RGASAGAFGHTGMFVDP DNIIHCNYGYNSITVNNHD EIWGYNGQPYVVYAYRYAR KQSNAKVDNQSVVSKFEK ELDVNTPLSNSNMPYYEA TISEDYYVESKPDVNSTDK ELLVAGTRVRVYEKVKGW ARIGAPQSNQWVEDAYLI DATDM (SEQ ID NO: 27)
S. agalactiae	LambdaSa2 prophage	LambdaSa2	Glycosidase and endopeptidase	MEINTEIAIAWMSARQGKV SYSMDYRDGPNSYDCSS SVYYALRSAGASSAGWA VNTEYMHDWLIKNGYELIA ENVDWNAVRGDIAIWGM RGHSSGAGGHVVMFIDPE NIIHCNWANNGITVNNYN QTAAASGWMYCYVYRLK SGASTQGKSLDTLVKETL AGNYGNGEARKAVLGNQ YEAVMSVINGKTTTNQKT VDQLVQEVIAGKHGNGEA

				RKKSLGSQYDAVQKRVTE LLKKQPSEPFKAQEVNKP TETKTSQTELTGQATATK EEGDLSFNGTILKKAVLDK ILGNCKKHDILPSYALTILH YEGLWGTSAVGKADNNW GGMTWTGQGNRPSGVTV TQGSARPSNEGGHYMHY ASVDDFLTDWFYLLRAGG SYKVSGAKTFSEAIKGMF KVGGAVYDYAASGFDSYI VGASSRLKAIEAENGSLD KFDKATDIGDGSKDKIDITI EGIEVTINGITYELTKKPV (SEQ ID NO: 28)
S. uberis	(ATCC700407) prophage	Ply700	Amidase	MTDSIQEMRKLQSIPVRY DMGDRYGNDADRDGRIE MDCSSAVSKALGISMTNN TETLQQALPAIGYGKIHDA VDGTFDMQAYDVIIWAPR DGSSSLGAFGHVLIATSPT TAIHCNYGSDGITENDYNY IWEINGRPREIVFRKGVTQ TQATVTSQFERELDVNAR LTVSDKPYYEATLSEDYY VEAGPRIDSQDKELIKAGT RVRVYEKLNGWSRINHPE SAQWVEDSYLVDATEM (SEQ ID NO: 29)
S. suis	SMP	LySMP	Glycosidase and endopeptidase	N/A
B. anthracis	Bcp1	PlyB	Muramidase	N/A
S. aureus	Phi11 and Phi12	Phi11 lysin	Amidase and endopeptidase	MQAKLTKNEFIEWLKTSE GKQFNVDLWYGFQCFDY ANAGWKVLFGLLLKGLGA KDIPFANNFDGLATVYQN TPDFLAQPGDMVVFGSNY

				GAGYGHVAWVIEATLDYII VYEQNWLGGGWTDGIEQ PGWGWEKVTRRQHAYDF PMWFIRPNFKSETAPRSV QSPTQAPKKETAKPQPKA VELKIIKDVVKGYDLPKRG SNPKGIVIHNDAGSKGATA EAYRNGLVNAPLSRLEAGI AHSYVSGNTVWQALDES QVGWHTANQIGNKYYYGI EVCQSMGADNATFLKNE QATFQECARLLKKWGLPA NRNTIRLHNEFTSTSCPH RSSVLHTGFDPVTRGLLP EDKRLQLKDYFIKQIRAYM DGKIPVATVSNESSASSN TVKPVASAWKRNKYGTYY MEESARFTNGNQPITVRK VGPFLSCPVGYQFQPGG YCDYTEVMLQDGHVWVG YTWEGQRYYLPIRTWNG SAPPNQILGDLWGEIS (SEQ ID NO: 30)
S. aureus	ΦH5	LysH5	Amidase and endopeptidase	MQAKLTKKEFIEWLKTSE GKQYNADGWYGFQCFDY ANAGWKALFGLLLKGVGA KDIPFANNFDGLATVYQN TPDFLAQPGDMVVFGSNY GAGYGHVAWVIEATLDYII VYEQNWLGGGWTDGVQ QPGSGWEKVTRRQHAYD FPMWFIRPNFKSETAPRS VQSPTQASKKETAKPQPK AVELKIIKDVVKGYDLPKR GSNPNFIVIHNDAGSKGAT AEAYRNGLVNAPLSRLEA GIAHSYVSGNTVWQALDE SQVGWHTANQIGNKYGY GIEVCQSMGADNATFLKN EQATFQECARLLKKWGLP

				ANRNTIRLHNEFTSTSCPH RSSVLHTGFDPVTRGLLP EDKRLQLKDYFIKQIRAYM DGKIPVATVSNDSSASSN TVKPVASAWKRNKYGTYY MEESARFTNGNQPITVRK VGPFLSCPVGYQFQPGG YCDYTEVMLQDGHVWVG YTWEGQRYYLPIRTWNG SAPPNQILGDLWGEIS (SEQ ID NO: 31)
S. warneri	ΦWMY	LysWMY	Amidase and endopeptidase	MKTKAQAKSWINSKIGKGI DWDGMYGYQCMDEAVD YIHHVTDGKVTMWGNAID APKNNFQGLCTVYTNTPE FRPAYGDVIVWSYGTFAT YGHIAIVVNPDPYGDLQYI TVLEQNWNGNGIYKTEFA TIRTHDYTGVSHFIRPKFA DEVKETAKTVNKLSVQKKI VTPKNSVERIKNYVKTSG YINGEHYELYNRGHKPKG VVIHNTAGTASATQEGQR LTNMTFQQLANGVAHVYI DKNTIYETLPEDRIAWHVA QQYGNTEFYGIEVCGSRN TDKEQFLANEQVAFQEAA RRLKSWGMRANRNTVRL HHTFSSTECPDMSMLLHT GYSMKNGKPTQDITNKCA DYFMKQINAYIDGKQPTST VVGSSSSNKLKAKNKDKS TGWNTNEYGTLWKKEHA TFTCGVRQGIVTRTTGPF TSCPQAGVLYYGQSVNY DTVCKQDGYVWISWTTS DGYDVWMPIRTWDRSTD KVSEIWGTIS (SEQ ID NO: 32)

Streptococci (GBS)	ΦNCTC 11261	PlyGBS	Muramidase and endopeptidase	MATYQEYKSRSNGNAYDI DGSFGAQCWDGYADYCK YLGLPYANCTNTGYARDI WEQRHENGILNYFDEVEV MQAGDVAIFMVVDGVTPY SHVAIFDSDAGGGYGWFL GQNQGGANGAYNIVKIPY SATYPTAFRPKVFKNAVT VTGNIGLNKGDYFIDVSAY QQADLTTTCQQAGTTKTII KVSESIAWLSDRHQQQAN TSDPIGYYHFGRFGGDSA LAQREADLFLSNLPSKKV SYLVIDYEDSASADKQAN TNAVIAFMDKIASAGYKPI YYSYKPFTLNNIDYQKIIAK YPNSIWIAGYPDYEVRTEP LWEFFPSMDGVRWWQFT SVGVAGGLDKNIVLLADD SSKMDIPKVDKPQELTFY QKLATNTKLDNSNVPYYE ATLSTDYYVESKPNASSA DKEFIKAGTRVRVYEKVN GWSRINHPESAQWVEDS YLVNATDM (SEQ ID NO: 33)
C. perfringens	Φ3626	Ply3626	Amidase	N/A
C. difficile	ΦCD27	CD27 lysin	Amidase	N/A
E. faecalis	Φ1	PlyV12	Amidase	N/A
A. naeslundii	ΦAv-1-	Av-1 lysin	Putative amidase/muramidase	N/A
L. gasseri	ΦgaY	LysgaY	Muramidase	N/A
S. aureus	ΦSA4	LysSA4	Amidase and endopeptidase	N/A
S. haemolyticus	ΦSH2	SH2	Amidase and endopeptidase	N/A
B. thuringiensis	ΦBtCS33	PlyBt33	Amidase	N/A

L. monocytogenes	ΦP40	PlyP40	Amidase	N/A
L. monocytogenes	ΦFWLLm3	LysZ5	Amidase	MVKYTVENKIIAGLPKGKLKGANFVIAHETANSKSTIDNEVSYMTRNWQNAFVTHFVGGGGRVVQVANVNYVSWGAGQYANSYSYAQVELCRTSNATTFKKDYEVYCQLLVDLAKKAGIPITLDSGSKTSDKGIKSHKWVADKLGGTTHQDPYAYLSSWGISKAQFASDLAKVSGGGNTGTAPAKPSTPSTNLDKLGLVDYMNAKKMDSSYSNRAKLAKQYGIANYSGTASQNTTLLSKIKGGAPKPSTPAPKPSTSTAKKIYFPPNKGNWSVYPTNKAPVKANAIGAINPTKFGGLTYTIQKDRGNGVYEIQTDQFGRVQVYGAPSTGAVIKK (SEQ ID NO: 34)
B. cereus	ΦBPS13	LysBPS13	Amidase	MAKREKYIFDVEAEVGKAAKSIKSLEAELSKLQKLNKEIDATGGDRTEKEMLATLKAAKEVNAEYQKMQRILKDLSKYSGKVSRKEFNDSKVINNAKTSVQGGKVTDSFGQMLKNMERQINSVNKQFDNHRKAMVDRGQQYTPHLKTNRKDSQGNSNPSMMGRNKSTTQDMEKAVDKFLNGQNEATTGLNQALYQLKEISKLNRRSESLSRRASASGYMSFQQYSNFTGDRRTVQQTYGGLKTANRERVLELSGQATGISKELDRLNSKKGLTAREGEERKKLMRQLEGIDAELTARKKLNSSLDETTSNMEKFNQSLVDAQV

				SVKPERGTMRGMMYERA PAIALAIGGAITATIGKLYS EGGNHSKAMRPDEMYVG QQTGAVGANWRPNRTAT MRSGLGNHLGFTGQEMM EFQSNYLSANGYHGAED MKAATTGQATFARATGLG SDEVKDFFNTAYRSGGID GNQTKQFQNAFLGAMKQ SGAVGREKDQLKALNGIL SSMSQNRTVSNQDMMRT VGLQSAISSSGVASLQGT KGGALMEQLDNGIREGFN DPQMRVLFGQGTKYQGM GGRAALRKQMEKGISDPD NLNTLIDASKASAGQDPA EQAEVLATLASKMGVNMS SDQARGLIDLQPSGKLTK ENIDKVMKEGLKEGSIESA KRDKAYSESKASIDNSSE AATAKQATELNDMGSKLR QANAALGGLPAPLYTAIAA VVAFTAAVAGSALMFKGA SWLKGGMASKYGGKGGK GGKGGGTGGGGGAGGA AATGAGAAAGAGGVGAA AAGEVGAGVAAGGAAAG AAAGGSKLAGVGKGFMK GAGKLMLPLGILMGASEIM QAPEEAKGSAIGSAVGGI GGGIAGGAATGAIAGSFL GPIGTAVGGIAGGIAGGFA GSSLGETIGGWFDSGPKE DASAADKAKADASAAALA AAAGTSGAVGSSALQSQ MAQGITGAPNMSQVGSM ASALGISSGAMASALGISS GQENQIQTMTDKENTNTK KANEAKKGDNLSYERENI SMYERVLTRAEQILAQAR

				AQNGIMGVGGGGTAGAG GGINGFTGGGKLQFLAAG QKWSSSNLQQHDLGFTD QNLTAEDLDKWIDSKAPQ GSMMRGMGATFLKAGQE YGLDPRYLIAHAAEESGW GTSKIARDKGNFFGIGAFD DSPYSSAYEFKDGTGSAA ERGIMGGAKWISEKYYGK GNTTLDKMKAAGYATNAS WAPNIASIMAGAPTGSGS GNVTATINVNVKGDEKVS DKLKNSSDMKKAGKDIGS LLGFYSREMTIA (SEQ ID NO: 35)
S. aureus	ΦGH15	LysGH15	Amidase and endopeptidase	MAKTQAEINKRLDAYAKG TVDSPYRIKKATSYDPSFG VMEAGAIDADGYYHAQC QDLITDYVLWLTDNKVRT WGNAKDQIKQSYGTGFKI HENKPSTVPKKGWIAVFT SGSYQQWGHIGIVYDGG NTSTFTILEQNWNGYANK KPTKRVDNYYGLTHFIEIP VKAGTTVKKETAKKSASK TPAPKKKATLKVSKNHINY TMDKRGKKPEGMVIHNDA GRSSGQQYENSLANAGY ARYANGIAHYYGSEGYVW EAIDAKNQIAWHTGDGTG ANSGNFRFAGIEVCQSMS ASDAQFLKNEQAVFQFTA EKFKEWGLTPNRKTVRLH MEFVPTACPHRSMVLHTG FNPVTQGRPSQAIMNKLK DYFIKQIKNYMDKGTSSST VVKDGKTSSASTPATRPV TGSWKKNQYGTWYKPEN ATFVNGNQPIVTRIGSPFL NAPVGGNLPAGATIVYDE

				VCIQAGHIWIGYNAYNGD RVYCPVRTCQGVPPNHIP GVAWGVFK (SEQ ID NO: 36)
S. aureus	ΦvB SauS-PLA88	HydH5	Endopeptidase and glycosidase	N/A
E. faecalis	ΦF168/08	Lys168	Endopeptidase	N/A
E. faecalis	ΦF170/08	Lys170	Amidase	N/A
S. aureus	ΦP-27/HP	P-27/HP	Nonspecified	N/A
C. perfringens	ΦSM101	Psm	Muramidase	N/A
C. sporogenes	Φ8074-B1	CS74L	Amidase	MKIGIDMGHTLSGADYGV VGLRPESVLTREVGTKVIY KLQKLGHVVVNCTVDKAS SVSESLYTRYYRANQANV DLFISIHFNATPGGTGTEV YTYAGRQLGEATRIRQEF KSLGLRDRGTKDGSGLAV IRNTKAKAMLVECCFCDN PNDMKLYNSESFSNAIVK GITGKLPNGESGNNNQG GNKVKAVVIYNEGADRRG AEYLADYLNCPTISNSRTF DYSCVEHVYAVGGKKEQ YTKYLKTLLSGANRYDTM QQILNFINGGK (SEQ ID NO: 37)
S. typhimurium	ΦSPN1S	SPN1S	Glycosidase	MDINQFRRASGINEQLAA RWFPHITTAMNEFGITKPD DQAMFIAQVGHESGGFTR LQENFNYSVNGLSGFIRA GRITPDQANALGRKTYEK SLPLERQRAIANLVYSKR MGNNGPGDGWNYRGRG LIQITGLNNYRDCGNGLKV DLVAQPELLAQDEYAARS AAWFFSSKGCMKYTGDL

				VRVTQIINGGQNGIDDRRT RYAAARKVLAL (SEQ ID NO: 38)
C. michiganensis	ΦCMP1	CMP1	Peptidase	N/A
C. michiganensis	ΦCN77	CN77	Peptidase	MGYWGYPNGQIPNDKMA LYRGCLLRADAAAQAYAL QDAYTRATGKPLVILEGY RDLTRQKYLRNLYLSGRG NIAAVPGLSNHGWGLACD FAAPLNSSGSEEHRWMR QNAPLFGFDWARGKADN EPWHWEYGNVPVSRWA SLDVTPIDRNDMADITEGQ MQRIAVILLDTEIQTPLGPR LVKHALGDALLLGQANAN SIAEVPDKTWDVLVDHPL AKNEDGTPLKVRLGDVAK YEPLEHQNTRDAIAKLGTL QFTDKQLATIGAGVKPIDE ASLVKKIVDGVRALFGRAA A (SEQ ID NO: 39)
A. baumannii	ΦAB2	LysAB2	Glycosidase	MILTKDGFSIIRNELFGGKL DQTQVDAINFIVAKATESG LTYPEAAYLLATIYHETGL PSGYRTMQPIKEAGSDSY LRSKKYYPYIGYGYVQLT WKENYERIGKLIGVDLIKN PEKALEPLIAIQIAIKGMLN GWFTGVGFRRKRPVSKY NKQQYVAARNIINGKDKA ELIAKYAIIFERALRSL (SEQ ID NO: 40)
B. cereus	ΦB4	LysB4	Endopeptidase	MAMALQTLIDKANRKLNV SGMRKDVADRTRAVITQM HAQGIYICVAQGFRSFAE QNALYAQGRTKPGSIVTN ARGGQSNHNYGVAVDLC

				LYTQDGSDVIWTVEGNFR KVIAAMKAQGFKWGGDW VSFKDYPHFELYDVVGGQ KPPADNGGAVDNGGGSG STGGSGGGSTGGGSTGG GYDSSWFTKETGTFVTNT SIKLRTAPFTSADVIATLPA GSPVNYNGFGIEYDGYV WIRQPRSNGYGYLATGES KGGKRQNYWGTFK (SEQ ID NO: 41)
P. aeruginosa	ΦKMV	KMV45	Nonspecified	N/A
C. tyrobutyricum	ΦCTP1	Ctp1l	Glycosidase	MKKIADISNLNGNVDVKLL FNLGYIGIIAKASEGGTFV DKYYKQNYTNTKAQGKIT GAYHFANFSTIAKAQQEA NFFLNCIAGTTPDFVVLDL EQQCTGDITDACLAFLNIV AKKFKCVVYCNSSFIKEHL NSKICAYPLWIANYGVATP AFTLWTKYAMWQFTEKG QVSGISGYIDFSYITDEFIK YIKGEDEVENLVVYNDGA DQRAAEYLADRLACPTIN NARKFDYSNVKNVYAVG GNKEQYTSYLTTLIAGSTR YTTMQAVLDYIKNLK (SEQ ID NO: 42)
P. aeruginosa	ΦEL	EL188	Transglycosylase	N/A
P. aeruginosa	ΦKZ	KZ144	Transglycosylase	N/A
S. aureus	Staph-ylococcus virus 187	Ply187	Cell Wall Hydrolase	MALPKTGKPTAKQVVDW AINLIGSGVDVDGYYGRQ CWDLPNYIFNRYWNFKTP GNARDMAWYRYPEGFKV FRNTSDFVPKPGDIAVWT GGNYNWNTWGHTGIVVG PSTKSYFYSVDQNWNNS

				NSYVGSPAAKIKHSYFGV THFVRPAYKAEPKPTPPA QNNPAPKDPEPSKKPESN KPIYKVVTKILFTTAHIEHV KANRFVHYITKSDNHNNK PNKIVIKNTNTALSTIDVYR YRDELDKDEIPHFFVDRLN VWACRPIEDSINGYHDSV VLSITETRTALSDNFKMNE IECLSLAESILKANNKKMS ASNIIVDNKAWRTFKLHTG KDSLKSSSFTSKDYQKAV NELIKLFNDKDKLLNNKPK DVVERIRIRTIVKENTKFVP SELKPRNNIRDKQDSKIDR VINNYTLKQALNIQYKLNP KPQTSNGVSWYNASVNQI KSAMDTTKIFNNNVQVYQ FLKLNQYQGIPVDKLNKLL VGKGTLANQGHAFADGC KKYNINEIYLIAHRFLESAN GTSFFASGKTGVYNYFGI GAFDNNPNNAMAFARSH GWTSPTKAIIGGAEFVGK GYFNVGQNTLYRMRWNP QKPGTHQYATDISWAKVQ AQMISAMYKEIGLTGDYFI YDQYKK (SEQ ID NO: 43)
P. uorescens	ΦOBP	OBPgp279	Glycosidase	N/A
L. monocytogenes	ΦP35	PlyP35	Amidase	MARKFTKAELVAKAEKKV GGLKPDVKKAVLSAVKEA YDRYGIGIIVSQGYRSIAE QNGLYAQGRTKPGNIVTN AKGGQSNHNFGVAVDFAI DLIDDGKIDSWQPSATIVN MMKRRGFKWGGDWKSF TDLPHFEACDWYRGERK YKVDTSEWKKKENINIVIK DVGYFQDKPQFLNSKSVR

				QWKHGTKVKLTKHNSHW YTGVVKDGNKSVRGYIYH SMAKVTSKNSDGSVNATI NAHAFCWDNKKLNGGDFI NLKRGFKGITHPASDGFY PLYFASRKKTFYIPRYMFD IKK (SEQ ID NO: 44)
L. fermentum	ΦPYB5	Lyb5	Muramidase	N/A
S. pneumoniae	ΦCP-7	Cpl-7	Muramidase	MVKKNDLFVDVASHQGY DISGILEEAGTTNTIIKVSE STSYLNPCLSAQVSQSNPI GFYHFAWFGGNEEEAEA EARYFLDNVPTQVKYLVL DYEDHASASVQRNTTACL RFMQIIAEAGYTPIYYSYK PFTLDNVDYQQILAQFPN SLWIAGYGLNDGTANFEY FPSMDGIRWWQYSSNPF DKNIVLLDDEKEDNINNEN TLKSLTTVANEVIQGLWG NGQERYDSLANAGYDPQ AVQDKVNEILNAREIADLT TVANEVIQGLWGNGQER YDSLANAGYDPQAVQDK VNEILNAREIADLTTVANE VIQGLWGNGQERYDSLA NAGYDPQAVQDKVNELLS (SEQ ID NO: 45)
P. chlororaphis201	Φ2-1	201φ2-1gp229	Glycosidase	N/A
S. enterica	ΦPVP-SE1)	PVP-SE1gp146	Glycosidase	N/A
Corynebacterium	ΦBFK20	BKF20	Amidase	N/A
E. faecalis	ΦEFAP-1	EFAL-1	Amidase	MKLKGILLSVVTTFGLLFG ATNVQAYEVNNEFNLQP WEGSQQLAYPNKIILHETA NPRATGRNEATYMKNNW FNAHTTAIVGDGGIVYKVA

				PEGNVSWGAGNANPYAP VQIELQHTNDPELFKANYK AYVDYTRDMGKKFGIPMT LDQGGSLWEKGVVSHQW VTDFVWGDHTDPYGYLA KMGISKAQLAHDLANGVS GNTATPTPKPDKPKPTQP SKPSNKKRFNYRVDGLEY VNGMWQIYNEHLGKIDFN WTENGIPVEVVDKVNPAT GQPTKDQVLKVGDYFNF QENSTGVVQEQTPYMGY TLSHVQLPNEFIWLFTDSK QALMYQ (SEQ ID NO: 46)
Lactobacilli	lambdaSA2	LysA, LysA2, and Lysga Y	Nonspecified	N/A
S. aureus		SAL-1	Nonspecified	N/A

In some instances, the lysin is a functionally active variant of the lysins described herein. In some instances, the variant of the lysin 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 lysin described herein or a naturally occurring lysin.

In some instances, the lysin may be bioengineered to modulate its bioactivity, e.g., increase or decrease or regulate, or to specify a target microorganism. In some instances, the lysin is produced by the translational machinery (e.g. a ribosome, etc.) of a microbial cell. In some instances, the lysin is chemically synthesized. In some instances, the lysin is derived from a polypeptide precursor. The polypeptide precursor can undergo cleavage (for example, processing by a protease) to yield the polypeptide of the lysin itself. As such, in some instances, the lysin is produced from a precursor polypeptide. In some instances, the lysin includes a polypeptide that has undergone post-translational modifications, for example, cleavage, or the addition of one or more functional groups.

The lysins described herein may be formulated in a composition for any of the uses described herein. The compositions disclosed herein may include any number or type (e.g., classes) of lysins, such as at least about any one of 1 lysin, 2, 3, 4, 5, 10, 15, 20, or more lysins. A suitable concentration of each lysin in the composition depends on factors such as efficacy, stability of the lysin, number of distinct lysin, the formulation, and methods of application of the composition. In some instances, each lysin in a liquid composition is from about 0.1 ng/mL to about 100 mg/mL. In some instances, each lysin in a solid

composition is from about 0.1 ng/g to about 100 mg/g. In some instances, wherein the composition includes at least two types of lysins, the concentration of each type of lysin may be the same or different.

A modulating agent including a lysin as described herein can be contacted with the target host in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of lysin concentration inside a target host; (b) reach a target level (e.g., a predetermined or threshold level) of lysin concentration inside a target host gut; (c) reach a target level (e.g., a predetermined or threshold level) of lysin concentration inside a target host bacteriocyte; (d) modulate the level, or an activity, of one or more microorganism (e.g., endosymbiont) in the target host; or/and (e) modulate fitness of the target host.

