

COMPOSITIONS AND RELATED METHODS FOR AGRICULTURE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/450,045, filed on January 24, 2017, and U.S. Provisional Application No. 62/583,763, filed on November 9, 2017, the contents of which are hereby incorporated herein by reference in their entireties.

BACKGROUND

Arthropod insects are pervasive in the human environment, and a multitude of means have been utilized for attempting to control infestations by these pests. The demand for pest control strategies is increasing. Thus, there is need in the art for new methods and compositions to control agricultural insect pests.

SUMMARY

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

In one aspect, the present disclosure refers to a method for agricultural pest control, comprising: altering an agricultural insect pest's microbiota in a manner that is detrimental to the agricultural insect pest, wherein altering comprises delivering a composition comprising an agent that targets a microorganism resident in the agricultural insect pest to the agricultural insect pest.

In one example, provided herein is a method of decreasing fitness of an agricultural insect pest, the method including delivering an antimicrobial peptide having at least 90% sequence identity (e.g., at least 90%, 92%, 94%, 96%, 98%, or 100% sequence identity) with one or more of the following: cecropin (SEQ ID NO: 82), melittin, copsin, drosomycin (SEQ ID NO: 93), dermcidin (SEQ ID NO: 81), andropin (SEQ ID NO: 83), moricin (SEQ ID NO: 84), ceratotoxin (SEQ ID NO: 85), abaecin (SEQ ID NO: 86), apidaecin (SEQ ID NO: 87), prophenin (SEQ ID NO: 88), indolicidin (SEQ ID NO: 89), protegrin (SEQ ID NO: 90), tachyplesin (SEQ ID NO: 91), or defensin (SEQ ID NO: 92) to the agricultural insect pest.

In some examples, the delivery may include delivering the antimicrobial peptide to at least one habitat where the agricultural insect pest grows, lives, reproduces, feeds, or infests.

In any of the above examples, the delivery may include spraying the antimicrobial peptide on an agricultural crop.

In any of the above examples, the antimicrobial peptide may be delivered as an insect comestible composition for ingestion by the agricultural insect pest.

In any of the above examples, the antimicrobial peptide may be formulated with an agriculturally acceptable carrier as a liquid, a solid, an aerosol, a paste, a gel, or a gas composition.

In any of the above examples, the agricultural insect pest may be an aphid.

In another example, provided herein is a composition including an antimicrobial peptide having at least 90% sequence identity (e.g., at least 90%, 92%, 94%, 96%, 98%, or 100% sequence identity) with one or more of the following: cecropin (SEQ ID NO: 82), melittin, copsin, drosomycin (SEQ ID NO: 93), dermcidin (SEQ ID NO: 81), andropin (SEQ ID NO: 83), moricin (SEQ ID NO: 84), ceratotoxin (SEQ ID NO: 85), abaecin (SEQ ID NO: 86), apidaecin (SEQ ID NO: 87), prophenin (SEQ ID NO: 88), indolicidin (SEQ ID NO: 89), protegrin (SEQ ID NO: 90), tachyplesin (SEQ ID NO: 91), or defensin (SEQ ID NO: 92) formulated for targeting a microorganism in an insect.

In some examples, the antimicrobial peptide may be at a concentration of about 0.1 ng/g to about 100 mg/g (about 0.1 ng/g to about 1 ng/g, about 1 ng/g to about 10 ng/g, about 10 ng/g to about 100 ng/g, about 100 ng/g to about 1000 ng/g, about 1 mg/g to about 10 mg/g, about 10 mg/g to about 100 mg/g) or about 0.1 ng/mL to about 100 mg/mL (about 0.1 ng/mL to about 1 ng/mL, about 1 ng/mL to about 10 ng/mL, about 10 ng/mL to about 100 ng/mL, about 100 ng/mL to about 1000 ng/mL, about 1 mg/mL to about 10 mg/mL, about 10 mg/mL to about 100 mg/mL) in the composition.

In some examples, the antimicrobial peptide may further include a targeting domain.

In some examples, the antimicrobial peptide may further include a cell penetrating peptide.

In 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 a decrease 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*, *Snirrhizobium 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 buchneri*, *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 examples, the bacteria is a *Buchnera spp.*, (e.g., *Buchnera aphidcola*, an endosymbiont of aphids).

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, *Gluboloviridae*, *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 alternative examples, the antibiotic is a narrow-spectrum antibiotic (e.g., rifampicin).

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*, *Gilliamella 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*, *Snirrhizobium 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 any of the above examples, the modulating agent may modulate the host's fitness by increasing pesticidal susceptibility of the host (e.g., susceptibility to a pesticide listed in Table 12). In some examples, the modulating agent modulates the host's fitness by increasing pesticidal susceptibility of the host. 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, the composition may include a modulating agent and an agent that increases crop growth.

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

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

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

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

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

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

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

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

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

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

In yet another 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 *Coleoptera*, *Diptera*, *Hemiptera*, *Lepidoptera*, *Orthoptera*, *Thysanoptera*, or *Acarina*. In some examples, the insect is a beetle, weevil, fly, aphid, whitefly, leafhopper, scale, moth, butterfly, grasshopper, cricket, thrip, or mite. In certain examples, the insect is an aphid.

