METHODS AND RELATED COMPOSITIONS FOR MANUFACTURING FOOD AND FEED

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/450,038, filed on January 24, 2017, and U.S. Provisional Application No. 62/584,011, filed on November 9, 2017, the contents of which are hereby incorporated herein by reference in their entireties.

BACKGROUND

Arthropods, such as crickets, cicadas, grasshoppers, ants, insect larvae, caterpillars, and scorpions, have many traditional and potential new uses in the production of food and feed for humans and animals, respectively. Insects as food and feed emerge as an especially relevant issue due to the rising cost of animal protein, food and feed insecurity, environmental pressures, population growth, and increasing demand for affordable and sustainable sources of nutrients for humans and animals (e.g., livestock). To cultivate beneficial arthropods for use in the food and feed industries, there is a need in the art for ways to promote the growth and fitness of such arthropods.

SUMMARY

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

In one aspect, the present disclosure refers to a method, comprising delivering to a host insect a composition comprising an amino acid-producing bacterium in an amount effective for increasing a nutritional profile of the host insect.

In one aspect, the present disclosure refers to use of an amino acid-producing bacterium in the manufacture of a composition to be delievered to a host insect for increasing a nutritional profile of the host insect.

In one aspect, the present disclosure refers to a method, comprising altering a host insect's microbiota wherein the host has a nutritional profile and wherein altering comprises delivering directly or indirectly into the host a composition comprising an amino acid-producing bacterium in an amount effective for increasing the nutritional profile of the host insect.

In one example, provided herein is a method for increasing a nutritional profile of an insect, the method including delivering an effective amount of methionine-producing bacteria to the insect.

In some examples, the insect is a cricket, a grasshopper, or a locust.

In some examples, the insect may be developmentally an embryo, larva, pupa, or adult.

In some examples, the delivery may include delivering the composition to at least one habitat where the insect grows, lives, reproduces, or feeds.

In some examples, the methionine-producing bacteria may be delivered in an insect comestible composition for ingestion by the insect.

In some examples, the methionine-producing bacteria may be formulated with an agriculturally acceptable carrier as a liquid, a solid, an aerosol, a paste, a gel, or a gas composition.

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

In a second example, provided herein is a modified insect comprising exogenous methionine-producing bacteria resident in the insect.

In some examples, the insect is developmentally an embryo, a larva, a pupa, or an adult.

In some examples, the methionine-producing bacteria alters microbiota in a gut and/or haemocoel of the insect.

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

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

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

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

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

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

In some examples, the small molecule is a metabolite.

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

In some examples, the modulating agent is a naturally occurring bacteria. In some examples, the bacteria is at least one selected from the group consisting of *Bartonella apis, Parasaccharibacter apium, Frischella perrara, Snodgrassella alvi, Gilliamela apicola, Bifidobacterium spp,* and *Lactobacillus spp.* In some examples, the bacterium is at least one selected from the group consisting of *Candidatus spp, Buchenera spp, Blattabacterium spp, Baumania spp, Wigglesworthia spp, Wolbachia spp, Rickettsia spp, Orientia spp, Sodalis spp, Burkholderia spp, Cupriavidus spp, Frankia spp, Snirhizobium spp, Streptococcus spp, Wolinella spp, Xylella spp, Erwinia spp, Agrobacterium spp,*

Bacillus spp, Paenibacillus spp, Streptomyces spp, Micrococcus spp, Corynebacterium spp, Acetobacter spp, Cyanobacteria spp, Salmonella spp, Rhodococcus spp, Pseudomonas spp, Lactobacillus spp, Enterococcus spp, Alcaligenes spp, Klebsiella spp, Paenibacillus spp, Arthrobacter spp, Corynebacterium spp, Brevibacterium spp, Thermus spp, Pseudomonas spp, Clostridium spp, and *Escherichia spp.*

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

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

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

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

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

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

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

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

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

In any of the above examples, the modulating agent of the composition may be effective to increase production of a nutrient in the host relative to a reference level. In some examples, the modulating agent is a microorganism that produces the nutrient. In some examples, the microorganism is a bacterium. In some examples, the nutrient is a vitamin, a carbohydrate, an amino acid, or a polypeptide. In certain examples, the amino acid is methionine.