(c) Antimicrobial Peptides

The modulating agent described herein may include an antimicrobial peptide (AMP). Any AMP suitable for inhibiting a microorganism resident in the host may be used. AMPs are a diverse group of molecules, which are divided into subgroups on the basis of their amino acid composition and structure. The AMP may be derived or produced from any organism that naturally produces AMPs, including AMPs derived from plants (e.g., copsin), insects (e.g., drosocin, scorpion peptide (e.g., Uy192, UyCT3, D3, D10, Uy17, Uy192), mastoparan, poneratoxin, cecropin, moricin, melittin), frogs (e.g., magainin, dermaseptin, aurein), and mammals (e.g., cathelicidins, defensins and protegrins). For example, the AMP may be a scorpion peptide, such as Uy192 (5'- FLSTIWNGIKGLL-3'; SEQ ID NO: 193), UyCT3 (5'- LSAIWSGIKSLF-3; SEQ ID NO: 194'), D3 (5'- LWGKLWEGVKSLI-3'; SEQ ID NO: 195), and D10 (5'- FPFLKLSLKIPKSAIKSAIKRL-3'; SEQ ID NO: 196), Uy17 (5'- ILSAIWSGIKGLL-3'; SEQ ID NO: 197). Other non-limiting examples of AMPs are listed in Table 6.

Table 6: Examples of Antimicrobial Peptides

Type	Characteristic	Example AMP	Sequence
Anionic peptides	rich in glutamic and aspartic acid	dermcidin	SSLLEKGLDGAKKAVGGLGKL GKDAVEDLESVGKGAVHDVKD VLDSVL (SEQ ID NO: 47)
Linear cationic α-helical peptides	lack cysteine	cecropin A	KWKLFKKIEKVGQNIRDGIIKAG PAVAVVGQATQIAK (SEQ ID NO: 48)
		andropin	MKYFSVLVVLTLILAIVDQSDAFI NLLDKVEDALHTGAQAGFKLIR PVERGATPKKSEKPEK (SEQ ID NO: 49)

		moricin	MNILKFFFVFIVAMSLVSCSTAA PAKIPIKAIKTVGKAVGKGLRAI NIASTANDVFNFLKPKKRKH (SEQ ID NO: 50)
		ceratotoxin	MANLKAVFLICIVAFIALQCVVA EPAAEDSVVVKRSIGSALKKAL PVAKKIGKIALPIAKAALPVAAG LVG (SEQ ID NO: 51)
Cationic peptide enriched for specific amino acid	rich in proline, arginine, phenylalanine, glycine, tryptophan	abaecin	MKVVIFIFALLATICAAFAYVPLP NVPQPGRRPFPTFPGQGPFNP KIKWPQGY (SEQ ID NO: 52)
		apidaecins	KNFALAILVVTFVVAVFGNTNLD PPTRPTRLRREAKPEAEPGNN RPVYIPQPRPPHPRLRREAEPE AEPGNNRPVYIPQPRPPHPRL RREAELEAEPGNNRPVYISQP RPPHPRLRREAEPEAEPGNNR PVYIPQPRPPHPRLRREAELEA EPGNNRPVYISQPRPPHPRLR REAEPEAEPGNNRPVYIPQPR PPHPRLRREAEPEAEPGNNRP VYIPQPRPPHPRLRREAEPEAE PGNNRPVYIPQPRPPHPRLRR EAKPEAKPGNNRPVYIPQPRP PHPRI (SEQ ID NO: 53)
		prophenin	METQRASLCLGRWSLWLLLLA LVVPSASAQALSYREAVLRAVD RLNEQSSEANLYRLLELDQPPK ADEDPGTPKPVSFTVKETVCP RPTRRPPELCDFKENGRVKQC VGTVTLDQIKDPLDITCNEGVR RFPWWWPFLRRPRLRRQAFP PPNVPGPRFPPPNVPGPRFPP PNFPGPRFPPPNFPGPRFPPP

			NFPGPPFPPPIFPGPWFPPPPP FRPPPFGPPRFPGRR (SEQ ID NO: 54)
		indolicidin	MQTQRASLSLGRWSLWLLLLG LVVPSASAQALSYREAVLRAVD QLNELSSEANLYRLLELDPPPK DNEDLGTRKPVSFTVKETVCP RTIQQPAEQCDFKEKGRVKQC VGTVTLDPSNDQFDLNCNELQ SVILPWKWPWWPWRRG (SEQ ID NO: 55)
Anionic and cationic peptides that contain cysteine and form disulfide bonds	contain 1-3 disulfide bond	protegrin	METQRASLCLGRWSLWLLLLA LVVPSASAQALSYREAVLRAVD RLNEQSSEANLYRLLELDQPPK ADEDPGTPKPVSFTVKETVCP RPTRQPPELCDFKENGRVKQC VGTVTLDQIKDPLDITCNEVQG VRGGRLCYCRRRFCVCVGRG (SEQ ID NO: 56)
		tachyplesins	KWCFRVCYRGICYRRCR (SEQ ID NO: 57)
		defensin	MKCATIVCTIAVVLAATLLNGSV QAAPQEEAALSGGANLNTLLD ELPEETHHAALENYRAKRATC DLASGFGVGSSLCAAHCIARR YRGGYCNSKAVCVCRN (SEQ ID NO: 58)
		drosomycin	MMQIKYLFALFAVLMLVVLGAN EADADCLSGRYKGPCAVWDN ETCRRVCKEEGRSSGHCSPSL KCWCEGC (SEQ ID NO: 59)

The AMP may be active against any number of target microorganisms. In some instances, the AMP may have antibacterial and/or antifungal activities. In some instances, the AMP may have a narrow-spectrum bioactivity or a broad-spectrum bioactivity. For example, some AMPs target and kill only a few

species of bacteria or fungi, while others are active against both gram-negative and gram-positive bacteria as well as fungi.

Further, the AMP may function through a number of known mechanisms of action. For example, the cytoplasmic membrane is a frequent target of AMPs, but AMPs may also interfere with DNA and protein synthesis, protein folding, and cell wall synthesis. In some instances, AMPs with net cationic charge and amphipathic nature disrupt bacterial membranes leading to cell lysis. In some instances, AMPs may enter cells and interact with intracellular target to interfere with DNA, RNA, protein, or cell wall synthesis. In addition to killing microorganisms, AMPs have demonstrated a number of immunomodulatory functions that are involved in the clearance of infection, including the ability to alter host gene expression, act as chemokines and/or induce chemokine production, inhibit lipopolysaccharide induced pro-inflammatory cytokine production, promote wound healing, and modulating the responses of dendritic cells and cells of the adaptive immune response.

In some instances, the AMP is a functionally active variant of the AMPs described herein. In some instances, the variant of the AMP 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 an AMP described herein or a naturally derived AMP.

In some instances, the AMP may be bioengineered to modulate its bioactivity, e.g., increase or decrease or regulate, or to specify a target microorganism. In some instances, the AMP is produced by the translational machinery (e.g. a ribosome, etc.) of a cell. In some instances, the AMP is chemically synthesized. In some instances, the AMP is derived from a polypeptide precursor. The polypeptide precursor can undergo cleavage (for example, processing by a protease) to yield the polypeptide of the AMP itself. As such, in some instances, the AMP is produced from a precursor polypeptide. In some instances, the AMP includes a polypeptide that has undergone post-translational modifications, for example, cleavage, or the addition of one or more functional groups.

The AMPs described herein may be formulated in a composition for any of the uses described herein. The compositions disclosed herein may include any number or type (e.g., classes) of AMPs, such as at least about any one of 1 AMP, 2, 3, 4, 5, 10, 15, 20, or more AMPs. A suitable concentration of each AMP in the composition depends on factors such as efficacy, stability of the AMP, number of distinct AMP in the composition, the formulation, and methods of application of the composition. In some instances, each AMP in a liquid composition is from about 0.1 ng/mL to about 100 mg/mL. In some instances, each AMP in a solid composition is from about 0.1 ng/g to about 100 mg/g. In some instances, wherein the composition includes at least two types of AMPs, the concentration of each type of AMP may be the same or different.

A modulating agent including an AMP as described herein can be contacted with the target host in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of AMP concentration inside a target host; (b) reach a target level (e.g., a predetermined or threshold level) of AMP concentration inside a target host gut; (c) reach a target level (e.g., a predetermined or threshold level) of AMP concentration inside a target host bacteriocyte; (d) modulate the level, or an

activity, of one or more microorganism (e.g., endosymbiont) in the target host; or/and (e) modulate fitness of the target host.

(d) Nodule C-rich Peptides

5 The modulating agent described herein may include a nodule C-rich peptide (NCR peptide). NCR peptides are produced in certain leguminous plants and play an important role in the mutualistic, nitrogen-fixing symbiosis of the plants with bacteria from the *Rhizobiaceae* family (rhizobia), resulting in the formation of root nodules where plant cells contain thousands of intracellular endosymbionts. NCR peptides possess anti-microbial properties that direct an irreversible, terminal differentiation process of
10 bacteria, e.g., to permeabilize the bacterial membrane, disrupt cell division, or inhibit protein synthesis. For example, in *Medicago truncatula* nodule cells infected with *Sinorhizobium meliloti*, hundreds of NCR peptides are produced which direct irreversible differentiation of the bacteria into large polyploid nitrogen-fixing bacteroids.). Non-limiting examples of NCR peptides are listed in Table 7.

15 Table 7: Examples of NCR Peptides

NAME	Peptide sequence	Producer
>gi|152218086|gb|ABS31477.1| NCR 340	MTKIVVFIYVVILLLTIFHVSAKKKRYIECETHEDCSQVFMPPFVMRCVIHECKIFNGEHLRY (SEQ ID NO: 60)	*Medicago truncatula*
>gi|152218084|gb|ABS31476.1| NCR 339	MAKIMKFVYNMIPFLSIFIITLQVNVVVCEIDADCPQICMPPYEVRCVNHRCGWVNTDDSLFLTQEFTRSKQYIIS (SEQ ID NO: 61)	*Medicago truncatula*
>gi|152218082|gb|ABS31475.1| NCR 338	MYKVVESIFIRYMHRKPNMTKFFKFVYTMFILISLFLVVTNANAHNCTDISDCSSNHCSYEGVSLCMNGQCICIYE (SEQ ID NO: 62)	*Medicago truncatula*
>gi|152218080|gb|ABS31474.1| NCR 337	MVETLRLFYIMILFVSLCLVVVDGESKLEQTCSEDFECYIKNPHVPFGHLRCFEGFCQQLNGPA (SEQ ID NO: 63)	*Medicago truncatula*
>gi|152218078|gb|ABS31473.1| NCR 336	MAKIVNFVYSMIVFLFLFLVATKAARGYLCVTDSHCPPHMCPPGMEPRCVRRMCKCLPIGWRKYFVP (SEQ ID NO: 64)	*Medicago truncatula*
>gi|152218076|gb|ABS31472.1| NCR 335	MQIGKNMVETPKLDYVIIFFFLYFFFRQMIILRLNTTFRPLNFKMLRFWGQNRNIMKHRGQKVHFSLILSDCKTNK	*Medicago truncatula*

	DCPKLRRANVRCRKSYCVPI (SEQ ID NO: 65)	
>gi|152218074|gb|ABS31471.1| NCR 334	MLRLYLVSYFLLKRTLLVSYFSYFST YIIECKTDNDCPISQLKIYAWKCVKN GCHLFDVIPMMYE (SEQ ID NO: 66)	*Medicago truncatula*
>gi|152218072|gb|ABS31470.1| NCR 333	MAEILKFVYIVILFVSLLLIVVASEREC VTDDDCEKLYPTNEYRMMCDSGYC MNLLNGKIIYLLCLKKKKFLIIISVLL (SEQ ID NO: 67)	*Medicago truncatula*
>gi|152218070|gb|ABS31469.1| NCR 332	MAEIIKFVYIMILCVSLLLIEVAGEECV TDADCDKLYPDIRKPLMCSIGECYSL YKGKFSLSIISKTSFSLMVYNVVTLVI CLRLAYISLLLKFL (SEQ ID NO: 68)	*Medicago truncatula*
>gi|152218068|gb|ABS31468.1| NCR 331	MAEILKDFYAMNLFIFLIILPAKIRGET LSLTHPKCHHIMLPSLFITEVFQRVT DDGCPKPVNHLRVVKCIEHICEYGY NYRPDFASQIPESTKMPRKRE (SEQ ID NO: 69)	*Medicago truncatula*
>gi|152218066|gb|ABS31467.1| NCR 330	MVEILKNFYAMNLFIFLIILAVKIRGAH FPCVTDDDCPKPVNKLRVIKCIDHIC QYARNLPDFASEISESTKMPCKGE (SEQ ID NO: 70)	*Medicago truncatula*
>gi|152218064|gb|ABS31466.1| NCR 329	MFHAQAENMAKVSNFVCIMILFLALF FITMNDAARFECREDSHCVTRIKCV LPRKPECRNYACGCYDSNKYR (SEQ ID NO: 71)	*Medicago truncatula*
>gi|152218062|gb|ABS31465.1| NCR 328	MQMRQNMATILNFVFVIILFISLLLVV TKGYREPFSSFTEGPTCKEDIDCPSI SCVNPQVPKCIMFECHCKYIPTTLK (SEQ ID NO: 72)	*Medicago truncatula*
>gi|152218060|gb|ABS31464.1| NCR 327	MATILMYVYITILFISILTVLTEGLYEPL YNFRRDPDCRRNIDCPSYLCVAPKV PRCIMFECHCKDIPSDH (SEQ ID NO: 73)	*Medicago truncatula*
>gi|152218058|gb|ABS31463.1| NCR 326	MTTSLKFVYVAILFLSLLLVVMGGIR RFECRQDSDCPSYFCEKLTVPKCF WSKCYCK (SEQ ID NO: 74)	*Medicago truncatula*
>gi|152218056|gb|ABS31462.1| NCR 325	MTTSLKFVYVAILFLSLLLVVMGGIR KKECRQDSDCPSYFCEKLTIAKCIHS TCLCK (SEQ ID NO: 75)	*Medicago truncatula*

<table>
<tr><td>>gi|152218054|gb|ABS31461.1| NCR 324</td><td>MQIGKNMVETPKLVYFIILFLSIFLCITVSNSSFSQIFNSACKTDKDCPKFGRVNVRCRKGNCVPI (SEQ ID NO: 76)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218046|gb|ABS31457.1| NCR 320</td><td>MTAILKKFINAVFLFIVLFLATTNVEDFVGGSNDECVYPDVFQCINNICKCVSHHRT (SEQ ID NO: 77)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218044|gb|ABS31456.1| NCR 319</td><td>MQKRKNMAQIIFYVYALIILFSPFLAARLVFVNPEKPCVTDADCDRYRHESAIYSDMFCKDGYCFIDYHHDPYP (SEQ ID NO: 78)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218042|gb|ABS31455.1| NCR 318</td><td>MQMRKNMAQILFYVYALLILFTPFLVARIMVVNPNNPCVTDADCQRYRHKLATRMICNQGFCLMDFTHDPYAPSLP (SEQ ID NO: 79)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218040|gb|ABS31454.1| NCR 317</td><td>MNHISKFVYALIIFLSIYLVVLDGLPISCKDHFECRRKINILRCIYRQEKPMCINSICTCVKLL (SEQ ID NO: 80)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218038|gb|ABS31453.1| NCR 316</td><td>MQREKNMAKIFEFVYAMIIFILLFLVEKNVVAYLKFECKTDDDCQKSLLKTYVWKCVKNECYFFAKK (SEQ ID NO: 81)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218036|gb|ABS31452.1| NCR 315</td><td>MAGIIKFVHVLIIFLSLFHVVKNDDGSFCFKDSDCPDEMCPSPLKEMCYFLQCKCGVDTIA (SEQ ID NO: 82)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218034|gb|ABS31451.1| NCR 314</td><td>MANTHKLVSMILFIFLFLASNNVEGYVNCETDADCPPSTRVKRFKCVKGECRWTRMSYA (SEQ ID NO: 83)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218032|gb|ABS31450.1| NCR 313</td><td>MQRRKKKAQVVMFVHDLIICIYLFIVITTRKTDIRCRFYYDCPRLEYHFCECIEDFCAYIRLN (SEQ ID NO: 84)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218030|gb|ABS31449.1| NCR 312</td><td>MAKVYMFVYALIIFVSPFLLATFRTRLPCEKDDDCPEAFLPPVMKCVNRFCQYEILE (SEQ ID NO: 85)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218028|gb|ABS31448.1| NCR 310</td><td>MIKQFSVCYIQMRRNMTTILKFPYIMVICLLLLHVAAYEDFEKEIFDCKKDGDCDHMCVTPGIPKCTGYVCFCFENL (SEQ ID NO: 86)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218026|gb|ABS31447.1| NCR 309</td><td>MQRSRNMTTIFKFAYIMIICVFLLNIAAQEIENGIHPCKKNEDCNHMCVMP</td><td>Medicago truncatula</td></tr>
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<tr><td></td><td>GLPWCHENNLCFCYENAYGNTR (SEQ ID NO: 87)</td><td></td></tr>
<tr><td>>gi|152218024|gb|ABS31446.1| NCR 304</td><td>MTIIIKFVNVLIIFLSLFHVAKNDDNKLLLSFIEEGFLCFKDSDCPYNMCPSPLKEMCYFIKCVCGVYGPIRERRLYQSHNPMIQ (SEQ ID NO: 88)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218022|gb|ABS31445.1| NCR 303</td><td>MRKNMTKILMIGYALMIFIFLSIAVSITGNLARASRKKPVDVIPCIYDHDCPRKLYFLERCVGRVCKYL (SEQ ID NO: 89)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218020|gb|ABS31444.1| NCR 301</td><td>MAHKLVYAITLFIFLFLIANNIEDDIFCITDNDCPPNTLVQRYRCINGKCNLSFVSYG (SEQ ID NO: 90)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218018|gb|ABS31443.1| NCR 300</td><td>MDETLKFVYILILFVSLCLVVADGVKNINRECTQTSDCYKKYPFIPWGKVRCVKGRCRLDM (SEQ ID NO: 91)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218016|gb|ABS31442.1| NCR 290</td><td>MAKIIKFVYVLAIFFSLFLVAKNVNGWTCVEDSDCPANICQPPMQRMCFYGECACVRSKFCT (SEQ ID NO: 92)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218014|gb|ABS31441.1| NCR 289</td><td>MVKIIKFVYFMTLFLSMLLVTTKEDGSVECIANIDCPQIFMLPFVMRCINFRCQIVNSEDT (SEQ ID NO: 93)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218012|gb|ABS31440.1| NCR 286</td><td>MDEILKFVYTLIIFFSLFFAANNVDANIMNCQSTFDCPRDMCSHIRDVICIFKKCKCAGGRYMPQVP (SEQ ID NO: 94)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218008|gb|ABS31438.1| NCR 278</td><td>MQRRKNMANNHMLIYAMIICLFPYLVVTFKTAITCDCNEDCLNFFTPLDNLKCIDNVCEVFM (SEQ ID NO: 95)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218006|gb|ABS31437.1| NCR 266</td><td>MVNILKFIYVIIFFILMFFVLIDVDGHVLVECIENRDCEKGMCKFPFIVRCLMDQCKCVRIHNLI (SEQ ID NO: 96)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218004|gb|ABS31436.1| NCR 265</td><td>MIIQFSIYYMQRRKLNMVEILKFSHALIIFLFLSALVTNANIFFCSTDEDCTWNLCRQPWVQKCRLHMCSCEKN (SEQ ID NO: 97)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152218002|gb|ABS31435.1| NCR 263</td><td>MDEVFKFVYVMIIFPFLILDVATNAEKIRRCFNDAHCPPDMCTLGVIPKCSRFTICIC (SEQ ID NO: 98)</td><td>Medicago truncatula</td></tr>
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<tr><td>>gi|152217998|gb|ABS31433.1| NCR 239</td><td>MQMKKMATILKFVYLIILLIYPLLVVTEESHYMKFSICKDDTDCPTLFCVLPNVPKCIGSKCHCKLMVN (SEQ ID NO: 100)</td><td>Medicago truncatula</td></tr>
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<tr><td>>gi|152217992|gb|ABS31430.1| NCR 224</td><td>MRINRTPAIFKFVYTIIIYLFLLRVVAKDLPFNICEKDEDCLEFCAHDKVAKCMLNICFCF (SEQ ID NO: 103)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217990|gb|ABS31429.1| NCR 221</td><td>MAEILKILYVFIIFLSLILAVISQHPFTPCETNADCKCRNHKRPDCLWHKCYCY (SEQ ID NO: 104)</td><td>Medicago truncatula</td></tr>
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<tr><td>>gi|152217984|gb|ABS31426.1| NCR 206</td><td>MAEIIKFVYIMILCVSLLLIAEASGKECVTDADCENLYPGNKKPMFCNNTGYCMSLYKEPSRYM (SEQ ID NO: 107)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217982|gb|ABS31425.1| NCR 201</td><td>MAKIIKFVYIMILCVSLLLIVEAGGKECVTDVDCEKIYPGNKKPLICSTGYCYSLYEEPPRYHK (SEQ ID NO: 108)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217980|gb|ABS31424.1| NCR 200</td><td>MAKVTKFGYIIIHFLSLFFLAMNVAGGRECHANSHCVGKITCVLPQKPECWNYACVCYDSNKYR (SEQ ID NO: 109)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217978|gb|ABS31423.1| NCR 192</td><td>MAKIFNYVVYALIMFLSLFLMGTSGMKNGCKHTGHCPRKMCGAKTTKCRNNKCQCV (SEQ ID NO: 110)</td><td>Medicago truncatula</td></tr>
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<tr><td>>gi|152217972|gb|ABS31420.1| NCR 181</td><td>MYREKNMAKTLKFVYVIVLFLSLFLAAKNIDGRVSYNSFIALPVCQTAADCPEGTRGRTYKCINNKCRYPKLLKPIQ (SEQ ID NO: 113)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217970|gb|ABS31419.1| NCR 176</td><td>MAHIFNYVYALLVFLSLFLMVTNGIHIGCDKDRDCPKQMCHLNQTPKCLKNICKCV (SEQ ID NO: 114)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217968|gb|ABS31418.1| NCR 175</td><td>MAEILKCFYTMNLFIFLIILPAKIREHIQCVIDDDCPKSLNKLLIIKCINHVCQYVGNLPDFASQIPKSTKMPYKGE (SEQ ID NO: 115)</td><td>Medicago truncatula</td></tr>
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<tr><td>>gi|152217964|gb|ABS31416.1| NCR 172</td><td>MANVTKFVYIAIYFLSLFFIAKNDATATFCHDDSHCVTKIKCVLPRTPQCRNEACGCYHSNKFR (SEQ ID NO: 117)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217962|gb|ABS31415.1| NCR 171</td><td>MGEIMKFVYVMIIYLFMFNVATGSEFIFTKKLTSCDSSKDCRSFLCYSPKFPVCKRGICECI (SEQ ID NO: 118)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217960|gb|ABS31414.1| NCR 169</td><td>MGEMFKFIYTFILFVHLFLVVIFEDIGHIKYCGIVDDCYKSKKPLFKIWKCVENVCVLWYK (SEQ ID NO: 119)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217958|gb|ABS31413.1| NCR 165</td><td>MARTLKFVYSMILFLSLFLVANGLKIFCIDVADCPKDLYPLLYKCIYNKCIVFTRIPFPFDWI (SEQ ID NO: 120)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217956|gb|ABS31412.1| NCR 159</td><td>MANITKFVYIAILFLSLFFIGMNDAAILECREDSHCVTKIKCVLPRKPECRNNACTCYKGGFSFHH (SEQ ID NO: 121)</td><td>Medicago truncatula</td></tr>
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>gi|152217954|gb|ABS31411.1| NCR 147	MQRVKKMSETLKFVYVLILFISIFHVVIVCDSIYFPVSRPCITDKDCPNMKHYKAKCRKGFCISSRVR (SEQ ID NO: 122)	*Medicago truncatula*
>gi|152217952|gb|ABS31410.1| NCR 146	MQIRKIMSGVLKFVYAIILFLFLFLVAREVGGLETIECETDGDCPRSMIKMWNKNYRHKCIDGKCEWIKKLP (SEQ ID NO: 123)	*Medicago truncatula*
>gi|152217950|gb|ABS31409.1| NCR 145	MFVYDLILFISLILVVTGINAEADTSCHSFDDCPWVAHHYRECIEGLCAYRILY (SEQ ID NO: 124)	*Medicago truncatula*
>gi|152217948|gb|ABS31408.1| NCR 144	MQRRKKSMAKMLKFFFAIILLLSLFLVATEVGGAYIECEVDDDCPKPMKNSHPDTYYKCVKHRCQWAWK (SEQ ID NO: 125)	*Medicago truncatula*
>gi|152217946|gb|ABS31407.1| NCR 140	MFVYTLIIFLFPSHVITNKIAIYCVSDDDCLKTFTPLDLKCVDNVCEFNLRCKGKCGERDEKFVFLKALKKMDQKLVLEEQGNAREVKIPKKLLFDRIQVPTPATKDQVEEDDYDDDDEEEEEEEDDVDMWFHLPDVVCH (SEQ ID NO: 126)	*Medicago truncatula*
>gi|152217944|gb|ABS31406.1| NCR 138	MAKFSMFVYALINFLSLFLVETAITNIRCVSDDDCPKVIKPLVMKCIGNYCYFFMIYEGP (SEQ ID NO: 127)	*Medicago truncatula*
>gi|152217942|gb|ABS31405.1| NCR 136	MAHKFVYAIILFIFLFLVAKNVKGYVVCRTVDDCPPDTRDLRYRCLNGKCKSYRLSYG (SEQ ID NO: 128)	*Medicago truncatula*
>gi|152217940|gb|ABS31404.1| NCR 129	MQRKKNMGQILIFVFALINFLSPILVEMTTTTIPCTFIDDCPKMPLVVKCIDNFCNYFEIK (SEQ ID NO: 129)	*Medicago truncatula*
>gi|152217938|gb|ABS31403.1| NCR 128	MAQTLMLVYALIIFTSLFLVVISRQTDIPCKSDDACPRVSSHHIECVKGFCTYWKLD (SEQ ID NO: 130)	*Medicago truncatula*
>gi|152217936|gb|ABS31402.1| NCR 127	MLRRKNTVQILMFVSALLIYIFLFLVITSSANIPCNSDSDCPWKIYYTYRCNDGFCVYKSIDPSTIPQYMTDLIFPR (SEQ ID NO: 131)	*Medicago truncatula*
>gi|152217934|gb|ABS31401.1| NCR 122	MAVILKFVYIMIIFLFLLYVVNGTRCNRDEDCPFICTGPQIPKCVSHICFCLSSGKEAY (SEQ ID NO: 132)	*Medicago truncatula*

