

Entomologists to develop special bacteria to combat spread of mosquito-borne diseases

June 14 2010

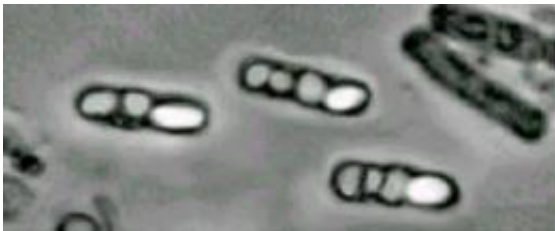


Photo shows genetically engineered *Bacillus thuringiensis* cells constructed in the laboratory to combine mosquito-killing proteins from different bacterial species. These new recombinant strains are much more potent than natural bacteria that are capable of killing the mosquitoes that transmit malaria, filariasis, and many viral diseases to humans. The bright oval structures are spores, and the angular gray structures to the left of the spores in each cell are crystals of the mosquito-killing proteins. Credit: Federici lab, UC Riverside.

Roughly half the world's population still lives in areas at risk of malaria transmission. Even in the United States, 1500 cases of malaria are reported annually on average.

One way to curtail the spread of malaria and other mosquito-borne diseases is to decrease the number of mosquitoes.

Now a five-year, \$1.86 million grant to the University of California, Riverside from the National Institute of Allergy and [Infectious Diseases](#) will help entomologists come closer to realizing this goal, potentially

benefiting millions of people worldwide.

Brian Federici, the principal investigator of the renewal grant, and colleagues will use the new funding to continue their work on developing genetically engineered bacteria for killing mosquitoes - specifically, mosquito larvae.

"Most new tactics today that use genetic engineering technology target a single species of mosquito," said Federici, a distinguished professor of entomology. "Our solution, however, is aimed at an enormous number of mosquito species, including those responsible for malaria, West Nile, [dengue fever](#) and filariasis."

Certain bacteria bear proteins that are highly toxic to only mosquitoes. In collaboration with industry, Federici's lab already has examined two different strains of such bacteria - *Bacillus sphaericus* (Bs) and *Bacillus thuringiensis* (Bt) - and engineered their mosquito-killing properties into a single new bacterial strain.

"This recombinant Bt/Bs strain is about ten times more effective than either one of the strains we used to combine the properties, and it is environmentally safe," he said. "Further, our recombinant is far more effective than bacterial strains used in commercially available products today."

Most available products exist in the form of a dried powder or liquid suspension in which the bacterium remains active. Widely used on vegetable crops, they are either sprayed from airplanes or hand-held sprayers. When mosquito larvae ingest the product, it destroys their stomachs and the larvae die within two hours. The proteins in the bacterium that kill the larvae are specific for mosquito larvae, attacking only these and certain other flies, nothing else.

Federici explained that his lab was able to accelerate the development of a recombinant [bacterial strain](#) by invoking genetic engineering into the research.

"It would probably be impossible to do what we did with just normal selection," he said. "It is far easier to take a gene from one bacterium and insert it into the DNA of another bacterium. Still, it took us ten years to get to this stage. We spent a good amount of time doing basic research to understand what controls the synthesis of mosquito-killing proteins."

To make the recombinant bacteria, Federici's lab constructed "plasmids" - small, circular pieces of DNA - using techniques developed in his lab as well as by other molecular biologists. The researchers cloned the genes coding for mosquito-killing bacteria from the two bacterial species, Bt and Bs, and then inserted them into the same new plasmid they constructed using genetic engineering techniques. They engineered the genes so that they would produce larger amounts of the desirable proteins. Finally, they injected the plasmid into a bacterium cell, rendering it recombinant (that is, containing genes from two or more different organisms).

Researchers in Federici's lab will use the grant to continue doing basic research on how the bacterium synthesizes the proteins. They also will explore fundamental questions about how the bacterium controls synthesis of these mosquito-killing proteins, as well as why these proteins are so specific for mosquitoes.

"Our work has implications for controlling many vector-borne diseases worldwide - filariasis in India, [malaria](#) in Africa, India, and Central and South America, and various viral diseases transmitted by mosquitoes around the world," Federici said. "Our recombinants are very good at killing *Culex* mosquitoes that occur in Brazil, India, Thailand, China and

Mexico. In preliminary field tests, we found that our recombinants kill 95-100 percent of the mosquitoes."

According to Federici, another significant advantage of using recombinant bacteria is that they come with built-in resistance management properties.

"Mosquitoes can become resistant to bacterial control agents," he explained. "Work in my lab has shown that it is possible to delay the evolution of mosquito resistance because this resistance takes much longer to evolve when recombinants are involved.

"Our preliminary efficacy data shows that our method works. We have a few more hurdles to jump over. But if all goes according to plan, we could have a product commercially available in 3-5 years. Certainly, our industry partners are eager to move forward."

The grant will support graduate students, specialists and postdoctoral researchers. Federici already is joined in the research by UCR's Margaret Wirth, a staff research associate in the Department of Entomology, and Mercedes Diaz-Mendoza, a postdoctoral researcher working in Federici's lab; and Dennis Bideshi and Hyun-Woo Park of California Baptist University, Riverside. Both Bideshi and Park also hold appointments as specialists in UCR's Department of Entomology.

In particular, Wirth will focus on the evolution of insecticide resistance to microbial toxins, the genetic basis of this resistance and evaluating the bacterial insecticides.

"We will also determine whether exposure to sublethal concentrations of microbial insecticides affects the adult longevity of female vector mosquitoes," said Wirth, who works in Federici's lab as well as the lab of William Walton, a professor of entomology.

Wirth explained that adult female mosquito longevity is the primary factor influencing the risk for disease transmission.

"This is because females must take a blood meal from an infected host, lay eggs, and survive long enough to take a second blood meal in order to transmit the disease agent," she said. "If adult female longevity is reduced in survivors of larval exposure to bacterial insecticides, then these can reduce the overall numbers of [mosquitoes](#) as well as the rate of disease transmission."

Provided by University of California - Riverside

Citation: Entomologists to develop special bacteria to combat spread of mosquito-borne diseases (2010, June 14) retrieved 24 April 2024 from <https://medicalxpress.com/news/2010-06-entomologists-special-bacteria-combat-mosquito-borne.html>

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