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

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

composition may be formulated for delivery to a plant. In some examples, the composition may be formulated for use in a host feeding station.

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

In a further example, also provided herein are hosts that include any of the above compositions. In some examples, the host is an insect. In some examples, the insect is a species belonging to the order *Anoplura, Araneae, Blattodea, Coleoptera, Dermaptera, Dictyoptera, Diplura, Diptera, Embioptera, Ephemeroptera, Grylloblatodea, Hemiptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Mantodea, Mecoptera, Neuroptera, Odonata, Orthoptera, Phasmida, Plecoptera, Protura, Psocoptera, Siphonaptera, Siphunculata, Thysanura, Strepsiptera, Thysanoptera, Trichoptera,* or *Zoraptera.* In certain examples, the insect is a cricket. In certain examples, the insect is a grasshopper. In certain examples, the insect is a locust.

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

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

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

In some examples of any of the above methods, the delivering step may include providing the modulating agent at a dose and time sufficient to effect the one or more microorganisms, thereby

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

In some examples of any of the above methods, the composition is effective to increase health and/or survival of the host. In some examples, the composition is effective to increase host fitness, increase host lifespan, increase effective pollination, increase generation of a host product, increase host reproduction, or a combination thereof. In some examples, the composition is effective to decrease the host's sensitivity to a pesticidal agent (e.g., a pesticide listed in Table 12). In some examples, the pesticidal agent is a neonicotinoid. In some examples, the composition is effective to increase the host's resistance to an allelochemical agent produced by a plant. In some examples, the allelochemical agent is toxic to the host prior to delivery of the composition. In some examples, the allelochemical agent is caffeine, soyacystatin N, monoterpenes, diterpene acids, or phenolic compounds. In some examples, the composition is effective to increase nutrient production in the host, thereby increasing the nutrient content in the product derived from the host. In some examples, the nutrient is a vitamin, a carbohydrate, an amino acid, or a polypeptide.

In some examples of any of the above methods, at least one part of the host may be used in the manufacture of a consumable product. In some examples of any of the above methods, the host is an insect. In some examples, the insect is a species belonging to the order *Anoplura, Araneae, Blattodea, Coleoptera, Dermaptera, Dictyoptera, Diplura, Diptera, Embioptera, Ephemeroptera, Grylloblatodea, Hemiptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Mantodea, Mecoptera, Neuroptera, Odonata, Orthoptera, Phasmida, Plecoptera, Protura, Psocoptera, Siphonaptera, Siphunculata, Thysanura, Strepsiptera, Thysanoptera, Trichoptera,* or *Zoraptera.* In certain examples, the insect is a cricket. In certain examples, the insect is a grasshopper. In certain examples, the insect is a locust. In some examples, the product includes a food product for humans. In some examples, the product includes a nutritional supplement that supplements an animal feed or human food product. In some examples, the product includes feed for animals. In some examples, the animals are livestock or farm animals.

In one aspect, the present disclosure refers to a method of making an animal or human food product, the method comprising: (a) delivering to a plurality of host insects a composition comprising an amino acid-producing bacteria formulated with an insect comestible carrier; and (b) processing the plurality of host insects into a food, food additive, or food supplement after at least a portion of the plurality of host insects have ingested the composition.

In one aspect, the present disclosure refers to a method of making an animal or human food product, the method comprising: (a) providing a plurality of host insects, wherein at least a portion of the plurality of host insects comprise a target entomopathogenic bacterium; (b) delivering to the plurality of host insects a composition comprising a phage that targets the entomopathogenic bacterium and an insect comestible carrier in an amount effective for increasing the fitness of the plurality of host insects; and (c) processing the plurality of host insects into a food, food additive, or food supplement.

In another example, provided herein is a method of making a human or animal food product, includes (a) providing a plurality (e.g., 2, >2, >5, >10, >100, >1000, >5,000, >10,000, > 50,000, >100,000) of host insects, (b) delivering an ingestible composition described herein to the plurality of host insects, in an amount effective to modulate one or more microorganisms resident in the plurality, and (c) processing the plurality (e.g., grinding, optionally admixing with a carrier or another food component) into a food, food additive or food supplement. In some examples, the ingestible composition comprises a microorganism. In some examples, the microorganism produces a nutrient, and the microorganism is effective to increase nutrient production in the host relative to a reference level. In some examples,

the microorganism is a bacterium. In some examples, the nutrient is a vitamin, a carbohydrate, an amino acid, or a polypeptide. In some examples, the amino acid is methionine.