>gi|152217932|gb|ABS31400.1| NCR 121	MDAILKFIYAMFLFLFLFVTTRNVEALFECNRDFVCGNDDECVYPYAVQCIHRYCKCLKSRN (SEQ ID NO: 133)	*Medicago truncatula*
>gi|152217930|gb|ABS31399.1| NCR 119	MQIGRKKMGETPKLVYVIILFLSIFLCTNSSFSQMINFRGCKRDKDCPQFRGVNIRCRSGFCTPIDS (SEQ ID NO: 134)	*Medicago truncatula*
>gi|152217928|gb|ABS31398.1| NCR 118	MQMRKNMAQILFYVYALLILFSPFLVARIMVVNPNNPCVTDADCQRYRHKLATRMVCNIGFCLMDFTHDPYAPSLP (SEQ ID NO: 135)	*Medicago truncatula*
>gi|152217926|gb|ABS31397.1| NCR 111	MYVYYIQMGKNMAQRFMFIYALIIFLSQFFVVINTSDIPNNSNRNSPKEDVFCNSNDDCPTILYYVSKCVYNFCEYW (SEQ ID NO: 136)	*Medicago truncatula*
>gi|152217924|gb|ABS31396.1| NCR 103	MAKIVNFVYSMIIFVSLFLVATKGGSKPFLTRPYPCNTGSDCPQNMCPPGYKPGCEDGYCNHCYKRW (SEQ ID NO: 137)	*Medicago truncatula*
>gi|152217922|gb|ABS31395.1| NCR 101	MVRTLKFVYVIILILSLFLVAKGGGKKIYCENAASCPRLMYPLVYKCLDNKCVKFMMKSRFV (SEQ ID NO: 138)	*Medicago truncatula*
>gi|152217920|gb|ABS31394.1| NCR 96	MARTLKFVYAVILFLSLFLVAKGDDVKIKCVVAANCPDLMYPLVYKCLNGICVQFTLTFPFV (SEQ ID NO: 139)	*Medicago truncatula*
>gi|152217918|gb|ABS31393.1| NCR 94	MSNTLMFVITFIVLVTLFLGPKNVYAFQPCVTTADCMKTLKTDENIWYECINDFCIPFPIPKGRK (SEQ ID NO: 140)	*Medicago truncatula*
>gi|152217916|gb|ABS31392.1| NCR 93	MKRVVNMAKIVKYVYVIIIFLSLFLVATKIEGYYYKCFKDSDCVKLLCRIPLRPKCMYRHICKCKVVLTQNNYVLT (SEQ ID NO: 141)	*Medicago truncatula*
>gi|152217914|gb|ABS31391.1| NCR 90	MKRGKNMSKILKFIYATLVLYLFLVVTKASDDECKIDGDCPISWQKFHTYKCINQKCKWVLRFHEY (SEQ ID NO: 142)	*Medicago truncatula*
>gi|152217912|gb|ABS31390.1| NCR 88	MAKTLNFMFALILFISLFLVSKNVAIDIFVCQTDADCPKSELSMYTWKCIDNECNLFKVMQQMV (SEQ ID NO: 143)	*Medicago truncatula*

>gi|152217910|gb|ABS31389.1| NCR 86	MANTHKLVSMILFIFLFLVANNVEGYVNCETDADCPPSTRVKRFKCVKGECRWTRMSYA (SEQ ID NO: 144)	*Medicago truncatula*
>gi|152217908|gb|ABS31388.1| NCR 77	MAHFLMFVYALITCLSLFLVEMGHLSIHCVSVDDCPKVEKPITMKCINNYCKYFVDHKL (SEQ ID NO: 145)	*Medicago truncatula*
>gi|152217906|gb|ABS31387.1| NCR 76	MNQIPMFGYTLIIFFSLFPVITNGDRIPCVTNGDCPVMRLPLYMRCITYSCELFFDGPNLCAVERI (SEQ ID NO: 146)	*Medicago truncatula*
>gi|152217904|gb|ABS31386.1| NCR 74	MRKDMARISLFVYALIIFFSLFFVLTNGELEIRCVSDADCPLFPLPLHNRCIDDVCHLFTS (SEQ ID NO: 147)	*Medicago truncatula*
>gi|152217902|gb|ABS31385.1| NCR 68	MAQILMFVYFLIIFLSLFLVESIKIFTEHRCRTDADCPARELPEYLKCQGGMCRLLIKKD (SEQ ID NO: 148)	*Medicago truncatula*
>gi|152217900|gb|ABS31384.1| NCR 65	MARVISLFYALIIFLFLFLVATNGDLSPCLRSGDCSKDECPSHLVPKCIGLTCYCI (SEQ ID NO: 149)	*Medicago truncatula*
>gi|152217898|gb|ABS31383.1| NCR 62	MQRRKNMAQILLFAYVFIISISLFLVVTNGVKIPCVKDTDCPTLPCPLYSKCVDGFCKMLSI (SEQ ID NO: 150)	*Medicago truncatula*
>gi|152217896|gb|ABS31382.1| NCR 57	MNHISKFVYALIIFLSVYLVVLDGRPVSCKDHYDCRRKVKIVGCIFPQEKPMCINSMCTCIREIVP (SEQ ID NO: 151)	*Medicago truncatula*
>gi|152217894|gb|ABS31381.1| NCR 56	MKSQNHAKFISFYKNDLFKIFQNNDSHFKVFFALIIFLYTYLHVTNGVFVSCNSHIHCRVNNHKIGCNIPEQYLLCVNLFCLWLDY (SEQ ID NO: 152)	*Medicago truncatula*
>gi|152217892|gb|ABS31380.1| NCR 54	MTYISKVVYALIIFLSIYVGVNDCMLVTCEDHFDCRQNVQQVGCSFREIPQCINSICKCMKG (SEQ ID NO: 153)	*Medicago truncatula*
>gi|152217890|gb|ABS31379.1| NCR 53	MTHISKFVFALIIFLSIYVGVNDCKRIPCKDNNDCNNNWQLLACRFEREVPRCINSICKCMPM (SEQ ID NO: 154)	*Medicago truncatula*
>gi|152217888|gb|ABS31378.1| NCR 43	MVQTPKLVYVIVLLLSIFLGMTICNSSFSHFFEGACKSDKDCPKLHRSNVRCRKGQCVQI (SEQ ID NO: 155)	*Medicago truncatula*
>gi|152217886|gb|ABS31377.1| NCR 28	MTKILMLFYAMIVFHSIFLVASYTDECSTDADCEYILCLFPIIKRCIHNHCKCV	*Medicago truncatula*

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<tr><td>>gi|152217882|gb|ABS31375.1| NCR 24</td><td>MQKEKNMAKTFEFVYAMIIFILLFLVENNFAAYIIECQTDDDCPKSQLEMFAWKCVKNGCHLFGMYEDDDDP (SEQ ID NO: 158)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217880|gb|ABS31374.1| NCR 21</td><td>MAATRKFIYVLSHFLFLFLVTKITDARVCKSDKDCKDIIIYRYILKCRNGECVKIKI (SEQ ID NO: 159)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217878|gb|ABS31373.1| NCR 20</td><td>MQRLDNMAKNVKFIYVIILLLFIFLVIIVCDSAFVPNSGPCTTDKDCKQVKGYIARCRKGYCMQSVKRTWSSYSR (SEQ ID NO: 160)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217876|gb|ABS31372.1| NCR 19</td><td>MKFIYIMILFLSLFLVQFLTCKGLTVPCENPTTCPEDFCTPPMITRCINFICLCDGPEYAEPEYDGPEPEYDHKGDFLSVKPKIINENMMMRERHMMKEIEV (SEQ ID NO: 161)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217874|gb|ABS31371.1| NCR 12</td><td>MAQFLMFIYVLIIFLYLFYVEAAMFELTKSTIRCVTDADCPNVVKPLKPKCVDGFCEYT (SEQ ID NO: 162)</td><td>Medicago truncatula</td></tr>
<tr><td>>gi|152217872|gb|ABS31370.1| NCR 10</td><td>MKMRIHMAQIIMFFYALIIFLSPFLVDRRSFPSSFVSPKSYTSEIPCKATRDCPYELYYETKCVDSLCTY (SEQ ID NO: 163)</td><td>Medicago truncatula</td></tr>
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Any NCR peptide known in the art is suitable for use in the methods or compositions described herein. NCR peptide-producing plants include but are not limited to *Pisum sativum* (pea), *Astragalus sinicus* (IRLC legumes), *Phaseolus vulgaris* (bean), *Vigna unguiculata* (cowpea), *Medicago truncatula* (barrelclover), and *Lotus japonicus*. For example, over 600 potential NCR peptides are predicted from the *M. truncatula* genome sequence and almost 150 different NCR peptides have been detected in cells isolated from root nodules by mass spectrometry.

The NCR peptides described herein may be mature or immature NCR peptides. Immature NCR peptides have a C-terminal signal peptide that is required for translocation into the endoplasmic reticulum and cleaved after translocation. The N-terminus of a NCR peptide includes a signal peptide, which may be cleavable, for targeting to a secretory pathway. NCR peptides are generally small peptides with disulfide bridges that stabilize their structure. Mature NCR peptides have a length in the range of about

20 to about 60 amino acids, about 25 to about 55 amino acids, about 30 to about 50 amino acids, about 35 to about 45 amino acids, or any range therebetween. NCR peptides may include a conserved sequence of cysteine residues with the rest of the peptide sequence highly variable. NCR peptides generally have about four or eight cysteines.

5 NCR peptides may be anionic, neutral, or cationic. In some instances, synthetic cationic NCR peptides having a pI greater than about eight possess antimicrobial activities. For example, NCR247 (pI = 10.15, RNGCIVDPRCPYQQCRRPLYCRRR; SEQ ID NO: 164) and NCR335 (pI = 11.22) are both effective against gram-negative and gram-positive bacteria as well as fungi. In some instances, neutral and/or anionic NCR peptides, such as NCR001

10 (MAQFLLFVYSLIIFLSLFFGEAAFERTETRMLTIPCTSDDNCPKVISPCHTKCFDGFCGWYIEGSYEGP; SEQ ID NO: 165), do not possess antimicrobial activities at a pI greater than about 8.

In some instances, the NCR peptide is effective to kill bacteria. In some instances, the NCR peptide is effective to kill *S. meliloti, Xenorhabdus spp, Photorhabdus spp, Candidatus spp, Buchnera spp, Blattabacterium spp, Baumania spp, Wigglesworthia spp, Wolbachia spp, Rickettsia spp, Orientia*

15 *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,*

20 *Thermus spp, Pseudomonas spp, Clostridium spp,* or *Escherichia spp.*

In some instances, the NCR peptide is a functionally active variant of a NCR peptide described herein. In some instances, the variant of the NCR peptide 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

25 a sequence of a NCR peptide described herein or naturally derived NCR peptide.

In some instances, the NCR peptide may be bioengineered to modulate its bioactivity, e.g., increase or decrease or regulate, or to specify a target microorganism. In some instances, the NCR peptide is produced by the translational machinery (e.g. a ribosome, etc.) of a cell. In some instances, the NCR peptide is chemically synthesized. In some instances, the NCR peptide is derived from a

30 polypeptide precursor. The polypeptide precursor can undergo cleavage (for example, processing by a protease) to yield the NCR peptide itself. As such, in some instances, the NCR peptide is produced from a precursor polypeptide. In some instances, the NCR peptide includes a polypeptide that has undergone post-translational modifications, for example, cleavage, or the addition of one or more functional groups.

The NCR peptide described herein may be formulated in a composition for any of the uses

35 described herein. The compositions disclosed herein may include any number or type of NCR peptides, such as at least about any one of 1 NCR peptide, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 100, or more NCR peptides. A suitable concentration of each NCR peptide in the composition depends on factors such as efficacy, stability of the NCR peptide, number of distinct NCR peptide, the formulation, and methods of application of the composition. In some instances, each NCR peptide in a liquid composition is from

40 about 0.1 ng/mL to about 100 mg/mL. In some instances, each NCR peptide in a solid composition is

from about 0.1 ng/g to about 100 mg/g. In some instances, wherein the composition includes at least two types of NCR peptides, the concentration of each type of NCR peptide may be the same or different.

A modulating agent including a NCR peptide as described herein can be contacted with the target host in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of NCR peptide concentration inside a target host; (b) reach a target level (e.g., a predetermined or threshold level) of NCR peptide concentration inside a target host gut; (c) reach a target level (e.g., a predetermined or threshold level) of NCR peptide concentration inside a target host bacteriocyte; (d) modulate the level, or an activity, of one or more microorganism (e.g., endosymbiont) in the target host; or/and (e) modulate fitness of the target host.

(e) Bacteriocyte Regulatory Peptides

The modulating agent described herein may include a bacteriocyte regulatory peptide (BRP). BRPs are peptides expressed in the bacteriocytes of insects. These genes are expressed first at a developmental time point coincident with the incorporation of symbionts and their bacteriocyte-specific expression is maintained throughout the insect's life. In some instances, the BRP has a hydrophobic amino terminal domain, which is predicted to be a signal peptide. In addition, some BRPs have a cysteine-rich domain. In some instances, the bacteriocyte regulatory peptide is a bacteriocyte-specific cysteine rich (BCR) protein. Bacteriocyte regulatory peptides have a length between about 40 and 150 amino acids. In some instances, the bacteriocyte regulatory peptide has a length in the range of about 45 to about 145, about 50 to about 140, about 55 to about 135, about 60 to about 130, about 65 to about 125, about 70 to about 120, about 75 to about 115, about 80 to about 110, about 85 to about 105, or any range therebetween. Non-limiting examples of BRPs and their activities are listed in Table 8.

Table 8: Examples of Bacteriocyte Regulatory Peptides

Name	**Peptide Sequence**
Bacteriocyte-specific cysteine rich proteins BCR family, peptide BCR1	MKLLHGFLIIMLTMHLSIQYAYGGPFLTKYLCDRVCHKLC GDEFVCSCIQYKSLKGLWFPHCPTGKASVVLHNFLTSP (SEQ ID NO: 166)
Bacteriocyte-specific cysteine rich proteins BCR family, peptide BCR2	MKLLYGFLIIMLTIHLSVQYFESPFETKYNCDTHCNKLCGK IDHCSCIQYHSMEGLWFPHCRTGSAAQMLHDFLSNP (SEQ ID NO: 167)
Bacteriocyte-specific cysteine rich proteins BCR family, peptide BCR3	MSVRKNVLPTMFVVLLIMSPVTPTSVFISAVCYSGCGSLA LVCFVSNGITNGLDYFKSSAPLSTSETSCGEAFDTCTDH CLANFKF (SEQ ID NO: 168)
Bacteriocyte-specific cysteine rich proteins BCR family, peptide BCR4	MRLLYGFLIIMLTIYLSVQDFDPTEFKGPFPTIEICSKYCAV VCNYTSRPCYCVEAAKERDQWFPYCYD (SEQ ID NO: 169)

Bacteriocyte-specific cysteine rich proteins BCR family, peptide BCR5	MRLLYGFLIIMLTIHLSVQDIDPNTLRGPYPTKEICSKYCEYNVVCGASLPCICVQDARQLDHWFACCYDGGPEMLM (SEQ ID NO: 170)
Secreted proteins SP family, peptide SP1	MKLFVVVVLVAVGIMFVFASDTAAAPTDYEDTNDMISLSSLVGDNSPYVRVSSADSGGSSKTSSKNPILGLLKSVIKLLTKIFGTYSDAAPAMPPIPPALRKNRGMLA (SEQ ID NO: 171)
Secreted proteins SP family, peptide SP2	MVACKVILAVAVVFVAAVQGRPGGEPEWAAPIFAELKSVSDNITNLVGLDNAGEYATAAKNNLNAFAESLKTEAAVFSKSFEGKASASDVFKESTKNFQAVVDTYIKNLPKDLTLKDFTEKSEQALKYMVEHGTEITKKAQGNTETEKEIKEFFKKQIENLIGQGKALQAKIAEAKKA (SEQ ID NO: 172)
Secreted proteins SP family, peptide SP3	MKTSSSKVFASCVAIVCLASVANALPVQKSVAATTENPIVEKHGCRAHKNLVRQNVVDLKTYDSMLITNEVVQKQSNEVQSSEQSNEGQNSEQSNEGQNSEQSNEVQSSEHSNEGQNSKQSNEGQNSEQSNEVQSSEHSNEGQNSEQSNEVQSSEHSNEGQNSKQSNEGQNSKQSNEVQSSEHWNEGQNSKQSNEDQNSEQSNEGQNSKQSNEGQNSKQSNEDQNSEQSNEGQNSKQSNEVQSSEQSNEGQNSKQSNEGQSSEQSNEGQNSKQSNEVQSPEEHYDLPDPESSYESEETKGSHESGDDSEHR (SEQ ID NO: 173)
Secreted proteins SP family, peptide SP4	MKTIILGLCLFGALFWSTQSMPVGEVAPAVPAVPSEAVPQKQVEAKPETNAASPVSDAKPESDSKPVDAEVKPTVSEVKAESEQKPSGEPKPESDAKPVVASESKPESDPKPAAVVESKPENDAVAPETNNDAKPENAAAPVSENKPATDAKAETELIAQAKPESKPASDLKAEPEAAKPNSEVPVALPLNPTETKATQQSVETNQVEQAAPAAAQADPAAAPAADPAPAPAAAPVAAEEAKLSESAPSTENKAAEEPSKPAEQQSAKPVEDAVPAASEISETKVSPAVPAVPEVPASPSAPAVADPVSAPEAEKNAEPAKAANSAEPAVQSEAKPAEDIQKSGAVVSAENPKPVEEQKPAEVAKPAEQSKSEAPAEAPKPTEQSAAEEPKKPESANDEKKEQHSVNKRDATKEKKPTDSIMKKQKQKKAN (SEQ ID NO: 174)
Secreted proteins SP family, peptide SP5a	MNGKIVLCFAVVFIGQAMSAATGTTPEVEDIKKVAEQMSQTFMSVANHLVGITPNSADAQKSIEKIRTIMNKGFTDMETEANKMKDIVRKNADPKLVEKYDELEKELKKHLSTAKDMFEDKVVKPIGEKVELKKITENVIKTTKDMEATMNKAIDGFKKQ (SEQ ID NO: 175)
Secreted proteins SP family, peptide SP6	MHLFLALGLFIVCGMVDATFYNPRSQTFNQLMERRQRSIPIPYSYGYHYNPIEPSINVLDSLSEGLDSRINTFKPIYQNV

	KMSTQDVNSVPRTQYQPKNSLYDSEYISAKDIPSLFPEE DSYDYKYLGSPLNKYLTRPSTQESGIAINLVAIKETSVFDY GFPTYKSPYSSDSVWNFGSKIPNTVFEDPQSVESDPNTF KVSSPTIKIVKLLPETPEQESIITTTKNYELNYKTTQETPTE AELYPITSEEFQTEDEWHPMVPKENTTKDESSFITTEEPL TEDKSNSITIEKTQTEDESNSIEFNSIRTEEKSNSITTEENQ KEDDESMSTTSQETTTAFNLNDTFDTNRYSSSHESLMLR IRELMKNIADQQNKSQFRTVDNIPAKSQSNLSSDESTNQ QFEPQLVNGADTYK (SEQ ID NO: 176)
Colepotericin A, ColA peptide	MTRTMLFLACVAALYVCISATAGKPEEFAKLSDEAPSND QAMYESIQRYRRFVDGNRYNGGQQQQQQPKQWEVRP DLSRDQRGNTKAQVEINKKGDNHDINAGWGKNINGPDS HKDTWHVGGSVRW (SEQ ID NO: 177)
RlpA type I	MKETTVVWAKLFLILIILAKPLGLKAVNECKRLGNNSCRSH GECCSGFCFIEPGWALGVCKRLGTPKKSDDSNNGKNIEK NNGVHERIDDVFERGVCSYYKGPSITANGDVFDENEMTA AHRTLPFNTMVKVEGMGTSVVVKINDRKTAADGKVMLLS RAAAESLNIDENTGPVQCQLKFVLDGSGCTPDYGDTCVL HHECCSQNCFREMFSDKGFCLPK (SEQ ID NO: 192)

In some instances, the BRP alters the growth and/or activity of one or more bacteria resident in the bacteriocyte of the host. In some instances, the BRP may be bioengineered to modulate its bioactivity (e.g., increase, decrease, or regulate) or to specify a target microorganism. In some instances,
5 the BRP is produced by the translational machinery (e.g. a ribosome, etc.) of a cell. In some instances, the BRP is chemically synthesized. In some instances, the BRP is derived from a polypeptide precursor. The polypeptide precursor can undergo cleavage (for example, processing by a protease) to yield the polypeptide of the BRP itself. As such, in some instances, the BRP is produced from a precursor polypeptide. In some instances, the BRP includes a polypeptide that has undergone post-translational
10 modifications, for example, cleavage, or the addition of one or more functional groups.

Functionally active variants of the BRPs as described herein are also useful in the compositions and methods described herein. In some instances, the variant of the BRP 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
15 entire sequence, to a sequence of a BRP described herein or naturally derived BRP.

The BRP described herein may be formulated in a composition for any of the uses described herein. The compositions disclosed herein may include any number or type (e.g., classes) of BRPs, such as at least about any one of 1 BRP, 2, 3, 4, 5, 10, 15, 20, or more BRPs. A suitable concentration of each BRP in the composition depends on factors such as efficacy, stability of the BRP, number of distinct
20 BRP, the formulation, and methods of application of the composition. In some instances, each BRP in a liquid composition is from about 0.1 ng/mL to about 100 mg/mL. In some instances, each BRP in a solid

composition is from about 0.1 ng/g to about 100 mg/g. In some instances, wherein the composition includes at least two types of BRPs, the concentration of each type of BRP may be the same or different.

