Trojan horse - eradicate mosquito-borne diseases using male engineered mosquito's microbiome

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Technology

Mosquitoes are significant vectors of severe and widespread diseases like Malaria, Dengue fever, Zika fever, West Nile virus, Lymphatic Filariasis and more. Current treatments are not specific unsafe and are directed only to large water sources with no regard for smaller water sources which are of great importance since mosquitoes can lay eggs in any small body of water. Unfortunately, the traditional methods, particularly insecticides, are problematic and account for the development of insecticide resistance and an environmental hazard. The present invention used the natural microbiome bacterium of *Aedes aegypti* mosquito, Serratia marcescens, is being genetically engineered to express *Bacillus thuringiensis israeliensis* (Bti) toxin, which through vertical inheritance will be delivered to egg-laying locations and reduce the population of mosquitoes. Bti toxins are selectively target the larval stages of insects. Few plasmid constructs were generated containing the most effective toxin combination against *A. aegypti*, producing the following sub-units: 'Cry4Ba', 'Cyt1Aa', 'Cry11Aa', 'P20'. Two fragmented toxin plasmids are created: the first one containing the 'Cry4Ba' subunit, and the other containing the rest of the subunits. To examine the feasibility of the method, *A. aegypti* was fed with GFP expressing *S. marcescens*. GFP was identified in the mosquito microbiome 3-8 days after mosquito feedings, and at the eggs after letting the female to lay eggs. It was also verified that these bacteria are been transferred to the larvae, which is the final target. The activity of the toxin and the efficacy of reduce viability of A. aegypti larvae was also examined. Three days old mosquito larvae were incubated with several *S. marcescens* variants. The inventors showed that the transgenic *S. marcescens* variants significantly reduced *A. aegypti* larvae survival, after 20, 40, and 80 hours, after incubation. Further, transgenic *S. marcescens* expressing the "final toxin" was shown to reduce *A. aegypti* larvae survival by staggering rates of more than 93% and 97% after 20 hours and 40 hours, respectively, and 100% mortality was observed after 80 hours. Therefore, the inventors conclude that transgenesis of non-pathogenic bacteria of an endogenous microbiome with a gene encoding a toxin or a combination thereof, may serve as an efficient and sustainable biocontrol agent and methodology to reduce or eradicate pests, such as exemplified herein with mosquitoes.

Application

Novel way to eradicate mosquito and mosquito-borne diseases, such as Malaria, Dengue fever, Zika fever, West Nile virus, Lymphatic Filariasis and more. The microbiome of male's mosquito is infected with the toxin–harbouring bacteria. The process is done in a laboratory and the male are released into nature. The bacterium reaches the stomach and from there passes through the body and reaches the male testicles. Following mating, infected females transfer the bacteria-infected eggs to the water reservoir. The larvae then hatch and die and the toxin is spread to the water reservoir.

Advantages

- Trojan horse – reach all water reservoir small and big in size
- The solution is specifically directed only to male mosquitoes, and it reduces the use of harmful chemicals
- Modular system that can be adapted to other mosquito species
- Past POC: Bacillus thuringiensis israelensis (Bti) Toxin is a validated and approved toxin for use

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