In 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 yet a further 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 may be effective to increase the host's sensitivity to a pesticidal agent (e.g., a pesticide listed in Table 12). In some examples, the host is resistant to the pesticidal agent prior to delivery of the modulating agent. In some examples, the pesticidal agent is an allelochemical agent. In some examples, the allelochemical agent is caffeine, soyacystatin N, monoterpenes, diterpene acids, or phenolic compounds. In some examples, the composition is effective to selectively kill the host. In some examples, the composition is effective to decrease host fitness. In some examples, the composition is effective to decrease the production of essential amino acids and/or vitamins in the host.

In some examples of any of the above methods, the host is an insect. In some examples, the insect is a species belonging to *Coleoptera*, *Diptera*, *Hemiptera*, *Lepidoptera*, *Orthoptera*, *Thysanoptera*, or *Acarina*. In some examples, the insect is a beetle, weevil, fly, aphid, whitefly, leafhopper, scale, moth, butterfly, grasshopper, cricket, thrip, or mite. In certain examples, the insect is an aphid.

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. In 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 a further 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 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, decreases host fitness. 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, rifampicin, 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 an aphid. In some examples, the insect is a mosquito. In some examples, the insect is a cricket.

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.

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 decrease or reduce 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 metabolic rate.

CLAIMS

1. A method for agricultural pest control, comprising: altering an agricultural insect pest's microbiota in a manner that is detrimental to the agricultural insect pest, wherein altering comprises delivering a composition comprising an agent that targets a microorganism resident in the agricultural insect pest to the agricultural insect pest.
2. The method of claim 1, wherein the microorganism is an obligate symbiont of the agricultural insect pest.
3. The method of claim 1 or 2, wherein the composition is formulated for delivery to a plant.
4. The method of claim 3, wherein the plant is an agricultural crop and delivering comprises spraying the agricultural crop with the composition.
5. The method of any one of claims 1-4, wherein delivering comprises delivering the composition to at least one habitat where the agricultural insect pest grows, lives, reproduces, feeds, or infests.
6. The method of any one of claims 1-5, wherein the composition is formulated as an insect comestible composition for ingestion by the agricultural insect pest.
7. The method of claim 3, wherein delivering comprises providing a plant that has been genetically engineered to produce the agent.
8. The method of any of claims 1-7, wherein the agent is chosen from an antimicrobial peptide, an antibiotic, a phage, a bacteriocin, chitosan, a vitamin analog, an amino acid transporter inhibitor, and combinations thereof.
9. The method of claim 8, wherein the agent is an antibiotic chosen from oxytetracycline, rifampicin, ciprofloxacin, doxycycline, ampicillin, polymyxin B, timentin, and azithromycin and combinations thereof.
10. The method of claim 8, wherein the agent is one or more antimicrobial peptides having at least 90% sequence identity with a peptide chosen from cecropin (SEQ ID NO: 82), melittin, copsin, drosomycin (SEQ ID NO: 93), dermcidin (SEQ ID NO: 81), andropin (SEQ ID NO: 83), moricin (SEQ ID NO: 84), ceratotoxin (SEQ ID NO: 85), abaecin (SEQ ID NO: 86), apidaecin (SEQ ID NO: 87), prophenin (SEQ ID NO: 88), indolicidin (SEQ ID NO: 89), protegrin (SEQ ID NO: 90), tachyplesin (SEQ ID NO: 91), or defensin (SEQ ID NO: 92).
11. The method of claim 10, wherein the antimicrobial peptide is chosen from moricin, abaecin, apidaecin, and defensin and combinations thereof.

12. The method of claim 8, wherein the agent is a phage chosen from Ampullaviridae, Bicaudaviridae, Clavaviridae, Gluboloviridae, and Guttaviridae and combinations thereof.
13. The method of claim 8, wherein the agent is a bacteriocin chosen from epidermin, pediocin PA-1, enterocin P, lactococcin G, lactacin-F, enterocin AS-48, aureocin A70, garvicin A, and colicin V and combinations thereof.
14. The method of claim 8, wherein the agent is chitosan.
15. The method of claim 1, wherein the agent is a vitamin analog, and the vitamin analog is pantothenol.
16. The method of claim 1, wherein the agent is an amino acid transporter inhibitor.
17. The method of claim 16, wherein the agent is a cocktail of amino acid transporter inhibitors.
18. The method of any one of claims 2-17, wherein the agricultural insect pest is chosen from aphids, psyllids, whiteflies, citrus mealybugs, scale bugs, pine bark adelgids, spittlebugs, cicadas, leafhoppers, grasshopper, beetles, tsetse flies, and mosquitos and the microorganism is the agricultural insect pest's corresponding obligate symbiont.
19. The method of claim 18, wherein the agricultural insect pest is an aphid and the microorganism is Buchnera.
20. The method of any one of claims 1-19, wherein the composition comprises at least two agents.