A modulating agent including a BRP as described herein can be contacted with the target host in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of BRP concentration inside a target host; (b) reach a target level (e.g., a predetermined or threshold level) of BRP concentration inside a target host gut; (c) reach a target level (e.g., a predetermined or threshold level) of BRP concentration inside a target host bacteriocyte; (d) modulate the level, or an activity, of one or more microorganism (e.g., endosymbiont) in the target host; or/and (e) modulate fitness of the target host.

iii. Small Molecules

Numerous small molecules (e.g., an antibiotic or a metabolite) may be used in the compositions and methods described herein. In some instances, an effective concentration of any small molecule described herein may alter the level, activity, or metabolism of one or more microorganisms (as described herein) resident in a host, the alteration resulting in an increase in the host's fitness.

A modulating agent comprising a small molecule as described herein can be contacted with the target host in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of a small molecule concentration inside a target host; (b) reach a target level (e.g., a predetermined or threshold level) of small molecule concentration inside a target host gut; (c) reach a target level (e.g., a predetermined or threshold level) of a small molecule concentration inside a target host bacteriocyte; (d) modulate the level, or an activity, of one or more microorganism (e.g., endosymbiont) in the target host; or/and (e) modulate fitness of the target host.

The small molecules discussed hereinafter, namely antibiotics and secondary metabolites, can be used to alter the level, activity, or metabolism of target microorganisms as indicated in the sections for increasing the fitness of instects, such as honeybees and silkworms.

(a) Antibiotics

The modulating agent described herein may include an antibiotic. Any antibiotic known in the art may be used. Antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity.

The antibiotic described herein may target any bacterial function or growth processes and may be either bacteriostatic (e.g., slow or prevent bacterial growth) or bactericidal (e.g., kill bacteria). In some instances, the antibiotic is a bactericidal antibiotic. In some instances, the bactericidal antibiotic is one that targets the bacterial cell wall (e.g., penicillins and cephalosporins); one that targets the cell membrane (e.g., polymyxins); or one that inhibits essential bacterial enzymes (e.g., rifamycins, lipiarmycins, quinolones, and sulfonamides). In some instances, the bactericidal antibiotic is an aminoglycoside. In some instances, the antibiotic is a bacteriostatic antibiotic. In some instances the bacteriostatic antibiotic targets protein synthesis (e.g., macrolides, lincosamides and tetracyclines). Additional classes of antibiotics that may be used herein include cyclic lipopeptides (such as daptomycin), glycylcyclines (such as tigecycline), oxazolidinones (such as linezolid), or lipiarmycins (such as

fidaxomicin). Examples of antibiotics include rifampicin, ciprofloxacin, doxycycline, ampicillin, and polymyxin B. Non-limiting examples of antibiotics are found in Table 9.

Table 9: Examples of Antibiotics

Antibiotics	**Action**
Penicillins, cephalosporins, vancomycin	Cell wall synthesis
Polymixin, gramicidin	Membrane active agent, disrupt cell membrane
Tetracyclines, macrolides, chloramphenicol, clindamycin, spectinomycin	Inhibit protein synthesis
Sulfonamides	Inhibit folate-dependent pathways
Ciprofloxacin	Inhibit DNA-gyrase
Isoniazid, rifampicin, pyrazinamide, ethambutol, (myambutol)l, streptomycin	Antimycobacterial agents

5

The antibiotic described herein may have any level of target specificity (e.g., narrow- or broad-spectrum). In some instances, the antibiotic is a narrow-spectrum antibiotic, and thus targets specific types of bacteria, such as gram-negative or gram-positive bacteria. Alternatively, the antibiotic may be a broad-spectrum antibiotic that targets a wide range of bacteria.

10 The antibiotics described herein may be formulated in a composition for any of the uses described herein. The compositions disclosed herein may include any number or type (e.g., classes) of antibiotics, such as at least about any one of 1 antibiotic, 2, 3, 4, 5, 10, 15, 20, or more antibiotics (e.g., a combination of rifampicin and doxycycline, or a combination of ampicillin and rifampicin). A suitable concentration of each antibiotic in the composition depends on factors such as efficacy, stability of the 15 antibiotic, number of distinct antibiotics, the formulation, and methods of application of the composition. In some instances, wherein the composition includes at least two types of antibiotics, the concentration of each type of antibiotic may be the same or different.

A modulating agent including an antibiotic as described herein can be contacted with the target host in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold 20 level) of antbiotic concentration inside a target host; (b) reach a target level (e.g., a predetermined or threshold level) of antibiotic concentration inside a target host gut; (c) reach a target level (e.g., a predetermined or threshold level) of antibiotic concentration inside a target host bacteriocyte; (d) modulate the level, or an activity, of one or more microorganism (e.g., endosymbiont) in the target host; or/and (e) modulate fitness of the target host.

25

(b) Secondary Metabolites

In some instances, the modulating agent of the compositions and methods described herein includes a secondary metabolite. Secondary metabolites are derived from organic molecules produced by an organism. Secondary metabolites may act (i) as competitive agents used against bacteria, fungi, 30 amoebae, plants, insects, and large animals; (ii) as metal transporting agents; (iii) as agents of symbiosis

between microbes and plants, nematodes, insects, and higher animals; (iv) as sexual hormones; and (v) as differentiation effectors. Non-limiting examples of secondary metabolites are found in Table 10.

Table 10: Examples of Secondary Metabolites

Phenyl-propanoids	**Alkaloids**	**Terpenoids**	**Quinones**	**Steroids**	**Polyketides**
Anthocyanins	Acridines	Carotenes	Anthro-quinones	Cardiac	Erythromycin
Coumarins	Betalaines	Monoterpenes	Bezo-quinones	Glycosides	Lovastatin and other statins
Flavonoids	Quinolo-zidines	Sesquiterpenes	Naphtho-quinones	Pregnenolo ne	Discodermolide
Hydroxy-cinnamoyl	Furono-quinones	Diterpenes		Derivatives	Aflatoxin B1
Derivatives	Harringtonines	Triterpenes			Avermectins
Isoflavonoids	Isoquinolines				Nystatin
Lignans	Indoles				Rifamycin
Phenolenones	Purines				
Proantho-cyanidins	Pyridines				
Stilbenes	Tropane				
Tanins	Alkaloids				

5

The secondary metabolite used herein may include a metabolite from any known group of secondary metabolites. For example, secondary metabolites can be categorized into the following groups: alkaloids, terpenoids, flavonoids, glycosides, natural phenols (e.g., gossypol acetic acid), enals (e.g., trans-cinnamaldehyde), phenazines, biphenols and dibenzofurans, polyketides, fatty acid synthase 10 peptides, nonribosomal peptides, ribosomally synthesized and post-translationally modified peptides, polyphenols, polysaccharides (e.g., chitosan), and biopolymers. For an in-depth review of secondary metabolites see, for example, Vining, *Annu. Rev. Microbiol.* 44:395-427, 1990.

Secondary metabolites useful for compositions and methods described herein include those that alter a natural function of an endosymbiont (e.g., primary or secondary endosymbiont), bacteriocyte, or 15 extracellular symbiont. In some instances, one or more secondary metabolites described herein is isolated from a high throughput screening (HTS) for antimicrobial compounds. For example, a HTS screen identified 49 antibacterial extracts that have specificity against gram positive and gram negative bacteria from over 39,000 crude extracts from organisms growing in diverse ecosystems of one specific region. In some instances, the secondary metabolite is transported inside a bacteriocyte.

20 In some instances, the small molecule is an inhibitor of vitamin synthesis. In some instances, the vitamin synthesis inhibitor is a vitamin precursor analog. In certain instances, the vitamin precursor analog is pantothenol.

In some instances, the small molecule is an amino acid analog. In certain instances, the amino acid analog is L-canvanine, D-arginine, D-valine, D-methionine, D-phenylalanine, D-histidine, D-25 tryptophan, D-threonine, D-leucine, L-NG-nitroarginine, or a combination thereof.

In some instances, the small molecule is a natural antimicrobial compound, such as propionic acid, levulinic acid, trans-cinnemaldehdye, nisin, or low molecular weight chitosan. The secondary metabolite described herein may be formulated in a composition for any of the uses described herein. The compositions disclosed herein may include any number or type (e.g., classes) of secondary
5 metabolites, such as at least about any one of 1 secondary metabolite, 2, 3, 4, 5, 10, 15, 20, or more secondary metabolites. A suitable concentration of each secondary metabolite in the composition depends on factors such as efficacy, stability of the secondary metabolite, number of distinct secondary metabolites, the formulation, and methods of application of the composition. In some instances, wherein the composition includes at least two types of secondary metabolites, the concentration of each type of
10 secondary metabolite may be the same or different.

A modulating agent including a secondary metabolite as described herein can be contacted with the target host in an amount and for a time sufficient to: (a) reach a target level (e.g., a predetermined or threshold level) of secondary metabolite concentration inside a target host; (b) reach a target level (e.g., a predetermined or threshold level) of secondary metabolite concentration inside a target host gut; (c) reach
15 a target level (e.g., a predetermined or threshold level) of secondary metabolite concentration inside a target host bacteriocyte; (d) modulate the level, or an activity, of one or more microorganism (e.g., endosymbiont) in the target host; or/and (e) modulate fitness of the target host.

iv. Bacteria as modulating agents

20 In some instances, the modulating agent described herein includes one or more bacteria. Numerous bacteria are useful in the compositions and methods described herein. In some instances, the agent is a bacterial species endogenously found in the host. In some instances, the bacterial modulating agent is an endosymbiotic bacterial species. Non-limiting examples of bacteria that may be used as modulating agents include all bacterial species described herein in Section II of the detailed description
25 and those listed in Table 1 starting at page 15. For example, the modulating agent may be a bacterial species from any bacterial phyla present in insect guts, including *Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Bacteroidetes, Firmicutes* (e.g., *Lactobacillus* and *Bacillus* spp.), *Clostridia, Actinomycetes, Spirochetes, Verrucomicrobia,* and *Actinobacteria*.

In some instances, the modulating agent is a bacterium that promotes microbial diversity or
30 otherwise alters the microbiota of the host in a favorable manner. In one instance, bacteria may be provided to promote microbiome development in honey bees. For example, the modulating agent may include, for example, *Bartonella apis, Parasaccharibacter apium, Frischella perrara, Snodgrassella alvi, Gilliamela apicola, Bifidobacterium spp,* or *Lactobacillus spp*.

The bacterial modulating agents discussed herein can be used to alter the level, activity, or
35 metabolism of target microorganisms as indicated in the sections for increasing the fitness of insects, such as, honeybees and silkworms.

In some instances, such bacterial modulating agents are bacteria which are capable of degrading pesticides as laid out in Table 12 including insecticides. Such insecticides include neonicotinoids such as imidacloprid, or organophosphorus insecticides, such as fenitrothion. In some instances, the pesticide-
40 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).

Examples 1 to 3, 5, and 6 describe how imidacloprid and fenitrothion degrading microorganisms can be identified which can then be used a modulating agents in insect hosts, such as honeybees, giving the treated insect hosts a competitive advantage. Administering such pesticide-degrading microorganisms, for example imidacloprid- or fenitrothion-degrading microorganisms to insect hosts such as honeybees is understood to be encompassed by the alteration of a level, activity, or metabolism of one or more microorganisms resident in the host.

In some instances, such bacterial modulating agents are bacteria which are capable of producing nutrients, including amino acids (e.g., methionine or glutamate). The nutrient-producing bacteria may be naturally occurring bacteria, e.g., naturally occurring bacteria exogenous to the insect host. Such bacteria may be isolated from a population of bacteria, such as that found in an environmental sample. Bacteria can be isolated that produce one or more amino acids in a manner that increases production of amino acids in the host relative to a host who has not been administered the amino-acid producing bacteria. Amino acids that can be produced by the bacteria in the host include methionine, alanine, arginine, asparagine, asparatic acid, cysteine, glutamine, glutamic acid, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, or valine. In certain instances, the amino acid-producing bacteria is a methionine-producing bacteria.

In some instances, the nutrient-producing bacteria (e.g., amino acid-producing bacteria, e.g., methionine-producing 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).

Examples 8, 9, and 10 describe how methionine-producing microorganisms can be identified which can then be used as modulating agents in insect hosts, such as honeybees, or in the model organism *Drosophila*, to increase the fitness of the hosts (e.g., increase amino acid content (e.g., methionine content or glutamate content).

v. Modifications to modulating agents

(a) Fusions

Any of the modulating agents described herein may be fused or linked to an additional moiety. In some instances, the modulating agent includes a fusion of one or more additional moieties (e.g., 1 additional moiety, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more additional moieties). In some instances, the additional moiety is any one of the modulating agents described herein (e.g., a peptide, polypeptide, small molecule, or antibiotic). Alternatively, the additional moiety may not act as modulating agent itself but may instead serve a secondary function. For example, the additional moiety may to help the modulating agent

access, bind, or become activated at a target site in the host (e.g., at a host gut or a host bacteriocyte) or at a target microorganism resident in the host (e.g., honeybee or silkworm).

In some instances, the additional moiety may help the modulating agent penetrate a target host cell or target microorganism resident in the host. For example, the additional moiety may include a cell penetrating peptide. Cell penetrating peptides (CPPs) may be natural sequences derived from proteins; chimeric peptides that are formed by the fusion of two natural sequences; or synthetic CPPs, which are synthetically designed sequences based on structure–activity studies. In some instances, CPPs have the capacity to ubiquitously cross cellular membranes (e.g., prokaryotic and eukaryotic cellular membranes) with limited toxicity. Further, CPPs may have the capacity to cross cellular membranes via energy-dependent and/or independent mechanisms, without the necessity of a chiral recognition by specific receptors. CPPs can be bound to any of the modulating agents described herein. For example, a CPP can be bound to an antimicrobial peptide (AMP), e.g., a scorpion peptide, e.g., UY192 fused to a cell penetrating peptide (e.g., YGRKKRRQRRRFLSTIWNGIKGLLFAM; SEQ ID NO: 198). Non-limiting examples of CPPs are listed in Table 11.

Table 11: Examples of Cell Penetrating Peptides (CPPs)

Peptide	Origin	Sequence
Protein-derived		
rit	Antennapedia	RQIKIWFQNRRMKWKK (SEQ ID NO: 178)
Tat peptide	Tat	GRKKRRQRRRPPQ (SEQ ID NO: 179)
pVEC	Cadherin	LLIILRRRIRKQAHAHSK (SEQ ID NO: 180)
Chimeric		
Transportan	Galanine/Mastoparan	GWTLNSAGYLLGKINLKALAALAKKIL (SEQ ID NO: 181)
MPG	HIV-gp41/SV40 T-antigen	GALFLGFLGAAGSTMGAWSQPKKKRKV (SEQ ID NO: 182)
Pep-1	HIV-reverse transcriptase/SV40 T-antigen	KETWWETWWTEWSQPKKKRKV (SEQ ID NO: 183)
Synthetic		
Polyarginines	Based on Tat peptide	$(R)_n$; $6 < n < 12$
MAP	de novo	KLALKLALKALKAALKLA (SEQ ID NO: 184)
R_6W_3	Based on penetratin	RRWWRRWRR (SEQ ID NO: 185)

In other instances, the additional moiety helps the modulating agent bind a target microorganism (e.g., a fungi or bacterium) resident in the host. The additional moiety may include one or more targeting domains. In some instances, the targeting domain may target the modulating agent to one or more microorganisms (e.g., bacterium or fungus) resident in the gut of the host. In some instances, the
5 targeting domain may target the modulating agent to a specific region of the host (e.g., host gut or bacteriocyte) to access microorganisms that are generally present in said region of the host. For example, the targeting domain may target the modulating agent to the foregut, midgut, or hindgut of the host. In other instances, the targeting domain may target the modulating agent to a bacteriocyte in the host and/or one or more specific bacteria resident in a host bacteriocyte. For example, the targeting
10 domain may be *Galanthus nivalis* lectin or agglutinin (GNA) bound to a modulating agent described herein, e.g., an AMP, e.g., a scorpion peptide, e.g., Uy192.

(b) Pre- or Pro-domains

In some instances, the modulating agent may include a pre- or pro- amino acid sequence. For
15 example, the modulating agent may be an inactive protein or peptide that can be activated by cleavage or post-translational modification of a pre- or pro-sequence. In some instances, the modulating agent is engineered with an inactivating pre- or pro-sequence. For example, the pre- or pro-sequence may obscure an activation site on the modulating agent, e.g., a receptor binding site, or may induce a conformational change in the modulating agent. Thus, upon cleavage of the pre- or pro-sequence, the
20 modulating agent is activated.

Alternatively, the modulating agent may include a pre- or pro-small molecule, e.g., an antibiotic. The modulating agent may be an inactive small molecule described herein that can be activated in a target environment inside the host. For example, the small molecule may be activated upon reaching a certain pH in the host gut.

25

(c) Linkers

In instances where the modulating agent is connected to an additional moiety, the modulating agent may further include a linker. For example, the linker may be a chemical bond, e.g., one or more covalent bonds or non-covalent bonds. In some instances, the linker may be a peptide linker (e.g., 2, 3,
30 4, 5, 6, 8, 10, 12, 14, 16, 20, 25, 30, 35, 40, or more amino acids longer). The linker maybe include any flexible, rigid, or cleavable linkers described herein.

A flexible peptide linker may include any of those commonly used in the art, including linkers having sequences having primarily Gly and Ser residues ("GS" linker). Flexible linkers may be useful for joining domains that require a certain degree of movement or interaction and may include small, non-
35 polar (e.g. Gly) or polar (e.g. Ser or Thr) amino acids.

Alternatively, a peptide linker may be a rigid linker. Rigid linkers are useful to keep a fixed distance between moieties and to maintain their independent functions. Rigid linkers may also be useful when a spatial separation of the domains is critical to preserve the stability or bioactivity of one or more components in the fusion. Rigid linkers may, for example, have an alpha helix-structure or Pro-rich
40 sequence, $(XP)_n$, with X designating any amino acid, preferably Ala, Lys, or Glu.

In yet other instances, a peptide linker may be a cleavable linker. In some instances, linkers may be cleaved under specific conditions, such as the presence of reducing reagents or proteases. *In vivo* cleavable linkers may utilize the reversible nature of a disulfide bond. One example includes a thrombin-sensitive sequence (e.g., PRS) between two Cys residues. *In vitro* thrombin treatment of CPRSC results in the cleavage of the thrombin-sensitive sequence, while the reversible disulfide linkage remains intact. Such linkers are known and described, e.g., in Chen et al., *Adv. Drug Deliv. Rev.* 65(10):1357-1369, 2013. Cleavage of linkers in fusions may also be carried out by proteases that are expressed *in vivo* under conditions in specific cells or tissues of the host or microorganisms resident in the host. In some instances, cleavage of the linker may release a free functional, modulating agent upon reaching a target site or cell.

Fusions described herein may alternatively be linked by a linking molecule, including a hydrophobic linker, such as a negatively charged sulfonate group; lipids, such as a poly (--CH2--) hydrocarbon chains, such as polyethylene glycol (PEG) group, unsaturated variants thereof, hydroxylated variants thereof, amidated or otherwise N-containing variants thereof, non-carbon linkers; carbohydrate linkers; phosphodiester linkers, or other molecule capable of covalently linking two or more molecules, e.g., two modulating agents. Non-covalent linkers may be used, such as hydrophobic lipid globules to which the modulating agent is linked, for example, through a hydrophobic region of the modulating agent or a hydrophobic extension of the modulating agent, such as a series of residues rich in leucine, isoleucine, valine, or perhaps also alanine, phenylalanine, or even tyrosine, methionine, glycine or other hydrophobic residue. The modulating agent may be linked using charge-based chemistry, such that a positively charged moiety of the modulating agent is linked to a negative charge of another modulating agent or an additional moiety.

IV. Formulations and Compositions

The compositions described herein may be formulated either in pure form (e.g., the composition contains only the modulating agent) or together with one or more additional agents (such as excipient, delivery vehicle, carrier, diluent, stabilizer, etc.) to facilitate application or delivery of the compositions. Examples of suitable excipients and diluents include, but are not limited to, lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, saline solution, syrup, methylcellulose, methyl- and propylhydroxybenzoates, talc, magnesium stearate, and mineral oil.

In some instances, the composition includes a delivery vehicle or carrier. In some instances, the delivery vehicle includes an excipient. Exemplary excipients include, but are not limited to, solid or liquid carrier materials, solvents, stabilizers, slow-release excipients, colorings, and surface-active substances (surfactants). In some instances, the delivery vehicle is a stabilizing vehicle. In some instances, the stabilizing vehicle includes a stabilizing excipient. Exemplary stabilizing excipients include, but are not limited to, epoxidized vegetable oils, antifoaming agents, e.g. silicone oil, preservatives, viscosity regulators, binding agents and tackifiers. In some instances, the stabilizing vehicle is a buffer suitable for the modulating agent. In some instances, the composition is microencapsulated in a polymer bead delivery vehicle. In some instances, the stabilizing vehicle protects the modulating agent against UV

and/or acidic conditions. In some instances, the delivery vehicle contains a pH buffer. In some instances, the composition is formulated to have a pH in the range of about 4.5 to about 9.0, including for example pH ranges of about any one of 5.0 to about 8.0, about 6.5 to about 7.5, or about 6.5 to about 7.0.

Depending on the intended objectives and prevailing circumstances, the composition may be formulated into emulsifiable concentrates, suspension concentrates, directly sprayable or dilutable solutions, coatable pastes, diluted emulsions, spray powders, soluble powders, dispersible powders, wettable powders, dusts, granules, encapsulations in polymeric substances, microcapsules, foams, aerosols, carbon dioxide gas preparations, tablets, resin preparations, paper preparations, nonwoven fabric preparations, or knitted or woven fabric preparations. In some instances, the composition is a liquid. In some instances, the composition is a solid. In some instances, the composition is an aerosol, such as in a pressurized aerosol can. In some instances, the composition is present in the waste (such as feces) of the pest. In some instances, the composition is present in or on a live pest.

In some instances, the delivery vehicle is the food or water of the host. In other instances, the delivery vehicle is a food source for the host. In some instances, the delivery vehicle is a food bait for the host. In some instances, the composition is a comestible agent consumed by the host. In some instances, the composition is delivered by the host to a second host, and consumed by the second host. In some instances, the composition is consumed by the host or a second host, and the composition is released to the surrounding of the host or the second host via the waste (such as feces) of the host or the second host. In some instances, the modulating agent is included in food bait intended to be consumed by a host or carried back to its colony.

In some instances, the modulating agent may make up about 0.1% to about 100% of the composition, such as any one of about 0.01% to about 100%, about 1% to about 99.9%, about 0.1% to about 10%, about 1% to about 25%, about 10% to about 50%, about 50% to about 99%, or about 0.1% to about 90% of active ingredients (such as phage, lysin or bacteriocin). In some instances, the composition includes at least any of 0.1%, 0.5%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more active ingredients (such as phage, lysin or bacteriocin). In some instances, the concentrated agents are preferred as commercial products, the final user normally uses diluted agents, which have a substantially lower concentration of active ingredient.

Any of the formulations described herein may be used in the form of a bait, a coil, an electric mat, a smoking preparation, a fumigant, or a sheet.

i. Liquid Formulations

The compositions provided herein may be in a liquid formulation. Liquid formulations are generally mixed with water, but in some instances may be used with crop oil, diesel fuel, kerosene or other light oil as a carrier. The amount of active ingredient often ranges from about 0.5 to about 80 percent by weight.

An emulsifiable concentrate formulation may contain a liquid active ingredient, one or more petroleum-based solvents, and an agent that allows the formulation to be mixed with water to form an emulsion. Such concentrates may be used in agricultural, ornamental and turf, forestry, structural, food processing, livestock, and public health pest formulations. These may be adaptable to application

equipment from small portable sprayers to hydraulic sprayers, low-volume ground sprayers, mist blowers, and low-volume aircraft sprayers. Some active ingredients are readily dissolve in a liquid carrier. When mixed with a carrier, they form a solution that does not settle out or separate, e.g., a homogenous solution. Formulations of these types may include an active ingredient, a carrier, and one or more other ingredients. Solutions may be used in any type of sprayer, indoors and outdoors.

