

# COMPOSITIONS AND RELATED METHODS FOR CONTROLLING VECTOR-BORNE DISEASES

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/450,032, filed on January 24, 2017, and U.S. Provisional Application No. 62/583,925, filed on November 9, 2017, the contents of which are hereby incorporated herein by reference in their entireties.

## BACKGROUND

Insects function as vectors for pathogens causing severe disease in humans and animals such as dengue, trypanosomiasis, and malaria. Vector-borne diseases that infect animals, such as livestock, represent a major global public health burden. Thus, there is need in the art for methods and compositions to control insects that carry vector-borne diseases.

## SUMMARY

Disclosed herein are compositions and methods for modulating the fitness of insects for controlling the spread of vector-borne diseases in animals. 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 of decreasing fitness of an insect vector for an animal pathogen, comprising: delivering to the insect vector a composition comprising an agent that targets a microorganism resident in the insect vector resulting in a decrease in fitness of the insect vector.

In one aspect, the present disclosure refers to use of an agent that targets a microorganism resident in an insect vector for an animal pathogen resulting in a decrease in fitness of the insect vector, in the manufacture of a composition for decreasing fitness of the insect vector.

In one example, provided herein is a method of decreasing fitness of a vector (e.g., insect vector) for an animal pathogen, 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 vector.

In some examples, the delivery includes delivering the antimicrobial peptide to at least one habitat where the vector grows, lives, reproduces, feeds, or infests.

In some examples, the antimicrobial peptide may be delivered in an insect comestible composition for ingestion by the vector.

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

In some examples, the insect may be at least one of a mosquito, midge, louse, sandfly, tick, triatomine bug, tsetse fly, or flea.

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 a vector (e.g., an insect vector) for an animal pathogen.

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 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 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 *Wolbachia spp*. (e.g., in a mosquito host). In certain emobdiments, the bacteria is a *Rickettsia spp*. (e.g., in a tick host).

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*.

5           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  
10   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*,  
15   *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*.

20           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  
25   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.

30           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  
35   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.

40           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  
5 chemoattractant.

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

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  
10 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  
15 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.  
20 In some examples, the host is an insect. In some examples, the insect is a mosquito, midge, louse, sandfly, tick, triatomine bug, tsetse fly, or flea. In certain examples, the insect is a mosquito. In certain examples, the insect is a tick. In certain examples, the insect is a mite. In certain examples, the insect is a louse.

Also provided herein is a system for modulating a host's fitness comprising a modulating agent  
25 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.  
30 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  
35 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.

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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 host is a vector for an animal pathogen. In some examples, the vector is a mosquito, midge, louse, sandfly, tick, triatomine bug, tsetse fly, or flea. In certain examples, the vector is a mosquito. In certain examples, the vector is a tick. In certain examples, the vector is a mite. In certain examples, the vector is a louse. In some examples, the animal pathogen is a virus, a protozoan, a bacterium, a protist, or a nematode. In some examples, the virus is one belonging to the group *Togaviridae*, *Flaviviridae*, *Bunyaviridae*, *Rhabdoviridae*, or *Orbiviridae*. In some examples, the bacterium is one belonging to the genus *Yersinia*, *Francisella*, *Rickettsia*, *Orientia*, or *Borrelia*. In some examples, the protozoan is one belonging to the genus *Plasmodium*, *Trypanosoma*, *Leishmania*, or *Babesia*. In some examples, the nematode is one belonging to the genus *Brugia*. In some examples, the composition is effective to prevent or decrease transmission of the pathogen to animals. In some examples, the composition is effective to prevent or decrease horizontal or vertical transmission of the pathogen between hosts. In some examples, the composition is effective to decrease host fitness, host development, or vectorial competence.

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 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 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 mosquito. In some examples, the insect is a tick. In certain examples, the insect is a mite. In certain examples, the insect is a louse.

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 “animals” refers to livestock or farm animals and other mammalian veterinary animals.

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, e.g., mosquito, tick, mite, louse); 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.

## CLAIMS

1. A method of decreasing fitness of an insect vector for an animal pathogen, comprising: delivering to the insect vector a composition comprising an agent that targets a microorganism resident in the insect vector resulting in a decrease in fitness of the insect vector.
2. Use of an agent that targets a microorganism resident in an insect vector for an animal pathogen resulting in a decrease in fitness of the insect vector, in the manufacture of a composition for decreasing fitness of the insect vector.
3. The method of claim 1 or use of claim 2, wherein the microorganism is an obligate symbiont of the insect vector.
4. The method or use of any of claims 1-3, 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.
5. The method or use of any of claims 1-4, wherein the agent is an antimicrobial peptide having at least 90% sequence identity with one or more of 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).
6. The method or use of claim 5, wherein the agent is an antimicrobial peptide chosen from moricin (SEQ ID NO: 84), abaecin (SEQ ID NO: 86), apidaecin (SEQ ID NO: 87), and defensin (SEQ ID NO: 92).
7. The method or use of any one of claims 1-4, wherein the agent is an antibiotic chosen from oxytetracycline, rifampicin, ciprofloxacin, doxycycline, ampicillin, polymyxin B, timentin, and azithromycin.
8. The method or use of any one of claims 1-4, wherein the agent is a phage chosen from Ampullaviridae, Bicaudaviridae, Clavaviridae, Gluboloviridae, and Guttaviridae.
9. The method or use of any one of claims 1-4, 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.
10. The method or use of claim 4, wherein the agent is chitosan.
11. The method or use of claim 4, wherein the agent is a vitamin analog.
12. The method or use of claim 11, wherein the vitamin analog is pantothenol.

13. The method or use of claim 4, wherein the agent is an amino acid transporter inhibitor.
14. The method or use of claim 13, wherein the agent is a cocktail of amino acid inhibitors.
15. The method or use of any one of claims 1-14, wherein the agent is at least two agents.
16. The method or use of any of claims 1-15, wherein targeting comprises altering at least one of a level, activity, or metabolism of the microorganism.
17. The method or use of any one of claims 1-16, wherein delivering comprises providing the composition to at least one habitat where the insect vector grows, lives, reproduces, feeds, or infests.
18. The method or use of any one of claims 1-17, wherein delivering comprises spraying the composition on an agricultural crop.
19. The method or use of any one of claims 1-17, wherein composition is formulated as an insect comestible composition for ingestion by the insect vector.