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

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

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

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

In some examples of the screening assay, the host may be an invertebrate. In some examples, the invertebrate is an insect. In some examples, the insect is a cricket. In certain examples, the insect is a grasshopper. In certain examples, the insect is a locust.

In any of the above examples of the screening assay, host fitness may be modulated by modulating 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, and derivatives thereof, such as engineered bacteriocins, recombinantly expressed bacteriocins, and 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.

CLAIMS

1. A method, comprising delivering to a host insect a composition comprising an amino acid-producing bacterium in an amount effective for increasing a nutritional profile of the host insect.

2. Use of an amino acid-producing bacterium in the manufacture of a composition to be delievered to a host insect for increasing a nutritional profile of the host insect.

3. A method, comprising altering a host insect's microbiota wherein the host has a nutritional profile and wherein altering comprises delivering directly or indirectly into the host a composition comprising an amino acid-producing bacterium in an amount effective for increasing the nutritional profile of the host insect.

4. A method according to claim 1 or 3, or the use according to claim 2, wherein the amino acid-producing bacterium produces the amino acid at a concentration of at least 0.1 mg/mL in a culture.

5. A method of making an animal or human food product, the method comprising:

(a) delivering to a plurality of host insects a composition comprising an amino acid-producing bacteria formulated with an insect comestible carrier; and

(b) processing the plurality of host insects into a food, food additive, or food supplement after at least a portion of the plurality of host insects have ingested the composition.

6. The method or use of any one of claims 1-4, wherein increasing the nutritional profile is determined by observing that production of the amino acid in the host insect to which the composition was delivered is greater than production of the amino acid in a host insect to which the same composition but without the amino acid-producing bacteria is delivered.

7. The method or use of any one of claims 1-4, wherein increasing the nutritional profile is determined by observing an increase in body mass of the host insect relative a host insect to which the same composition but without the amino acid-producing bacteria is delivered.

8. The method or use of any one of claims 1-7, wherein the amino acid-producing bacteria is a methionine-producing bacteria.

9. The method or use of any one of claims 1-7, wherein the amino acid-producing bacteria is a glutamate-producing bacteria.

10. A method of making an animal or human food product, the method comprising:

(a) providing a plurality of host insects, wherein at least a portion of the plurality of host insects comprise a target entomopathogenic bacterium;

(b) delivering to the plurality of host insects a composition comprising a phage that targets the entomopathogenic bacterium and an insect comestible carrier in an amount effective for increasing the fitness of the plurality of host insects; and

(c) processing the plurality of host insects into a food, food additive, or food supplement.

11. The method of claim 10, wherein the phage is chosen from Myoviridae, Siphoviridae, Podoviridae, Lipothrixviridae, Rudiviridae, Ampullaviridae, Bicaudaviridae, Clavaviridae, Corticoviridae, Cystoviridae, Fuselloviridae, Glubuloviridae, Guttaviridae, Inoviridae, Leviviridae, Microviridae, Plasmaviridae, and Tectiviridae.

12. The method of claim 10 or 11, wherein fitness is measured as mobility, body weight, survival, reproduction, or metabolism.

13. The method or use of any one of claims 1-12, wherein the host insect is a cricket, a grasshopper, or a locust.

14. The method or use of any one of claims 1-12, wherein the host insect is a dipteran insect.

15. The method or use of claim 14, wherein the dipteran insect is a black soldier fly.

16. The method or use of any one of claims 1-12, wherein the host insect is a lesser mealworm.

17. The method or use of any one of claims 1-16, wherein the host insect is developmentally an embryo, larva, pupa, or adult.

18. The method or use of any one of claims 1-17, wherein delivering comprises delivering the composition to at least one habitat where the host insect grows, lives, reproduces, or feeds.

19. The method or use of any one of claims 1-18, wherein the composition is a liquid, a solid, an aerosol, a paste, a gel, or a gas.