In some instances, the composition may be formulated as an invert emulsion. An invert emulsion is a water-soluble active ingredient dispersed in an oil carrier. Invert emulsions require an emulsifier that allows the active ingredient to be mixed with a large volume of petroleum-based carrier, usually fuel oil. Invert emulsions aid in reducing drift. With other formulations, some spray drift results when water droplets begin to evaporate before reaching target surfaces; as a result the droplets become very small and lightweight. Because oil evaporates more slowly than water, invert emulsion droplets shrink less and more active ingredient reaches the target. Oil further helps to reduce runoff and improve rain resistance. It further serves as a sticker-spreader by improving surface coverage and absorption. Because droplets are relatively large and heavy, it is difficult to get thorough coverage on the undersides of foliage. Invert emulsions are most commonly used along rights-of-way where drift to susceptible non-target areas can be a problem.

A flowable or liquid formulation combines many of the characteristics of emulsifiable concentrates and wettable powders. Manufacturers use these formulations when the active ingredient is a solid that does not dissolve in either water or oil. The active ingredient, impregnated on a substance such as clay, is ground to a very fine powder. The powder is then suspended in a small amount of liquid. The resulting liquid product is quite thick. Flowables and liquids share many of the features of emulsifiable concentrates, and they have similar disadvantages. They require moderate agitation to keep them in suspension and leave visible residues, similar to those of wettable powders.

Flowables/liquids are easy to handle and apply. Because they are liquids, they are subject to spilling and splashing. They contain solid particles, so they contribute to abrasive wear of nozzles and pumps. Flowable and liquid suspensions settle out in their containers. Because flowable and liquid formulations tend to settle, packaging in containers of five gallons or less makes remixing easier.

Aerosol formulations contain one or more active ingredients and a solvent. Most aerosols contain a low percentage of active ingredients. There are two types of aerosol formulations—the ready-to-use type commonly available in pressurized sealed containers and those products used in electrical or gasoline-powered aerosol generators that release the formulation as a smoke or fog.

Ready to use aerosol formulations are usually small, self-contained units that release the formulation when the nozzle valve is triggered. The formulation is driven through a fine opening by an inert gas under pressure, creating fine droplets. These products are used in greenhouses, in small areas inside buildings, or in localized outdoor areas. Commercial models, which hold five to 5 pounds of active ingredient, are usually refillable.

Smoke or fog aerosol formulations are not under pressure. They are used in machines that break the liquid formulation into a fine mist or fog (aerosol) using a rapidly whirling disk or heated surface.

ii. Dry or Solid Formulations

Dry formulations can be divided into two types: ready-to-use and concentrates that must be mixed with water to be applied as a spray. Most dust formulations are ready to use and contain a low percentage of active ingredients (less than about 10 percent by weight), plus a very fine, dry inert carrier made from talc, chalk, clay, nut hulls, or volcanic ash. The size of individual dust particles varies. A few dust formulations are concentrates and contain a high percentage of active ingredients. Mix these with dry inert carriers before applying. Dusts are always used dry and can easily drift to non-target sites.

iii. Granule or Pellet Formulations

In some instances, the composition is formulated as granules. Granular formulations are similar to dust formulations, except granular particles are larger and heavier. The coarse particles may be made from materials such as clay, corncobs, or walnut shells. The active ingredient either coats the outside of the granules or is absorbed into them. The amount of active ingredient may be relatively low, usually ranging from about 0.5 to about 15 percent by weight. Granular formulations are most often used to apply to the soil, insects or nematodes living in the soil, or absorption into plants through the roots. Granular formulations are sometimes applied by airplane or helicopter to minimize drift or to penetrate dense vegetation. Once applied, granules may release the active ingredient slowly. Some granules require soil moisture to release the active ingredient. Granular formulations also are used to control larval mosquitoes and other aquatic pests. Granules are used in agricultural, structural, ornamental, turf, aquatic, right-of-way, and public health (biting insect) pest-control operations.

In some instances, the composition is formulated as pellets. Most pellet formulations are very similar to granular formulations; the terms are used interchangeably. In a pellet formulation, however, all the particles are the same weight and shape. The uniformity of the particles allows use with precision application equipment.

iv. Powders

In some instances, the composition is formulated as a powder. In some instances, the composition is formulated as a wettable powder. Wettable powders are dry, finely ground formulations that look like dusts. They usually must be mixed with water for application as a spray. A few products, however, may be applied either as a dust or as a wettable powder—the choice is left to the applicator. Wettable powders have about 1 to about 95 percent active ingredient by weight; in some cases more than about 50 percent. The particles do not dissolve in water. They settle out quickly unless constantly agitated to keep them suspended. They can be used for most pest problems and in most types of spray equipment where agitation is possible. Wettable powders have excellent residual activity. Because of their physical properties, most of the formulation remains on the surface of treated porous materials such as concrete, plaster, and untreated wood. In such cases, only the water penetrates the material.

In some instances, the composition is formulated as a soluble powder. Soluble powder formulations look like wettable powders. However, when mixed with water, soluble powders dissolve readily and form a true solution. After they are mixed thoroughly, no additional agitation is necessary. The amount of active ingredient in soluble powders ranges from about 15 to about 95 percent by weight;

in some cases more than about 50 percent. Soluble powders have all the advantages of wettable powders and none of the disadvantages, except the inhalation hazard during mixing.

In some instances, the composition is formulated as a water-dispersible granule. Water-dispersible granules, also known as dry flowables, are like wettable powders, except instead of being dust-like, they are formulated as small, easily measured granules. Water-dispersible granules must be mixed with water to be applied. Once in water, the granules break apart into fineparticles similar to wettable powders. The formulation requires constant agitation to keep it suspended in water. The percentage of active ingredient is high, often as much as 90 percent by weight. Water-dispersible granules share many of the same advantages and disadvantages of wettable powders, except they are more easily measured and mixed. Because of low dust, they cause less inhalation hazard to the applicator during handling

v. Bait

In some instances, the composition includes a bait. The bait can be in any suitable form, such as a solid, paste, pellet or powdered form.The bait can also be carried away by the host back to a population of said host (e.g., a colony or hive). The bait can then act as a food source for other members of the colony, thus providing an effective modulating agent for a large number of hosts and potentially an entire host colony.

The baits can be provided in a suitable "housing" or "trap." Such housings and traps are commercially available and existing traps can be adapted to include the compositions described herein. The housing or trap can be box-shaped for example, and can be provided in pre-formed condition or can be formed of foldable cardboard for example. Suitable materials for a housing or trap include plastics and cardboard, particularly corrugated cardboard. The inside surfaces of the traps can be lined with a sticky substance in order to restrict movement of the host once inside the trap. The housing or trap can contain a suitable trough inside which can hold the bait in place. A trap is distinguished from a housing because the host cannot readily leave a trap following entry, whereas a housing acts as a "feeding station" which provides the host with a preferred environment in which they can feed and feel safe from predators.

vi. Attractants

In some instances, the composition includes an attractant (e.g., a chemoattractant). The attractant may attract an adult host or immature host (e.g., larva) to the vicinity of the composition. Attractants include pheromones, a chemical that is secreted by an animal, especially an insect, which influences the behavior or development of others of the same species. Other attractants include sugar and protein hydrolysate syrups, yeasts, and rotting meat. Attractants also can be combined with an active ingredient and sprayed onto foliage or other items in the treatment area.

Various attractants are known which influence host behavior as a host's search for food, oviposition or mating sites, or mates. Attractants useful in the methods and compositions described herein include, for example, eugenol, phenethyl propionate, ethyl dimethylisobutyl-cyclopropane carboxylate, propyl benszodioxancarboxylate, cis-7,8-epoxy-2-methyloctadecane, trans-8,trans-0-dodecadienol, cis-9-tetradecenal (with cis-11-hexadecenal), trans-11-tetradecenal, cis-11-hexadecenal,

(Z)-11,12-hexadecadienal, cis-7-dodecenyl acetate, cis-8-dodecenyul acetate, cis-9-dodecenyl acetate, cis-9-tetradecenyl acetate, cis-11-tetradecenyl acetate, trans-11-tetradecenyl acetate (with cis-11), cis-9,trans-11-tetradecadienyl acetate (with cis-9,trans-12), cis-9,trans-1 2-tetradecadienyl acetate, cis-7,cis-11- hexadecadienyl acetate (with cis-7,trans-11), cis-3,cis-13-octadecadienyl acetate, trans-3,cis-13-
5 octadecadienyl acetate, anethole and isoamyl salicylate.

Means other than chemoattractants may also be used to attract insects, including lights in various wavelengths or colors.

vii. Nanocapsules/Microencapsulation/Liposomes

10 In some instances, the composition is provided in a microencapsulated formulation. Microencapsulated formulations are mixed with water and sprayed in the same manner as other sprayable formulations. After spraying, the plastic coating breaks down and slowly releases the active ingredient.

15 *viii. Carriers*

Any of the compositions described herein may be formulated to include the modulating agent described herein and an inert carrier. Such carrier can be a solid carrier, a liquid carrier, a gel carrier, and/or a gaseous carrier. In certain instances, the carrier can be a seed coating. The seed coating is any non-naturally occurring formulation that adheres, in whole or part, to the surface of the seed. The
20 formulation may further include an adjuvant or surfactant. The formulation can also include one or more modulating agents to enlarge the action spectrum.

A solid carrier used for formulation includes finely-divided powder or granules of clay (e.g. kaolin clay, diatomaceous earth, bentonite, Fubasami clay, acid clay, etc.), synthetic hydrated silicon oxide, talc, ceramics, other inorganic minerals (e.g., sericite, quartz, sulfur, activated carbon, calcium carbonate,
25 hydrated silica, etc.), a substance which can be sublimated and is in the solid form at room temperature (e.g., 2,4,6-triisopropyl-1,3,5-trioxane, naphthalene, p-dichlorobenzene, camphor, adamantan, etc.); wool; silk; cotton; hemp; pulp; synthetic resins (e.g., polyethylene resins such as low-density polyethylene, straight low-density polyethylene and high-density polyethylene; ethylene-vinyl ester copolymers such as ethylene-vinyl acetate copolymers; ethylene-methacrylic acid ester copolymers such as ethylene-methyl
30 methacrylate copolymers and ethylene-ethyl methacrylate copolymers; ethylene-acrylic acid ester copolymers such as ethylene-methyl acrylate copolymers and ethylene-ethyl acrylate copolymers; ethylene-vinylcarboxylic acid copolymers such as ethylene-acrylic acid copolymers; ethylene-tetracyclododecene copolymers; polypropylene resins such as propylene homopolymers and propylene-ethylene copolymers; poly-4-methylpentene-1, polybutene-1, polybutadiene, polystyrene; acrylonitrile-
35 styrene resins; styrene elastomers such as acrylonitrile-butadiene-styrene resins, styrene-conjugated diene block copolymers, and styrene-conjugated diene block copolymer hydrides; fluororesins; acrylic resins such as poly(methyl methacrylate); polyamide resins such as nylon 6 and nylon 66; polyester resins such as polyethylene terephthalate, polyethylene naphthalate, polybutylene terephthalate, and polycyclohexylenedimethylene terephthalate; polycarbonates, polyacetals, polyacrylsulfones,
40 polyarylates, hydroxybenzoic acid polyesters, polyetherimides, polyester carbonates, polyphenylene ether

resins, polyvinyl chloride, polyvinylidene chloride, polyurethane, and porous resins such as foamed polyurethane, foamed polypropylene, or foamed ethylene, etc.), glasses, metals, ceramics, fibers, cloths, knitted fabrics, sheets, papers, yarn, foam, porous substances, and multifilaments.

A liquid carrier may include, for example, aromatic or aliphatic hydrocarbons (e.g., xylene, toluene, alkylnaphthalene, phenylxylylethane, kerosine, gas oil, hexane, cyclohexane, etc.), halogenated hydrocarbons (e.g., chlorobenzene, dichloromethane, dichloroethane, trichloroethane, etc.), alcohols (e.g., methanol, ethanol, isopropyl alcohol, butanol, hexanol, benzyl alcohol, ethylene glycol, etc.), ethers (e.g., diethyl ether, ethylene glycol dimethyl ether, diethylene glycol monomethyl ether, diethylene glycol monoethyl ether, propylene glycol monomethyl ether, tetrahydrofuran, dioxane, etc.), esters (e.g., ethyl acetate, butyl acetate, etc.), ketones (e.g., acetone, methyl ethyl ketone, methyl isobutyl ketone, cyclohexanone, etc.), nitriles (e.g., acetonitrile, isobutyronitrile, etc.), sulfoxides (e.g., dimethyl sulfoxide, etc.), amides (e.g., N,N-dimethylformamide, N,N-dimethylacetamide, cyclic imides (e.g. N-methylpyrrolidone) alkylidene carbonates (e.g., propylene carbonate, etc.), vegetable oil (e.g., soybean oil, cottonseed oil, etc.), vegetable essential oils (e.g., orange oil, hyssop oil, lemon oil, etc.), or water.

A gaseous carrier may include, for example, butane gas, flon gas, liquefied petroleum gas (LPG), dimethyl ether, and carbon dioxide gas.

ix. Adjuvants

In some instances, the composition provided herein may include an adjuvant. Adjuvants are chemicals that do not possess activity. Adjuvants are either pre-mixed in the formulation or added to the spray tank to improve mixing or application or to enhance performance. They are used extensively in products designed for foliar applications. Adjuvants can be used to customize the formulation to specific needs and compensate for local conditions. Adjuvants may be designed to perform specific functions, including wetting, spreading, sticking, reducing evaporation, reducing volatilization, buffering, emulsifying, dispersing, reducing spray drift, and reducing foaming. No single adjuvant can perform all these functions, but compatible adjuvants often can be combined to perform multiple functions simultaneously.

Among nonlimiting examples of adjuvants included in the formulation are binders, dispersants and stabilizers, specifically, for example, casein, gelatin, polysaccharides (e.g., starch, gum arabic, cellulose derivatives, alginic acid, etc.), lignin derivatives, bentonite, sugars, synthetic water-soluble polymers (e.g., polyvinyl alcohol, polyvinylpyrrolidone, polyacrylic acid, etc.), PAP (acidic isopropyl phosphate), BHT (2,6-di-t-butyl-4-methylphenol), BHA (a mixture of 2-t-butyl-4-methoxyphenol and 3-t-butyl-4-methoxyphenol), vegetable oils, mineral oils, fatty acids and fatty acid esters.

x. Surfactants

In some instances, the composition provided herein includes a surfactant. Surfactants, also called wetting agents and spreaders, physically alter the surface tension of a spray droplet. For a formulation to perform its function properly, a spray droplet must be able to wet the foliage and spread out evenly over a leaf. Surfactants enlarge the area of formulation coverage, thereby increasing the pest's exposure to the chemical. Surfactants are particularly important when applying a formulation to waxy or

hairy leaves. Without proper wetting and spreading, spray droplets often run off or fail to cover leaf surfaces adequately. Too much surfactant, however, can cause excessive runoff and reduce efficacy.

Surfactants are classified by the way they ionize or split apart into electrically charged atoms or molecules called ions. A surfactant with a negative charge is anionic. One with a positive charge is cationic, and one with no electrical charge is nonionic. Formulation activity in the presence of a nonionic surfactant can be quite different from activity in the presence of a cationic or anionic surfactant. Selecting the wrong surfactant can reduce the efficacy of a pesticide product and injure the target plant. Anionic surfactants are most effective when used with contact pesticides (pesticides that control the pest by direct contact rather than being absorbed systemically). Cationic surfactants should never be used as stand-alone surfactants because they usually are phytotoxic.

Nonionic surfactants, often used with systemic pesticides, help pesticide sprays penetrate plant cuticles. Nonionic surfactants are compatible with most pesticides, and most EPA-registered pesticides that require a surfactant recommend a nonionic type. Adjuvants include, but are not limited to, stickers, extenders, plant penetrants, compatibility agents, buffers or pH modifiers, drift control additives, defoaming agents, and thickeners.

Among nonlimiting examples of surfactants included in the compositions described herein are alkyl sulfate ester salts, alkyl sulfonates, alkyl aryl sulfonates, alkyl aryl ethers and polyoxyethylenated products thereof, polyethylene glycol ethers, polyvalent alcohol esters and sugar alcohol derivatives.

xi. Combinations

In formulations and in the use forms prepared from these formulations, the modulating agent may be in a mixture with other active compounds, such as pesticidal agents (e.g., insecticides, sterilants, acaricides, nematicides, molluscicides, or fungicides; see, e.g., pesticides listed in Table 12), attractants, growth-regulating substances, or herbicides. As used herein, the term "pesticidal agent" refers to any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. A pesticide can be a chemical substance or biological agent used against pests including insects, mollusks, pathogens, weeds, nematodes, and microbes that compete with humans for food, destroy property, spread disease, or are a nuisance. The term "pesticidal agent" may further encompass other bioactive molecules such as antibiotics, antivirals pesticides, antifungals, antihelminthics, nutrients, pollen, sucrose, and/or agents that stun or slow insect movement.

In instances where the modulating agent is applied to plants, a mixture with other known compounds, such as herbicides, fertilizers, growth regulators, safeners, semiochemicals, or else with agents for improving plant properties is also possible.

V. Delivery

A host described herein can be exposed to any of the compositions described herein in any suitable manner that permits delivering or administering the composition to the insect. The modulating agent may be delivered either alone or in combination with other active or inactive substances and may be applied by, for example, spraying, microinjection, through plants, pouring, dipping, in the form of

concentrated liquids, gels, solutions, suspensions, sprays, powders, pellets, briquettes, bricks and the like, formulated to deliver an effective concentration of the modulating agent.

Amounts and locations for application of the compositions described herein are generally determined by the habits of the host, the lifecycle stage at which the microorganisms of the host can be targeted by the modulating agent, the site where the application is to be made, and the physical and functional characteristics of the modulating agent. The modulating agents described herein may be administered to the insect by oral ingestion, but may also be administered by means which permit penetration through the cuticle or penetration of the insect respiratory system.

In some instances, the insect can be simply “soaked” or “sprayed” with a solution including the modulating agent. Alternatively, the modulating agent can be linked to a food component (e.g., comestible) of the insect for ease of delivery and/or in order to increase uptake of the modulating agent by the insect. Methods for oral introduction include, for example, directly mixing a modulating agent with the insect's food, spraying the modulating agent in the insect's habitat or field, as well as engineered approaches in which a species that is used as food is engineered to express a modulating agent, then fed to the insect to be affected. In some instances, for example, the modulating agent composition can be incorporated into, or overlaid on the top of, the insect's diet. For example, the modulating agent composition can be sprayed onto a field of crops which an insect inhabits.

In some instances, the composition is sprayed directly onto a plant e.g., crops, by e.g., backpack spraying, aerial spraying, crop spraying/dusting etc. In instances where the modulating agent is delivered to a plant, the plant receiving the modulating agent may be at any stage of plant growth. For example, formulated modulating agents can be applied as a seed-coating or root treatment in early stages of plant growth or as a total plant treatment at later stages of the crop cycle. In some instances, the modulating agent may be applied as a topical agent to a plant, such that the host insect ingests or otherwise comes in contact with the plant upon interacting with the plant.

Further, the modulating agent may be applied (e.g., in the soil in which a plant grows, or in the water that is used to water the plant) as a systemic agent that is absorbed and distributed through the tissues (e.g., stems or leafs) of a plant or animal host, such that an insect feeding thereon will obtain an effective dose of the modulating agent. In some instances, plants or food organisms may be genetically transformed to express the modulating agent such that a host feeding upon the plant or food organism will ingest the modulating agent.

Delayed or continuous release can also be accomplished by coating the modulating agent or a composition with the modulating agent(s) with a dissolvable or bioerodable coating layer, such as gelatin, which coating dissolves or erodes in the environment of use, to then make the modulating agent available, or by dispersing the agent in a dissolvable or erodable matrix. Such continuous release and/or dispensing means devices may be advantageously employed to consistently maintain an effective concentration of one or more of the modulating agents described herein in a specific host habitat.

The modulating agent can also be incorporated into the medium in which the insect grows, lives, reproduces, feeds, or infests. For example, a modulating agent can be incorporated into a food container, feeding station, protective wrapping, or a hive. For some applications the modulating agent may be bound to a solid support for application in powder form or in a “trap” or “feeding station.” As an

example, for applications where the composition is to be used in a trap or as bait for a particular host insect, the compositions may also be bound to a solid support or encapsulated in a time-release material. For example, in instances where the host is a honeybee, the compositions described herein can be administered by delivering the composition to a honeybee hive or at least one habitat where a honeybee grows, lives, reproduces, or feeds.

VI. Screening

Included herein are screening assays for identifying a modulating agent, wherein the modulating agent is effective to alter the microbiota of a host and thereby increase host fitness (e.g., insect or nematode fitness). For example, the screening assay may be used to identify one or more modulating agents that target specific microorganisms and/or specific hosts. Further, the screening assays may be used to identify one or more microorganisms with enhanced functionalities. For example, the screening assay may be effective to isolate one or more microorganisms with an enhanced ability to metabolize (e.g., degrade) a pesticide (e.g., a pesticide listed in Table 12 or an insecticide known in the art, e.g., a neonicotinoid (e.g., imidacloprid) or an organophosphorus insecticide (e.g., a phosphorothioate, e.g., fenitrothion)) or plant allelochemical (e.g., caffeine, soyacystatin N, monoterpenes, diterpene acids, or phenolic compounds). Delivery and colonization of an isolated microorganism in the host may increase the host's resistance to the pesticide (e.g., a pesticide listed in Table 12) or plant allelochemical, thereby increasing host fitness. The methods may also be useful for the isolation of microorganisms with an enhanced ability to colonize any of the hosts described herein.

For example, to screen for microorganisms that increase a host's resistance to a pesticide (e.g., a pesticide listed in Table 12), a starting culture may be used that includes microorganisms (e.g., bacteria) and high concentrations of a pesticide (e.g., a pesticide listed in Table 12 or an insecticide known in the art, e.g., a neonicotinoid (e.g., imidacloprid) or an organophosphorus insecticide (e.g., a phosphorothioate, e.g., fenitrothion)). In some instances, the pesticide may be provided in the form of an environmental sample enriched with the pesticide (e.g., a soil sample). Alternatively, the pesticide (e.g., a pesticide listed in Table 12) may be provided in pure form or in combination with other carriers. Further, the one or more microorganism isolates may be inoculated directly into the media (e.g., from a laboratory strain) or may be an environmental sample including one or more microorganism species. The growth media may be either liquid or solid. In some instances, the pesticide of interest is the sole carbon or nitrogen source for the microorganisms in the media. The culture may be sub-cultured (e.g., inoculated into fresh media with high levels of the pesticide) any number of times to enrich for and/or isolate microbial strains (e.g., bacterial strains) capable of metabolizing the pesticide. The original culture or the subcultures may be assessed using any methods known in the art to test for alterations (e.g., decrease) in the levels of the pesticide in the sample (e.g., using HPLC). Isolates that reduce the concentration of the pesticide (e.g., a pesticide listed in Table 12 or a pesticide known in the art, e.g., a neonicotinoid (e.g., imidacloprid) or an organophosphorus insecticide (e.g., a phosphorothioate, e.g., fenitrothion)) may be isolated for use as a modulating agent in any of the methods or compositions described herein.

The methods may be used to further select for microorganisms described herein, including those isolated from a screening assay, with an enhanced ability to colonize and survive in a host (e.g., insect,

e.g., bee). For example, a host may be inoculated with a bacterial isolate (e.g., one with the ability to degrade a pesticide). The host may then be tested at regular intervals for the presence of the bacterial isolate (e.g., via culturing or 16s RNA from guts isolated from the host (e.g., honeybee)). Bacterial isolates that survive in the host (e.g., the midgut of the honeybee) may be isolated for use as a
5 modulating agent in any of the methods or compositions described herein.

Table 12. Pesticides

Aclonifen	Fenchlorazole-ethyl	Pendimethalin
Acetamiprid	Fenothiocarb	Penflufen
Alanycarb	Fenitrothion	Penflufen
Amidosulfuron	Fenpropidin	Pentachlorbenzene
Aminocyclopyrachlor	Fluazolate	Penthiopyrad
Amisulbrom	Flufenoxuron	Penthiopyrad
Anthraquinone	Flumetralin	Pirimiphos-methyl
Asulam, sodium salt	Fluxapyroxad	Prallethrin
Benfuracarb	Fuberidazole	Profenofos
Bensulide	Glufosinate-ammonium	Proquinazid
beta-HCH; beta-BCH	Glyphosate	Prothiofos
Bioresmethrin	Group: Borax, borate salts (see	Pyraclofos
Blasticidin-S	Group: Paraffin oils, Mineral	Pyrazachlor
Borax; disodium tetraborate	Halfenprox	Pyrazophos
Boric acid	Imiprothrin	Pyridaben
Bromoxynil heptanoate	Imidacloprid	Pyridalyl
Bromoxynil octanoate	Ipconazole	Pyridiphenthion
Carbosulfan	Isopyrazam	Pyrifenox
Chlorantraniliprole	Isopyrazam	Quinmerac
Chlordimeform	Lenacil	Rotenone
Chlorfluazuron	Magnesium phosphide	Sedaxane
Chlorphropham	Metaflumizone	Sedaxane
Climbazole	Metazachlor	Silafluofen
Clopyralid	Metazachlor	Sintofen
Copper (II) hydroxide	Metobromuron	Spinetoram
Cyflufenamid	Metoxuron	Sulfoxaflor
Cyhalothrin	Metsulfuron-methyl	Temephos
Cyhalothrin, gamma	Milbemectin	Thiocloprid
Decahydrate	Naled	Thiamethoxam
Diafenthiuron	Napropamide	Tolfenpyrad
Dimefuron	Nicosulfuron	Tralomethrin
Dimoxystrobin	Nitenpyram	Tributyltin compounds
Dinotefuran	Nitrobenzene	Tridiphane
Diquat dichloride	o-phenylphenol	Triflumizole
Dithianon	Oils	Validamycin
E-Phosphamidon	Oxadiargyl	Zinc phosphide

EPTC	Oxycarboxin	
Ethaboxam	Paraffin oil	
Ethirimol	Penconazole	

EXAMPLES

The following is an example of the methods of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

5

Example 1: Isolation of microorganisms that degrade imidacloprid, a nicotinoid

This example demonstrates the acquisition of a library of microorganisms able to degrade imidacloprid, a neonicotinoid.

10 *Experimental design:*

Four soil samples with elevated concentrations of imidacloprid are collected as described in Bonmatin et al. (*Environ. Sci. Pollut. Res. Int.* 22:35-67, 2015) and diluted in Kaufman and Kearney's minimal salts media (MSM) to enrich for imidacloprid-degrading microorganisms. Three variations of cultures for each soil sample include: 50 mL carbon-limited MSM with 83 mg/L imidacloprid as the sole 15 carbon source; 50 mL nitrogen-limited MSM with imidacloprid as the sole nitrogen source and sodium citrate and sucrose added as supplemental carbon sources; and 50 mL MSM broth, with all components plus sodium citrate, sucrose, and imidacloprid. Each culture is inoculated with 2 g of the collected soil.

Cultures are maintained on a shaker at 100 rpm and 27℃. Subcultures (1 mL of the cultured solution diluted in 25 mL of new medium) are made monthly in order to obtain soil-free enrichments. 20 Aliquots of the cultures are periodically removed from the shaker flasks for HPLC analysis as described in Tago et al. (*Microbes Environ.* 21:58-64, 2006). For the HPLC assay, $\approx 7.0 \times 10^7$ microorganisms are incubated in 1.0 mL of 20 mM sodium-potassium phosphate buffer (pH 7.0) with 1.0 mM imidacloprid. After 20 min of incubation at 30℃, 1.0 mL of methanol is added. Imidacloprid and products of its partial degradation are quantitatively analyzed on a HPLC system (a 600E pump and 2487 dual absorbance 25 detector; Waters). Retention times and peak areas of the HPLC profiles are compared with those of known standards Bonmatin et al. (*Environ. Sci. Pollut. Res. Int.* 22:35-67, 2015).

Mixed enrichment cultures from the soil that show a decrease of imidacloprid via HPLC analysis during incubation are spread-plated using dilutions of 10^{-1} to 10^{-6} on nitrogen-limited agar plates with 36 mg/kg imidacloprid and streptomycin.

30 After a one-week incubation at 27℃, the plates are screened for colonies that morphologically appear different from one another (size differences). Individual colonies are grown into new agar plates with imidacloprid as the sole nitrogen source. After three weeks, single colonies are put into 25 mL of tryptic soy broth (TSB) with 25 mg/L imidacloprid.

After three days' growth, each of the isolates is centrifuged in conical Falcon tubes for 10 min at 35 6700 x g. The supernatant is removed, and the isolates are re-suspended in sterile phosphate buffer for a total volume of 10 mL. A 2-mL sample of each isolate is inoculated into a nitrogen-limited MSM and a carbon-limited MSM cultures; both cultures with 30 mg/L of imidacloprid in 25 mL total. Non-inoculated

controls are also made by inoculating 2 mL of phosphate buffer into each of the media types. All samples are wrapped in aluminum foil and placed on a shaker operated at 27℃ and 100 rpm. The samples are then analyzed for imidacloprid concentration by HPLC.

After seven days of incubation, 25 mL of methanol is added to the flasks. The samples are then sonicated for six minutes each at 50% duty cycle and centrifuged at 6700 x g. The supernatant is then analyzed and the imidacloprid is quantitatively measured by HPLC as described herein.

Isolates that reduce the concentration of imidacloprid are stored at -80C in a solution of 50% glycerol.

Example 2: Selection of imidacloprid degrading isolates that survive in bees' midgut

This example demonstrates the ability to select for an imidacloprid-degrading microorganism from isolates described in Example 1 that are able to survive in the bee midgut.

Therapeutic design: Ten isolates described in Example 1 are formulated with 0 (negative control), 10^2, 10^5, or 10^8 cfu/ml in 10 mL of 50% sucrose syrup.

Experimental design:

About fifty bees per bacterial isolate are collected and kept in a wooden cage. Bacterial strains isolated in the previous example are cultivated and prepared for feeding as followed. Cell from each isolate are washed and diluted to an OD600 at 0.1 in MSM supplemented with 50% (w/v) sucrose syrup and incubated at 30 ℃ for cell growth. The four concentrations of fresh bacterial cultures are administered to bees as feed (between 0-10^8 cfu/ml) as follows. The different concentrations of isolates are placed in a 15 ml tube with six small pinched holes in which the bees project their proboscis and consume the isolate-containing solutions. Fresh isolate-containing cultures are prepared daily for use as feed and mortality of bees is scored every day during the entire course (7 days) of the experiment by counting and removing dead bees. After 7 days, the isolate-containing solution is replaced with 15% (w/v) sucrose for 48 h.

Ten bees are removed from each replicate every 24 hours for bacterial analysis; the first sample is removed before experiment onset, followed by seven additional sampling points. After each sampling, the bees are anaesthetized on ice and the midgut is removed. Replicates for each time point are pooled and homogenized in 150 µl 1×PBS, then serially diluted and plated on nitrogen-limited agar plates with 36 mg/kg imidacloprid to grow out bacteria under selective growth conditions at 30℃ for 2 days.

The bacterial isolates that survive in the bee midgut are selected for administration to increase bees' resistance to neonicotinoids.

Example 3: Increase bees' resistance to neonicotinoids through the administration of imidacloprid-degrading bacteria

This example demonstrates the ability to increase the resistance of bees to insecticides, and more specifically to neonicotinoids. Bees play an important role in pollinating flowering plants, and are the major type of pollinator in many ecosystems that contain flowering plants. It is estimated that one third of the human food supply depends on pollination by insects, birds and bats, most of which is

accomplished by bees, especially the domesticated European honey bee. The number of colonies kept by beekeepers has declined, through urbanization, systematic pesticide use, and tracheal and Varroa mites. Through direct consumption of contaminated nectar and pollen from insecticide treated plants, neonicotinoids in the insecticides affect foraging, learning, and memory in worker bees. By introducing imidacloprid degrading bacteria into their microbiome, bees are expected to increase their resistance to neonicotinoids through improved behavior, such as increased foraging, hygienic behavior, and survival.

Therapeutic design: The bacterial isolate selected in Example 2 and identified as surviving in bees' midgut is formulated at 10^8 organisms in 10 mL of 50% sucrose syrup.

Experimental design:

The isolate selected in Example 2 is prepared at a concentration of 10^8 cells/mL (see Example 2) and placed in a 15 ml tube with six small pinched holes in which the bees project their proboscis and consume the isolate-containing solution. Fresh isolate-containing cultures are prepared daily for use as feed. The pretreatment lasts for seven days in total.

Approximately 1500, 3000, or 7000 worker hives estimated by weight of worker bees, *Apis mellifera*, (0.5, 1, and 2 lbs., respectively, or 0.23, 0.45, and 0.1 kg) are pretreated with the isolate selected in Example 2 prepared at a concentration of 10^8 cells/mL and placed in a 15 ml tube with six small pinched holes in which the bees project their proboscis and consume the isolate-containing solution. Fresh isolate-containing cultures are prepared daily for use as feed. The pretreatment lasts for seven days in total.

After the 7 day pretreatment, each worker hive is fed 2 to 4 grams of pollen supplement and sugar syrup (1:1) for 1-2 days before imidacloprid treatment. Smaller colonies (1500- and 3000-bees) are placed in glass-walled observation hives. The larger colonies (7000-bees) are placed in Ulster observation hives (Brushy Mountain Bee Supply, NC) containing a bottom box holding 4 standard frames and a division board feeder where treatment syrup is provided. All observation hives are housed in sheds maintained at constant temperature and relative humidity (23–25 °C and 70%). Additionally, all hives contain an entrance leading to the outside allowing bees to freely forage in surrounding agricultural fields and urban residential neighborhoods.

When egg laying is visually confirmed, each colony is randomly assigned an imidacloprid treatment (0, 10, 20, 50, and 100 ppm) provided in 50% sucrose syrup. Colonies are given through a dispenser system similar to the one described in Example 2 with proportional amounts of sucrose solution with imidacloprid: 80, 160 and 320 mL for 1500-, 3000-, and 7000-bee colonies, respectively. Syrup is replenished every other day for 3 weeks. Syrup quantities are designed to supplement, but not sustain, the colonies, so bees are required to freely forage on other resources. Stock solutions of imidacloprid (100 ppm) are prepared using 99.5 ± 0.5% technical grade imidacloprid purchased from Chem Service, Inc (PS-2086) dissolved with agitation in 50% sucrose overnight. Stock solutions are prepared every two weeks and treatment solutions are prepared every week. Samples of treatment solutions (3–6 per dose) are randomly selected and tested for residue concentrations to confirm accuracy of dosage.

Measurements of behavioral phenotypes:

Foraging activity is measured by recording the number of workers entering and exiting the entrance of the bee colony during one-minute observations twice a day. Hygienic behavior is used as a measure to assess worker activity inside the hive, and is defined as the ability of worker bees to detect and remove diseased and mite-infested brood thereby limiting within-colony transmission of pathogens and parasites. Hygienic behavior is measured using a freeze-killed brood assay, in which a 3-inch (7.6 cm) polyvinyl chloride tube is gently pushed into a section of comb containing a large area of sealed pupal cells (taken from non-experimental field colonies). The number of empty cells is counted and recorded before pouring 400 ml of liquid nitrogen to freeze-kill roughly 160 pupae. The frame is then temporarily put into the bee colony and the proportion of pupae completely or partially removed from the cells is quantified after 24 hours to assess hygienic behavior. The removal of freeze-killed brood is correlated with the removal of diseased and parasitized brood.

Bees pretreated with the bacterial isolate selected in Example 2 demonstrate higher activity than bees treated with the control.

Example 4: Treatment of Varroa mites that infect bees with oxytetracycline solutions

This example demonstrates the ability to kill, decrease the fitness of Varroa mites by treating them with an antibiotic solution. This example demonstrates that the effect of oxytetracycline on Varroa mites is mediated through the alteration of bacterial populations endogenous, such as *Bacillus*, to the Varroa mites that are sensitive to oxytetracycline.

Varroa mites are thought to be a leading cause for the wide spread Colony Collapse Disorder (CCD) that decimates domesticated honey bee colonies of *Apis mellifera*, around the world. They attach to the bees' abdomen and suck on their blood, depriving them of nutrients, and eventually killing them. While Varroa mites can be killed with chemically synthesized miticides, these types of chemicals must be kept away from comestible honey.

Therapeutic design: Oxytetracycline solution is formulated with 0 (negative control), 1, 10, or 50 µg/ml in 10 mL of sterile water with 0.5% sucrose and essential amino acids.

Experimental design:

To determine whether adult Varroa mites at the reproductive stage have different susceptibility compared to phoretic mites or their offspring because their cuticle is not hardened, Varroa mites living on adult bees, *Apis mellifera,* and mites associated with larvae and pupae are collected. This assay tests antibiotic solutions on different types of mites and determines how their fitness is altered by targeting endogenous microbes, such as *Bacillus.*

The brood mites are collected from combs (or pieces of combs) of Varroa mite-infested bee colonies. The mites are collected from cells where bee larvae develop.

Varroa mites are grouped per age of their brood host and assayed separately. The age of the brood is determined based on the morphology and pigmentation of the larva or the pupa as follows: Varroa mites collected from spinning larvae that are small enough to move around their cell are grouped into Group 1; Varroa mites collected from stretched larvae, which are too big to lay in the cell and start to

stretch upright with their mouth in the direction of the cell opening, are grouped into Group 2; and Varroa mites collected from pupae are grouped into Group 3. Mites are stored on their host larva or pupa in glass Petri dishes until 50 units are collected. This ensures their feeding routine and physiological status remains unchanged. To prevent mites from straying from their host larva or pupa or climbing onto one another, only hosts at the same development stage are kept in the same dish.

The equipment – a stainless steel ring (56 mm inner diameter, 2–3 mm height) and 2 glass circles (62 mm diameter) – is cleaned with acetone and hexane or pentane to form the testing arena. The oxytetracycline solutions and control solution are applied on the equipment by spraying the glass disks and ring of the arena homogeneously. For this, a reservoir is loaded with 1 ml of the solutions; the distance of the sprayed surface from the bottom end of the tube is set at 11 mm and a 0.0275 inch nozzle is used. The pressure is adjusted (usually in the range 350–500 hPa) until the amount of solution deposited is 1 ± 0.05 mg/cm^2. The antibiotic solutions are poured in their respective dishes, covering the whole bottom of the dishes, and residual liquid is evaporated under a fume hood. The ring is placed between the glass circles to build a cage. The cages are used within 60 hr of preparation, for not more than three assays, in order to control the exposure of mites to antibiotic solutions. 10 to 15 Varroa mites are introduced in this cage and the equipment pieces are bound together with droplets of melted wax. Mites collected from spinning larvae, stretched larvae, white eyed pupae and dark eyed with white and pale body are used.

After 4 hours, mites are transferred into a clean glass Petri dish (60 mm diameter) with two or three white eye pupae (4-5 days after capping) to feed on. The mites are observed under a dissecting microscope at 4hr, 24hr, and 48hr after being treated with the antibiotic or the control solutions, and classified according to the following categories:

- Mobile: they walk around when on their legs, whether after being poked by a needle.
- Paralyzed: they move one or more appendages, unstimulated or after stimulation, but they cannot move around.
- Dead: immobile and do not react to 3 subsequent stimulations.

A sterile toothpick or needle is used to stimulate the mites by touching their legs. New tooth picks or sterile needles are used for stimulating each group to avoid contamination between mite groups.

The assays are carried out at 32.5℃ and 60-70% relative humidity. If the mortality in the controls exceeds 30%, the replicate is excluded. Each experiment is replicated with four series of cages.

The status of *Bacillus* in Varroa mite groups is assessed by PCR. Total DNA is isolated from control (non-oxytetracycline treated) and oxytetracyline treated individuals (whole body) using a DNA Kit (OMEGA, Bio-tek) according to the manufacturer's protocol. The primers for *Bacillus*, forward primer 5'-GAGGTAGACGAAGCGACCTG -3' (SEQ ID NO: 186) and reverse primer 5'-TTCCCTCACGGTACTGGTTC -3' (SEQ ID NO: 187), are designed based on *23S-5S rRNA* sequences obtained from the *Bacillus* genome (Accession Number: AP007209.1) (Takeno et al., *J. Bacteriol.* 194(17):4767-4768, 2012) using Primer 5.0 software (Primer-E Ltd., Plymouth, UK). The PCR amplification cycles included an initial denaturation step at 95 ℃ for 5 min, 35 cycles of 95 ℃ for 1min,

59 °C for 1min, and 72 °C for 2min, and a final extension step of 5min at 72 °C. Amplification products from oxytetracyline treated and control samples are analyzed on 1% agarose gels, stained with SYBR safe, and visualized using an imaging System.

The survival rates of Varroa mites treated with an oxytetracyline solution are compared to the Varroa mites treated with the negative control.

The survival rate and the mobility of Varroa mites treated with oxytetracyline solution are decreased compared to the control.

Example 5: Isolation of microorganisms that degrade fenitrothion, an organophosphorus insecticide

This Example demonstrates the acquisition of a library of microorganisms able to degrade fenitrothion, an organophosphorus insecticide.

Experimental design

Soil samples are obtained from various regions where fenitrothion was previously utilized for insect control. Fenitrothion degrading bacteria will be isolated from the soil samples as described in (Microbes Environ. Vol. 21, No. 1, 58–64, 2006). Briefly, 1 g of the soil sample is mixed thoroughly with 9 ml of sterile distilled water. The soil particles are then centrifuged at 1000 rcf for 5 min, and 100µl of the supernatant is then plated onto fenitrothion with mineral salts medium (0.4 g/l of yeast extract, 0.4 g/l fenitrothion, 15 g/l bacteriological agar). The plates are cloudy when prepared as the fenitrothion is an emulsion.

Colonies that develop clear zones around them and are likely to be degrading or metabolizing fenitrothion, and these colonies are isolated and regrown on LB agar, nutrient agar, yeast glucose agar, TSA agar, BHI agar, or MRS agar. Once unique bacterial colonies are identified, their genomes are extracted using a genomic DNA extraction kit, (Qiagen, DNeasy Blood and Tissue Kit) as per the manufacturer's protocol.

The 16S rRNA gene is amplified using universal bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3'; SEQ ID NO: 188) and 1492R (5'-TACCTTGTTACGACTT-3'; SEQ ID NO: 189), and using PCR conditions of 94°C for 2 min, 30 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min, and a final extension of 72°C for 5min. Gel electrophoresis is used to confirm that the amplicons are of the correct size (~1500 bp), and the products are excised from the gel and purified using a gel extraction kit (Qiagen, QIAquick) as per the manufacturer's protocol. The correct size amplicons are Sanger sequenced and BLAST is used to match the sequence against various repositories of 16s rRNA gene sequences to identify the bacteria.

To determine whether the isolated bacteria is degrading fenitrothion, ~10^7 bacterial cells are incubated in 1 ml of 20mM sodium-potassium phosphate buffer (pH 7) with 1mM fenitrothion, as described in PNAS, Vol. 109, No. 22, 8618-8622, 2012. After 30 min of incubation at 30°C, the reaction is stopped by adding an equal volume of methanol. Then fenitrothion and its metabolite, 3-methyl-4-nitrophenol, are analyzed by HPLC. The retention times and peak areas of the HPLC profiles are compared to known standards.

Unique bacterial isolates that have fenitrothion degrading capabilities are then stored as frozen glycerol at -80℃.

Example 6: Increasing *Drosophila melanogaster's* resistance to fenitrothion through the administration of fenitrothion-degrading bacteria

This Example demonstrates the ability to protect an insect model, *Drosophila melanogaster*, from one or more negative effects of insecticides in their diet, more specifically fenitrothion. The following approach is a surrogate for other insects, such as bees or other insects disclosed herein, e.g., insects that are important pollinators for many flowering plant crops and vegetables. Many insecticides including fenitrothion have been shown to be toxic to bees.

Experimental design:

Therapeutic design: The bacterial isolates selected in Example 5 are formulated at 10^9 organisms in 100 µl of fly food medium with and without fenitrothion.

The media used to rear flies is cornmeal, molasses and yeast medium (11 g/l yeast, 54 g/l yellow cornmeal, 5 g/l agar, 66 ml/l molasses, and 4.8 ml/l propionic acid). For experimental procedures, fenitrothion at 0, 10, and 100 p.p.m. or phosphate-buffered saline as negative controls are infused into sterile fly food medium.

Development rate assay

On day one, 10^9 fenitrothion-degrading bacteria described in Example 5 are suspended in 100 µl phosphate-buffered saline or equal volumes of saline (negative controls) are added to sterile fly food medium with or without fenitrothion. All are left to dry for a day at 25℃ as described in *Appl. Environ. Microbiol.* Vol. 82, No. 20, 6204-6213, 2016.

On day two, fertilized embryos collected from flies are treated with 2% hypochlorite solution for 5 min and then washed with sterile water to remove any extracellular microbes from the embryos. 10µl of the embryo suspension in water (1:3 embryo:water suspension) is added to the bacteria-seeded or negative control fly food with or without the fenitrothion. The fly food cohorts with the embryos are maintained at 25℃ with 12h light and 12 dark cycle for the rest of the experiment.

The time to puparium formation and the number of pupa formed is measured in each cohort. The time to adult emergence and the rate of emergence is measured in each sample. From the time the first adult emerges from the pupa, the number of emerging adult flies are counted every 12 hours and rate of emergence is computed.

Embryos in the fenitrothion infused fly food seeded with Fenitrothion-degrading bacteria are expected to develop faster than the the embryos on fenitrothion infused food without the fenitrothion-degrading bacteria.

Survival assay

About 12 days before day one, sterile embryos are generated as described previously and raised on sterile fly food. Adults start to emerge from their pupae 11 days from embryo collection when raised in sterile fly food without fenitrothion at 25℃ with 12 h light and 12 h dark cycle.
5 On day one, 10^9 of the fenitrothion-degrading bacteria in phosphate-buffered saline are added to sterile fly food medium as described in a previous Example.

On day two, 10 newly emerged sterile adult males and females are introduced to bacteria-seeded or negative control fly food with or without fenitrothion. The fly food with the flies is maintained at 25℃ with 12h light and 12 dark cycle for the rest of the experiment. The number of surviving male and female
10 flies are counted every day until all the flies are dead. Survival analysis is performed to assess the fitness benefit of fenitrothion-degrading bacteria on the fly survival.

Flies raised on fenitrothion infused fly food seeded with fenitrothion-degrading bacteria are expected to survive longer than flies raised on fenitrothion infused food without the fenitrothion-degrading bacteria.

15

Example 7: Elimination of entomopathogenic bacteria from *Drosophila melanogaster* using naturally occurring phages

This Example demonstrates the ability to eliminate insect bacterial pathogens (such as *Serratia marcescens*, *Erwinia carotovora*, and *Pseudomonas entomophila*) from *Drosophila melanogaster* using
20 naturally occurring phages. This procedure can be useful as a surrogate assay for eliminating bacteria in other insect species, such as in bees.

Experimental design:

Therapeutic design: Phage library collections are used having the following phage families:
25 *Myoviridae, Siphoviridae, Podoviridae, Lipothrixviridae, Rudiviridae, Ampullaviridae, Bicaudaviridae, Clavaviridae, Corticoviridae, Cystoviridae, Fuselloviridae, Gluboloviridae, Guttaviridae, Inoviridae, Leviviridae, Microviridae, Plasmaviridae, Tectiviridae.*

Multiple environmental samples (soil, pond sediments, and sewage water) are collected in sterile 1L flasks over a period of 2 weeks and are immediately processed after collection and stored thereafter at
30 4 ℃. Solid samples are homogenized in sterile double-strength difco luria broth (LB) or tryptic soy broth (TSB) supplemented with 2mM CaCl2 to a final volume of 100mL. The pH and phosphate levels are measured using phosphate test strips. For purification, all samples are centrifuged at 3000–6000 g for 10–15 min at 4 ℃, and filtered through 0.2-µm low protein filters to remove all remaining bacterial cells. The supernatant that contains the phage library is then stored at 4 ℃ in the presence of chloroform in a
35 glass bottle.

20–30 ml of the phage library is diluted to a volume of 30–40 ml with LB-broth. The target bacterial strain (e.g., *Serratia marcescens*, *Erwinia carotovora*, and *Pseudomonas entomophila*) is added (50–200 µl overnight culture grown in LB-broth) to enrich phages that target this specific bacterial strain in the culture. This culture is incubated overnight at 37 ℃, shaken at 230 rpm. Bacteria from this
40 enrichment culture are removed by centrifugation (3000–6000 g 15–20 min, 4 ℃) and filtered (0.2 or 0.45

µm filter). 2.5 ml of the bacteria free culture is added to 2.5 ml of LB-broth and 50–100 µl of the target bacteria are added back to the culture to further enrich the phages. The enrichment culture is grown overnight as above. A sample from this enrichment culture is centrifuged at 13,000 g for 15 min at room temperature and 10 µl of the supernatant is plated on an LB-agar petri dish along with 100–300 µl of the target bacteria and 3 ml of melted 0.7% soft-agar. The plates are incubated overnight at 37 °C.

Each of the plaques observed on the bacterial lawn are picked and transferred into 500 µl of LB-broth. A sample from this plaque-stock is further plated on the target bacteria. Plaque-purification is performed three times for all discovered phages in order to isolate a single homogenous phage from the heterogeneous phage mix.

Lysates from plates with high-titer phages (>1x 10^10 PFU/ml) are prepared by harvesting overlay plates of a host bacterium strain exhibiting confluent lysis. After being flooded with 5 ml of buffer, the soft agar overlay is macerated, clarified by centrifugation, and filter sterilized. The resulting lysates are stored at 4 °C. High-titer phage lysates are further purified by isopycnic CsCl centrifugation, as described in Summer et al., *J. Bacteriol.* 192:179-190, 2010.

DNA is isolated from CsCl-purified phage suspensions as described in Summer, *Methods Mol. Biol.* 502:27-46, 2009. An individual isolated phage is sequenced as part of two pools of phage genomes by using a 454 pyrosequencing method. Briefly, phage genomic DNA is mixed in equimolar amounts to a final concentration of about 100 ng/L. The pooled DNA is sheared, ligated with a multiplex identifier (MID) tag specific for each of the pools, and sequenced by pyrosequencing using a full-plate reaction on a sequencer (Roche) according to the manufacturer's protocols. The pooled phage DNA is present in two sequencing reactions. The output corresponding to each of the pools is assembled individually by using software (454 Life Sciences), by adjusting the settings to include only reads with a single MID per assembly. The identity of individual contigs is determined by PCR using primers generated against contig sequences and individual phage genomic DNA preparations as the template. Sequence software (Gene Codes Corporation) is used for sequence assembly and editing.

Phage chromosomal end structures are determined experimentally. Cohesive (cos) ends for phages are determined by sequencing off the ends of the phage genome and sequencing the PCR products derived by amplification through the ligated junction of circularized genomic DNA, as described in Summer, *Methods Mol. Biol.* 502:27-46, 2009. Protein-coding regions are initially predicted using gene prediction software (Lukashin et al. *Nucleic Acids Res.* 26:1107-1115, 1998), refined through manual analysis in Artemis (Rutherford et al., *Bioinformatics* 16:944-945, 2000), and analyzed through the use of BLAST (E value cutoff of 0.005) (Camacho et al., *BMC Bioinformatics* 10:421, 2009). Proteins of particular interest are additionally analyzed by sequence searching software (Hunter et al., *Nucleic Acids Res.* 40:D306-D312, 2012).

Electron microscopy of CsCl-purified phage (>1 x10^11 PFU/ml) that lysed the *Drosophila*'s pathogenic bacterial species is performed by diluting phage stock with the tryptic soy broth buffer. Phages are applied onto thin 400-mesh carbon-coated grids, stained with 2% (wt/vol) uranyl acetate, and air dried. Specimens are observed on a transmission electron microscope operating at an acceleration voltage of 100 kV. Five virions of each phage are measured to calculate mean values and standard deviations for dimensions of capsid and tail, where appropriate.

Incorporating phages into a meal

The media used to rear flies is cornmeal, molasses and yeast medium (11 g/l yeast, 54 g/l yellow cornmeal, 5 g/l agar, 66 ml/l molasses, and 4.8 ml/l propionic acid). Phage solutions are infused into the fly food to obtain final concentrations of phages between 0 and 10^8 pfu/ml.

S. Marcescens, *Erwinia carotovora*, and *Pseudomonas entomphila* bacteria are grown at 30℃ in nutrient broth or LB broth.

Sterile fly embryos are generated by treating fertilized embryos collected from flies with 2% hypochlorite solution for 5 min and then washed with sterile water to remove any extracellular microbes. Fly larvae with the target bacteria are generated by seeding 10^9 CFUs of bacteria in sterile fly food and adding sterile fly embryos to this food. After 2 days, ten *S. marcescens* infected first instar fly larvae are added to the fly food with a range (0-10^8 pfu/ml) of the phage concentrations. The larvae are left to grow in the food with the phages for 3 days until they become third instars. The larvae are then collected and individually homogenized in nutrient broth or LB broth, and plated on nutrient agar or LB agar plates, and incubated at 30℃. The number of CFUs of *S. marcescens* obtained from larvae in fly food with varying phage concentrations are recorded. This shows the number of live bacteria that were present in the flies.

The number of live bacteria are expected to be reduced in the larvae grown on fly food with the phages against the bacteria.

Example 8: Generation of a library of natural microbes

This Example demonstrates the isolation of bacteria from soil that naturally produce the amino acid, methionine.

The medium used for isolation of microorganisms is Starch-Casein-Nitrate agar (Starch, 10.0 g; Casein, 0.003 g; KNO_3, 0.02 g; NaCl, 0.02 g; $MgSO_4$, 0.5 mg; $CaCO_3$, 0.2 mg; $FeSO_4$, 0.1 mg; Agar, 12.0 g; H_2O, 1L; pH 7.0) (Kuster and Williams, 1964). Each environmental soil sample (1.0 g) is suspended in 9 ml of sterile water, and 1 ml of the suspension is serially diluted ten-fold in sterile distilled water. One milliliter of the 10^{-5} dilution is inoculated onto the agar medium and incubated for 7 days at 30℃. At the end of this period, the plates are observed for growth. White discrete and leathery colonies are picked and grown on new Starch-Casein-Nitrate agar plates to create a library of isolates. After 7 days of growth at 30℃, the plates are kept at 4℃.

Example 9: Screen for isolates that produce methionine

This Example demonstrates the screening assay of isolates from Example 8 that naturally produce the amino acid, methionine.

Screening for methionine production:

A modified basal medium (K_2HPO_4, 0.3 g; KH_2PO_4, 0.7 g; Na_2CO_3, 1.0 g; $CaCl_2$, 5.0 mg; $MgSO_4$, 0.3 g; $FeSO_4$, 1.0 mg; H_2O, 1L) with sucrose (20.0 g) and NH_4Cl (10.0 g) is used for fermentation (Chay, B.P., Galvez, F.C.F., and Padolina, W.G.P.U.L.B.P. (1992). Methionine production by batch fermentation from various carbohydrates. ASEAN Food Journal (Malaysia)). The pH of the medium is 7.2.

Culture conditions: Two loops of the 7 day isolate culture of Example 8 are inoculated into a 250 ml Erlenmeyer flask with 30 ml of the fermentation medium. Methionine production is assayed after incubation of the flask for 5 days on a rotary shaker (160 rpm) at 30℃. Duplicate flasks are prepared and non-inoculated flasks served as control in all experiments.

5 The presence of methionine in the culture broths of the isolates is examined by paper chromatography following a modified method of Khanna and Nag (Khanna et al., “Production of amino acids in vitro tissue culture,” *Indian Journal of Experimental Biology* (1973)). The broth culture is centrifuged at 5000 x g for 20 min and 2 µL of the supernatant is applied 1.5 cm above one edge of Whatman No.1 filter paper, with dimensions of 18 cm x 22 cm. 1 µL of volume of a standard methionine 10 solution (0.1 mg/mL) is applied alongside with the supernatant, and the chromatogram is developed in a solvent mixture of n-butanol, acetic acid and water (4: 1: 1) for 18 h. The chromatogram is air-dried at room temperature, sprayed with 0.15% ninhydrin solution in butanol and dried again before heating at 60℃ for 5 min in an oven. The value of the ninhydrin-positive spot (bluish-violet) of the supernatant that corresponds with the value of the standard methionine solution indicates presence of methionine in the 15 broth culture. The concentration of methionine produced in the broth culture of the isolate is estimated as follows. The ninhydrin-positive spot of the supernatant of the isolate on the chromatogram is eluted in 10% ethanol and the spectrophotometric reading of the eluate at 520 nm recorded. The methionine concentration in the supernatant is determined from a standard curve. A plot of the values of optical densities against varying concentrations (0.1 to 0.9 mg/ml) of a methionine solution serve as the standard 20 methionine curve.

Isolates that produce methionine are kept on fresh agar plates and a stock solution is created by suspending two loopfuls of microorganism in an aliquot of 50% glycerol solution.

25 **Example 10: Administration of amino acid producing strain of bacteria to *Drosophila melanogaster* through diet to increase their development rate**

This example demonstrates the ability to treat *Drosophila melanogaster* with amino acid producing bacteria to increase their development rate when raised on nutrient deprived diets. This experimental design can be extended to aid in the development of other insects, such as honeybees, 30 which may lack nutrients in their diets.

Therapeutic design:

Isolated bacteria *Corynebacterium glutamicum* that are glutamate or methionine producing, are formulated with a solution of 10^9 colony forming units (CFUs) mixed to the feeding substrate for 35 *Drosophila* flies.

Experimental design:

Corynebacterium glutamicum strains that produce glutamate or methionine were grown in nutrient broth at 30℃.

The media used to rear flies is cornmeal, molasses and yeast medium (11 g/l yeast, 54 g/l yellow cornmeal, 5 g/l agar, 66 ml/l molasses, and 4.8 ml/l propionic acid). All the components of the diet except propionic acid are heated together to 80℃ in deionized water with constant mixing for 30 minutes and let to cool to 60℃. Propionic acid is then mixed in and 50ml of the diet is aliquoted into individual bottles
5 and allowed to cool down and solidify. The flies are raised at 26℃, 16:8 hour light:dark cycle, at around 60% humidity.

For the experimental setup to measure the larval growth rate, defined diet was used (Piper et al., 2014, Nature Methods). Defined diet eliminates the effects of batch to batch variation in the ingredients used for the cornmeal based diet. In addition, the defined diet allows for the exclusion of individual
10 components to test their effects on fly development.

Development rate assay

On day one, 100 ul of a *Corynebacterium glutamicum* suspension in nutrient broth consisting of 10^9 colony forming units (CFUs) were added to five replicates of fly food. As controls, nutrient broth
15 without the bacteria was added to five more bottles of fly food. Fertilized embryos collected from fruit flies were treated with 2% hypochlorite solution for five minutes and then washed with sterile water to remove any extracellular microbes from the embryos. 10ul of the embryo suspension in water (one:three embryo:water suspension) was added to all the bacteria seeded and control fly food bottles. The fly food with the embryos was maintained at 26℃, 16:8 hour light:dark cycle, at around 60% humidity for the rest
20 of the experiment. The time to adult emergence and the rate of emergence was measured in every replicate. From the time the first adult emerges from the pupa, the number of adult flies emerging was counted every 12 hours and rate of emergence was be computed.

Larval mass assay

25 To test whether the presence of bacteria producing amino acids can increase the body mass of developing larvae when raised on defined diet, we produced larvae that are axenic, and mono-associated with a single strain of bacterium. For these assays, three different bacteria were used, Corynebacterium glutamicum - a strain that produces glutamate, Corynebacterium glutamicum – a strain that produces methionine, and *E. coli*.

30 First, axenic embryos were generated. Fertilized embryos were collected from fruit flies over a 6 hour period on grape juice agar plates with yeast. To eliminate any bacterial contamination, the embryos were treated with 2% hypochlorite solution for five minutes and then washed thrice with sterile water. One volume of embryos was then suspended in 3 volumes of water.

The defined diet was aliquoted into vials and nine replicates were used for every condition being
35 tested. The conditions were:

1. No bacteria added to the food
2. Food containing C. glutamicum, strain that produces glutamate (C.glu-Glu)
3. Food containing C. glutamicum, a strain that produces methionine (C.glu-Met)
4. Food containing *E. coli*

To each vial of the food that were in conditions 2, 3, and 4, 100ul of overnight stationary phase cultures was added.

To each of the nine replicates in every condition, 10ul of the sterile embryo+water suspension was added. The vials were then incubated at 26℃, 60% humidity, 16:8 light:dark cycle.

5 After 13 days, 10-15 randomly chosen larvae from each replicate were sampled, and their areas were measured, as a proxy to their biomass and weight. The larvae were scooped out from the food with a sterile spatula, rinsed in water to clean the food from their bodies, and an image of every larvae sampled was acquired individually for every replicate in each condition. An Image J script was used to identify, outline, and measure the area of the larva in every image.

10 *Amino acid producing bacteria treatment increases insect development rate.*

Embryos that developed on diet that was seeded with the amino acid producing strain of bacterium reached adulthood significantly faster than those that were raised on the sterile diet (Fig. 1). Further, this effect was slightly stronger in female flies than in male files (Figs. 2A and 2B).

15 *Amino acid producing bacteria treatment increases larval body mass.*

Larvae from the diet supplemented with C. glu-Met had the largest body size on average, followed by those in diet with C.glu-Glu, *E.coli*, and no bacteria (Fig. 3). This shows that augmenting the diet of insects with bacteria that produce amino acids produced insect biomass faster than un-supplemented diet.

20 Together this data demonstrates that augmenting the diet of insects with bacteria that were capable of producing amino acids produced insect biomass faster than un-supplemented diet. Extending this to other insects such as bees, supplementing their diet with bacteria that are capable of producing methionine can increase their fitness.

25 **Example 11: Insects treated with a solution of purified phage**

This Example demonstrates the isolation and purification of phages from environmental samples that targeted specific insect bacteria. This Example also demonstrates the efficacy of isolated phages against the target bacteria in vitro by plaque assays, by measuring their oxygen consumption rate, and the extracellular acidification rate. Finally, this Example demonstrates the efficacy of the phages in vivo, 30 by measuring the ability of the phage to the target bacteria from flies by treating them with a phage isolated against the bacteria. This Eample demonstrates that a pathogenic bacterium that decreased the fitness of an insect can be cleared using a phage to target the bacteria. Specifically, *Serratia marcescens* which is a pathogenic bacterium in flies can be cleared with the use of a phage that was isolated from garden compost.

35 There are several beneficial and commercially useful insects that are affected by naturally occurring bacterial pathogens. One such example is the bacterium *Paenibacillus larvae* which is the

cause of foul brood disease in honey bees. Using a phage to treat foul brood disease would be of great value in mitigating huge losses due to foul brood disease.

Experimental design

5 *Isolation of specific bacteriophages from natural samples:*

Bacteriophages against target bacteria were isolated from environmental source material. Briefly, a saturated culture of *Serratia marcescens* was diluted into fresh double-strength tryptic soy broth (TSB) and grown for ~120 minutes to early log-phase at 24-26°C, or into double-strength Luria-Bertani (LB) broth and grown for ~90 min at 37°C. Garden compost was prepared by homogenization in PBS and 10 sterilized by 0.2 µm filtration. Raw sewage was sterilized by 0.2 µm filtration. One volume of filtered source material was added to log-phase bacterial cultures and incubation was continued for 24 h. Enriched source material was prepared by pelleting cultures and filtering supernatant fluid through 0.45 µm membranes.

Phages were isolated by plating samples onto double-agar bacterial lawns. Stationary bacterial 15 cultures were combined with molten 0.6% agar LB or TSB and poured onto 1.5% agar LB or TSB plates. After solidification, 2.5 µL of phage sample dilutions were spotted onto the double-agar plates and allowed to absorb. Plates were then wrapped and incubated overnight at 25°C (TSA) or 37°C (LB), then assessed for the formation of visible plaques. Newly isolated plaques were purified by serial passaging of individual plaques on the target strain by picking plaques into SM Buffer (50 mM Tris-HCl [pH 7.4], 10 mM 20 $MgSO4$, 100 mM NaCl) and incubating for 15 min at 55°C, then repeating the double-agar spotting method from above using the plaque suspension.

Bacteriophages were successfully isolated from both sewage and compost, as detailed above. Plaque formation was clearly evident after spotting samples onto lawns of the *S. marcescens* bacteria used for the enrichments.

25

Passaging, quantification, and propagation of bacteriophages:

Propagation and generation of phage lysates for use in subsequent experiments was performed using bacteriophages isolated and purified as above. Briefly, saturated bacterial cultures were diluted 100-fold into fresh medium and grown for 60-120 minutes to achieve an early-logarithmic growth state for 30 effective phage infection. Phage suspensions or lysates were added to early log phase cultures and incubation was continued until broth clearing, indicative of phage propagation and bacterial lysis, was observed, or until up to 24 h post-infection. Lysates were harvested by pelleting cells at 7,197 x g for 20 min, then filtering the supernatant fluid through 0.45 or 0.2 µm membranes. Filtered lysates were stored at 4°C.

35 Enumeration of infective phage particles was performed using the double-agar spotting method. Briefly, a 1:10 dilution series of samples was performed in PBS and dilutions were spotted onto solidified

double-agar plates prepared with the host bacteria as above. Plaque-forming units (PFU) were counted after overnight incubation to determine the approximate titer of samples.

In vitro analysis of isolated phages measuring bacterial respiration:

5 A Seahorse XFe96 Analyzer (Agilent) was used to measure the effects of phages on bacteria by monitoring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) during infection. XFe96 plates were coated the day prior to experiments by 15 µL of a 1 mg/mL poly-L-lysine stock per well and dried overnight at 28°C and XFe96 probes were equilibrated by placing into wells containing 200 µL of XF Calibrant and incubating in the dark at room temperature. The following day, poly-L-lysine coated 10 plates were washed twice with ddH2O. Saturated overnight cultures of *E. coli* BL21 (LB, 37°C) or *S. marcescens* (TSB, 25°C) were subcultured at 1:100 into the same media and grown with aeration for ~2.5 h at 30°C. Cultures were then diluted to O.D.600nm ~ 0.02 using the same media. Treatments were prepared by diluting stocks into SM Buffer at 10x final concentration and loading 20 µL of the 10x solutions into the appropriate injection ports of the probe plate. While the probes were equilibrating in the 15 XFe96 Flux Analyzer, bacterial plates were prepared by adding 90 µL of bacterial suspensions or media controls and spun at 3,000 rpm for 10 min. Following centrifugation, an additional 90 µL of the appropriate media were added gently to the wells so as not to disturb bacterial adherence, bringing the total volume to 180 µL per well.

The XFe96 Flux Analyzer was run at ~30°C, following a Mix, Wait, Read cycling of 1:00, 0:30, 20 3:00. Four cycles were completed to permit equilibration/normalization of bacteria, then the 20 µL treatments were injected and cycling continued as above, for a total time of approximately 6 h. Data were analyzed using the Seahorse XFe96 Wave software package.

The effects of isolated bacteriophages were assayed by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of bacteria with a Seahorse XFe96 Analyzer. When 25 *E. coli* was infected with phage T7 and *S. marcescens* infected with the newly isolated ΦSmVL-C1, dramatic decreases in OCR were observed following brief bursts in this rate (Fig. 4). For both phages with both host organisms, the Seahorse assay permitted the detection of successful phage infection without the need for plaque assays. Thus, this method is applicable for detecting phage infection of a host organism not amenable to traditional phage detection methods.

30

SYBR Gold transduction assay for infection identification:

Bacteriophage preparations were prepared for staining by pretreating with nucleases to remove extraviral nucleic acids that could interfere with fluorescent signal interpretation. Briefly, MgCl2 was added to 10 mL of phage lysate at 10 mM final concentration, and RNase A (Qiagen) and DNase I 35 (Sigma) were both added to final concentrations of 10 µg/mL. Samples were incubated for 1 h at room temperature. After nuclease treatment, 5 mL of lysates were combined with 1 µL of SYBR Gold (Thermo, 10,000x) and incubated at room temperature for ~1.5 h. Excess dye was subsequently removed from samples using Amicon ultrafiltration columns. Briefly, Amicon columns (15 mL, 10k MWCO) were washed by adding 10 mL of SM Buffer and spinning at 5,000 x g, 4°C for 5 min. Labeled phage samples 40 were then spun through the columns at 5,000 x g, 4°C until the volume had decreased by approximately

10-fold (15-30 min). To wash samples, 5 mL SM Buffer was added to each reservoir and the spin repeated, followed by two additional washes. After the third wash, the retained samples were pipetted out from the Amicon reservoirs and brought up to approximately 1 mL using SM Buffer. To remove larger contaminants, washed and labeled phage samples were spun at 10,000 x g for 2 min, and the
5 supernatants were subsequently filtered through 0.2 µm membranes into black microtubes and stored at 4℃.

Saturated bacterial cultures (*E. coli* MG1655 grown in LB at 37℃, *S. marcescens* and S. *symbiotica* grown in TSB at 26℃) were prepared by spinning down 1 mL aliquots and washing once with 1 mL PBS before a final resuspension using 1 mL PBS. Positive control labeled bacteria were
10 stained by combining 500 µL of washed bacteria with 1 µL of SYBR Gold and incubating for 1 h in the dark at room temperature. Bacteria were pelleted by spinning at 8,000 x g for 5 min and washed twice with an equal volume of PBS, followed by resuspension in a final volume of 500 µL PBS. A volume of 25 µL of stained bacteria was combined with 25 µL of SM Buffer in a black microtube, to which 50 µL of 10% formalin (5% final volume, ~2% formaldehyde) was added and mixed by flicking. Samples were fixed at
15 room temperature for ~3 h and then washed using Amicon ultrafiltration columns. Briefly, 500 µL of picopure water was added to Amicon columns (0.5 mL, 100k MWCO) and spun at 14,000 x g for 5 min to wash membranes. Fixed samples were diluted by adding 400 µL of PBS and then transferred to pre-washed spin columns and spun at 14,000 x g for 10 min. Columns were transferred to fresh collection tubes, and 500 µL of PBS was added to dilute out fixative remaining in the retentate. Subsequently, two
20 additional PBS dilutions were performed, for a total of three washes. The final retentates were diluted to roughly 100 µL, then columns were inverted into fresh collection tubes and spun at 1,000 x g for 2 min to collect samples. Washed samples were transferred to black microtubes and stored at 4℃.

For transduction experiments and controls, 25 µL of bacteria (or PBS) and 25 µL of SYBR Gold labeled phage (or SM Buffer) were combined in black microtubes and incubated static for 15-20 min at
25 room temperature to permit phage adsorption and injection into recipient bacteria. Immediately after incubation, 50 µL of 10% formalin was added to samples and fixation was performed at room temperature for ~4 h. Samples were washed with PBS using Amicon columns, as above.

Injection of bacteriophage nucleic acid was required for a phage to successfully infect a host bacterial cell. Coliphage P1kc labeled with SYBR Gold and co-incubated with *S. marcescens* revealed
30 the presence of fluorescent bacteria by microscopy, validating the use of this assay in a phage isolation pipeline. As with the Seahorse assay, this approach provided an alternative to traditional phage methods to permit expansion to organisms not amenable to plaque assay. Additionally, the SYBR Gold transduction assay did not require bacterial growth, so is applicable to analysis of phages targeting difficult or even non-culturable organisms, including endosymbionts such as *Buchnera*.

35

Testing in vivo efficacy of the phages against S. marcescens in Drosophila melanogaster flies

S. marcescens cultures were grown in Tryptic Soy Broth (TSB) at 30℃ with constant shaking at 200rpm.

The media used to rear fly stocks was cornmeal, molasses and yeast medium (11 g/l yeast, 54 g/l
40 yellow cornmeal, 5 g/l agar, 66 ml/l molasses, and 4.8 ml/l propionic acid). All the components of the diet

except propionic acid were heated together to 80°C in deionized water with constant mixing for 30 minutes and let to cool to 60°C. Propionic acid was then mixed in and 50ml of the diet was aliquoted into individual bottles and allowed to cool down and solidify. The flies were raised at 26°C, 16:8 hour light:dark cycle, at around 60% humidity.

5 To infect the flies with *S. marcescens*, a fine needle (About 10um wide tip) was dipped in a dense overnight stationary phase culture and the thorax of the flies was punctured. For this experiment, four replicates of 10 males and 10 females each were infected with *S. marcescens* using the needle puncturing method as the positive control for fly mortality. For the treatment group, four replicates of 10 males and 10 females each were pricked with *S. marcescens* and a phage solution containing about 108 10 phage particles/ml. Finally, two replicates of 10 males and 10 females each that were not pricked or treated in anyway were used as a negative control for mortality.

Flies in all conditions were placed in food bottles and incubated at 26°C, 16:8 light:dark cycle, at 60% humidity. The number of alive and dead flies were counted every day for four days after the pricking. All The flies pricked with *S. marcescens* alone were all dead within 24 hours of the treatment. 15 In comparison, more than 60% of the flies in the phage treatment group, and all the flies in the untreated control group were alive at that time point (Fig. 5). Further, most of the flies in the phage treatment group and the negative control group went on to survive for four more days when the experiment was terminated.

To ascertain the reason of death of the flies, dead flies from both *the S. marcescens* and 20 *S. marcescens* + phage pricked flies were homogenized and plated out. Four dead flies from each of the four replicates of both the *S. marcescens* and the *S. marcescens* + phage treatment were homogenized in 100ul of TSB. A 1:100 dilution was also produced by diluting the homogenate in TSB. 10ul of the concentrated homogenate as well as the 1:100 dilution was plated out onto TSA plates, and incubated overnight at 30°C. Upon inspection of the plates for bacteria growth, all the plates from the dead 25 *S. marcescens* pricked flies had a lawn of bacteria growing on them, whereas the plates from the dead *S. marcescens* + phage pricked flies had no bacteria on them. This shows that in the absence of the phage, *S. marcescens* likely induced septic shock in the flies leading to their fatality. However, in the presence of the phage, the mortality may have been due to injury caused by the pricking with the needle.

30

OTHER EMBODIMENTS

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

35

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.

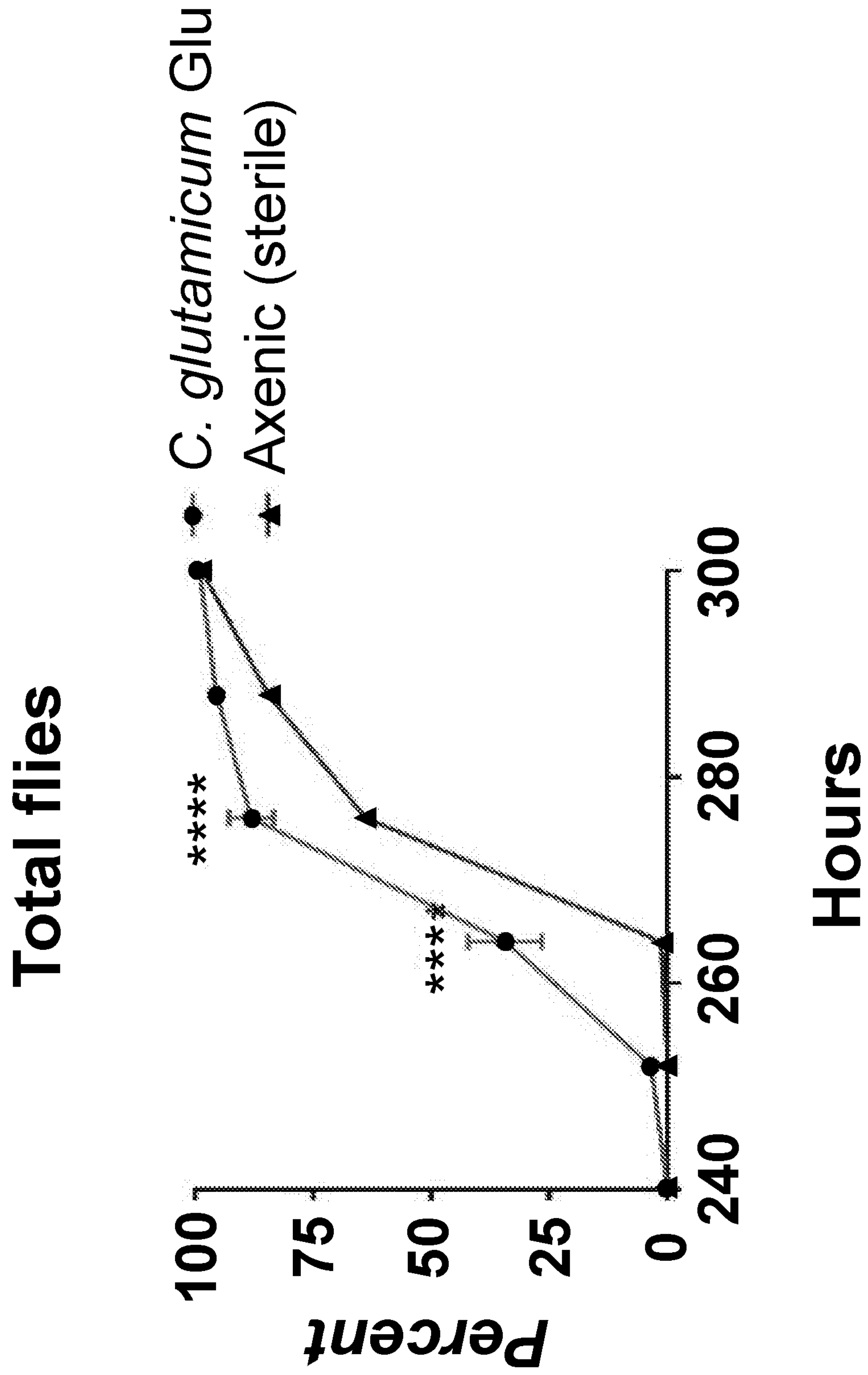
7. A composition comprising an effective amount of organophosphorous insecticide-metabolizing bacteria formulated with an insect comestible carrier as a liquid, a solid, an aerosol, a paste, a gel, or a gas.

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.

Fig. 1



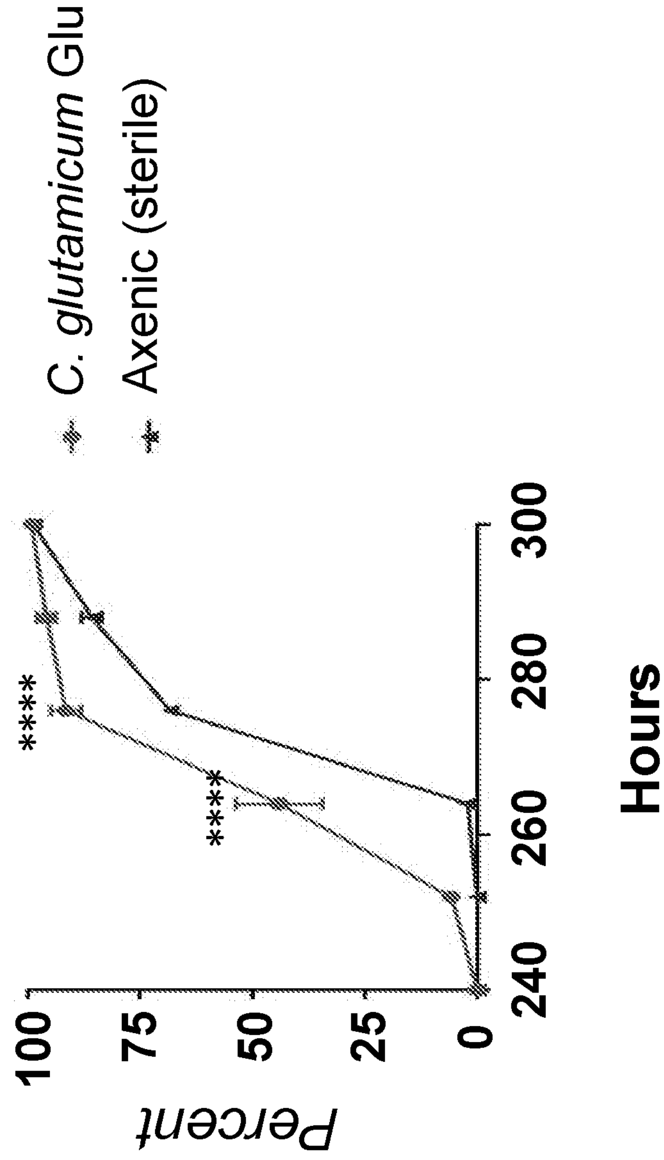


Fig. 2B
Females
Percent
100
75
50
25
0
240
260
280
300
Hours
****
****
C. glutamicum Glu
Axenic (sterile)

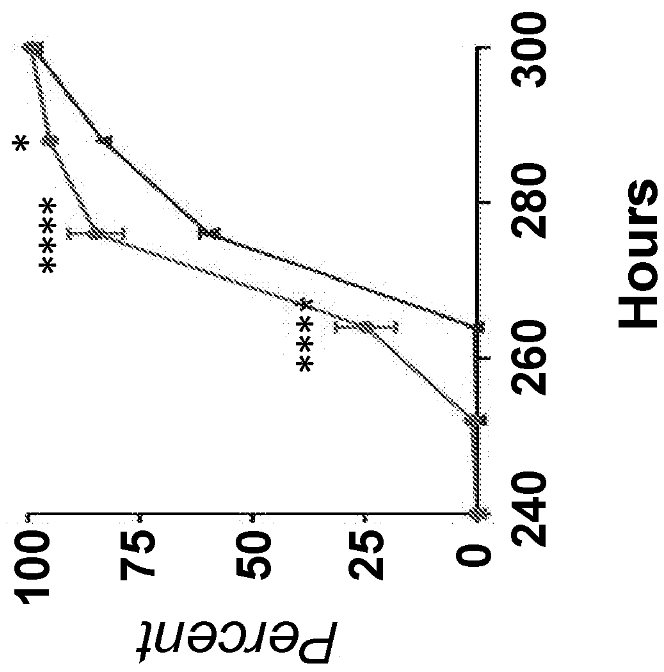


Fig. 2A
Males
Percent
100
75
50
25
0
240
260
280
300
Hours
***
****
*

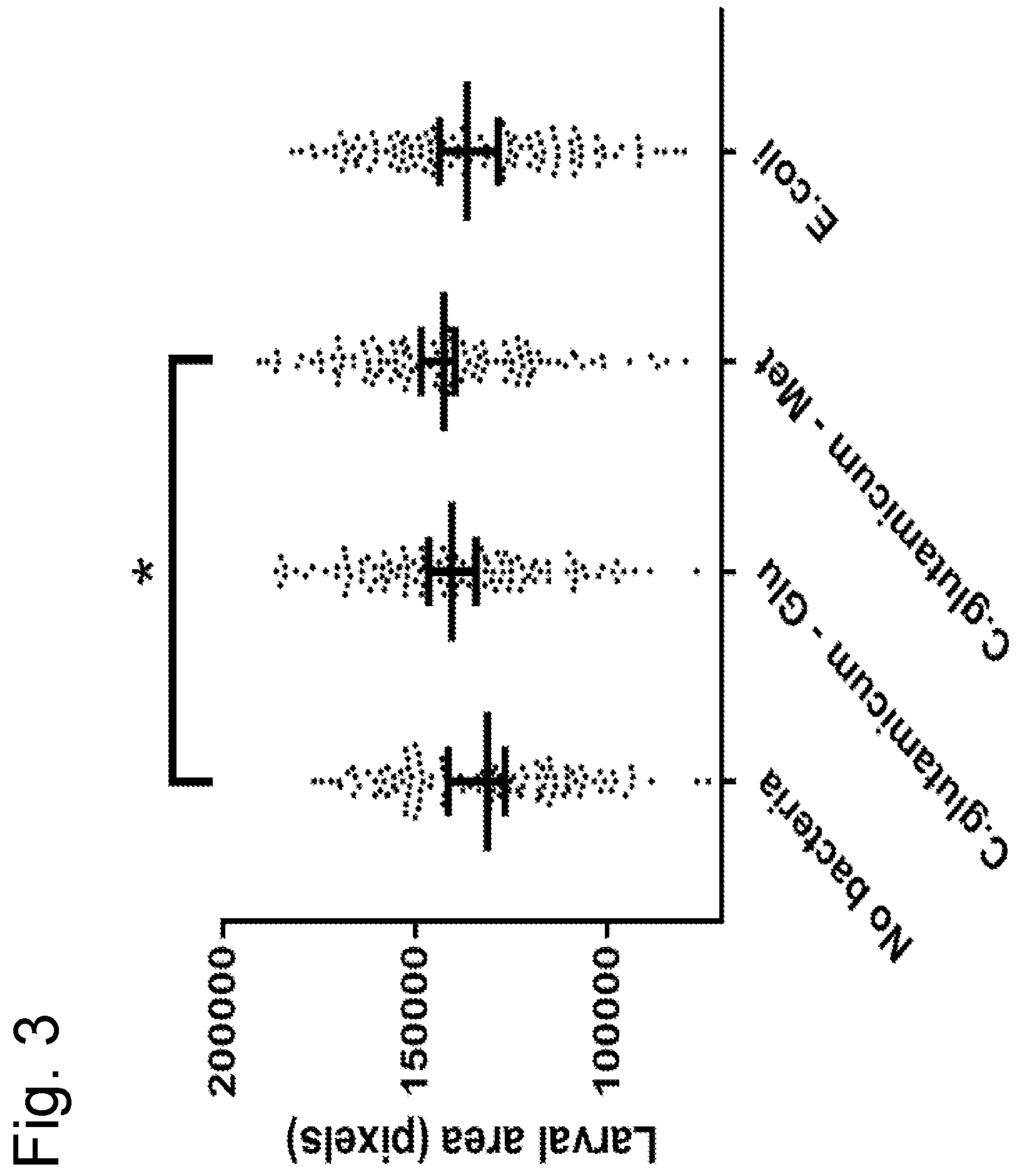


Fig. 3

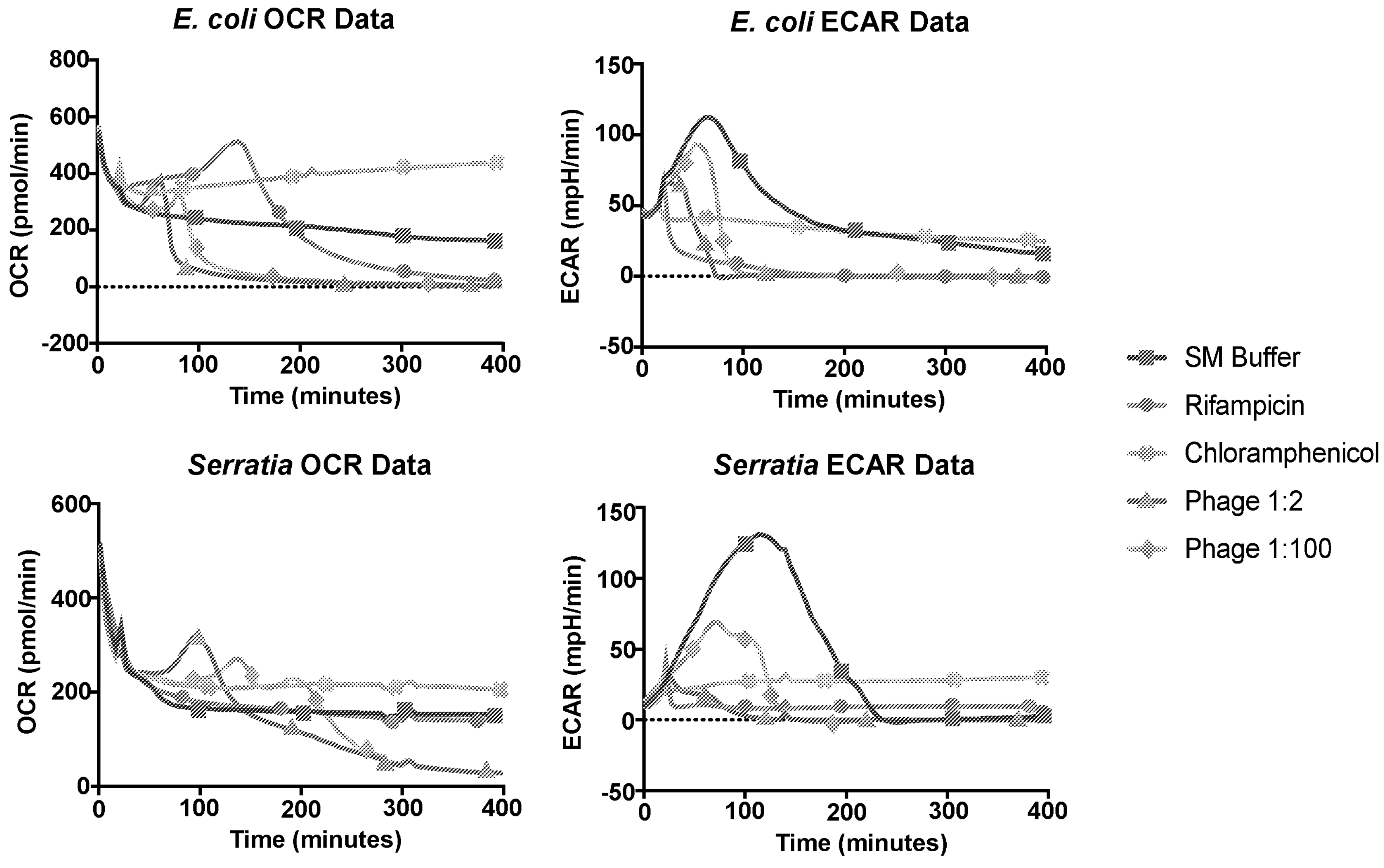


Fig. 4
E. coli OCR Data
OCR (pmol/min)
800
600
400
200
0
-200
0
100
200
300
400
Time (minutes)
E. coli ECAR Data
ECAR (mpH/min)
150
100
50
0
-50
0
100
200
300
400
Time (minutes)
Serratia OCR Data
OCR (pmol/min)
600
400
200
0
0
100
200
300
400
Time (minutes)
Serratia ECAR Data
ECAR (mpH/min)
150
100
50
0
-50
0
100
200
300
400
Time (minutes)
SM Buffer
Rifampicin
Chloramphenicol
Phage 1:2
Phage 1:100

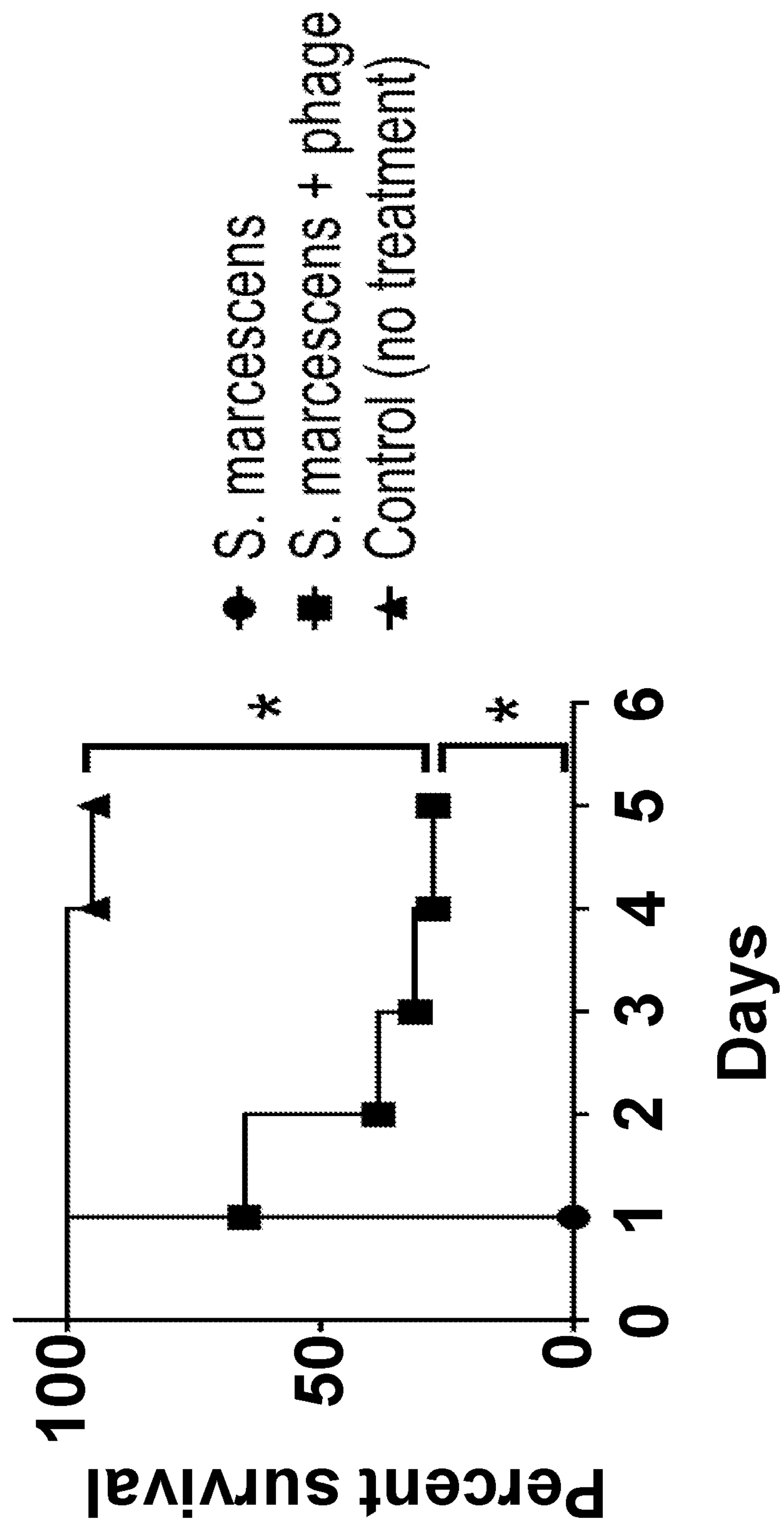


Fig. 5
Percent survival
100
50
0
0
1
2
3
4
5
6
Days
*
*
S. marcescens
S. marcescens + phage
Control (no treatment)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/015025

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01G 31/00; A01N 25/26; A61K 35/74; A61K 35/745; A61K 35/747; C02F 3/32 (2018.01)

CPC - A01G 2/00; A01G 31/00; A01N 63/00; A01N 63/02; A01N 63/04; A61K 35/74; A61K 35/745; A61K 35/747; A61K 35/748 (2018.02)

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
USPC - 424/93.45 (keyword delimited)

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.
Y	AMOS, H. "UBC Students Give Bees a Chance," The University of British Columbia, 18 September 2015 (18.09.2015), Pgs. 1-4. Retrieved from the Internet: <http://news.ubc.ca/2015/09/18/ubc-students-give-bees-a-chance/> on 10 March 2018 (10.03.2018). entire document	1-3, 7-9
Y	KIKUCHI et al. "Symbiont-Mediated Insecticide Resistance," Proceedings of the National Academy of Sciences of the United States of America, 29 May 2012 (29.05.2012), Vol. 109, Iss. 22, Pgs. 8618-8622. entire document	1-3, 7-9
Y	WO 2015/100432 A2 (SYMBIOTA, INC. et al) 02 July 2015 (02.07.2015) entire document	9
A	SHARMA et al. "Metabolism of 1 -Naphthyl-N-Methyl Carbamate (Carbaryl) by Bacterial Isolates from Honey Bees and the Effect of Bacterial Inoculations on Carbaryl Tolerance in Bees," Journal of Applied Bacteriology, 01 September 1996 (01.09.1996), Vol. 81, No. 3, Pgs. 235-241. entire document	1-3, 7-9
A	TRINDER et al. "Probiotic Lactobacillus rhamnosus Reduces Organophosphate Pesticide Absorption and Toxicity to Drosophila melanogaster," Applied and Environmental Microbiology, 12 August 2016 (12.08.2016), Vol. 82, No. 20, Pgs. 6204-6213. entire document	1-3, 7-9

☐ 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

"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
10 March 2018	13 APR 2018

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/015025

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.: 4-6, 10
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:

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.:

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.