Pitting Microbes against One Another

To quell infectious disease, investigators are testing whether other microbes can help to hold pathogens in check

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To quell a variety of infectious diseases, scientists are engaging nonpathogenic bacteria and viruses to counteract the culprit microbial pathogens that so far resist more conventional control approaches. This strategy is being pursued against a range of pathogens, including those responsible for causing dengue fever in humans, American foulbrood in bees, and bleaching in coral, according to several investigators who described their respective approaches during the 2012 ASM General Meeting, held in San Francisco, Calif., last June.

Although microorganisms traditionally were viewed as the problem, now they are being eyed at least sometimes as potential allies in the face of the complex challenges that infectious diseases can pose. Not so long ago, that challenge was framed almost exclusively in bellicose metaphors. More recently, that tone is softening as new alliances take shape, and investigators explore a more subtle set of possibilities for treating medical, agricultural, and ecological problems with infectious components.

Bacteria Burden Insects That Carry the Dengue Virus

The virus responsible for causing dengue fever in humans infects as many as 100 million individuals per year, according to Scott O’Neill of Monash University in Australia, who spoke during the opening session symposium, “Harnessing the Power of Microbes for Biomedicine.” The numbers of people who develop dengue fever exceed those who become infected with other arthropod-borne viruses, called arboviruses, including the West Nile and yellow fever viruses. Dengue fever thus is the leading cause of illnesses and death among arbovirus-caused infections, and its impact is second only to that of malaria among tropical diseases.

Although endemic to the tropics, the dengue virus recently began moving into more temperate areas, carried along with its main vector host, the *Aedes aegypti* mosquito. “Dengue hemorrhagic fever has expanded from Southeast Asia to 28 countries in the Western Hemisphere,” says O’Neill. “The severity and frequency of dengue outbreaks is rapidly increasing across the world.” Indeed, incidence of dengue has risen 30-fold in the past 50 years, despite various attempts to slow the spread of disease. Vaccine development, antiviral drugs, and strategies aimed at reducing mosquito populations all failed to prevent, treat, or control dengue thus far, leaving 40% of the world’s population at risk.

O’Neill is taking a novel approach, harnessing bacteria to thwart the dengue virus. Specifically, he and his collaborators infect the mosquitoes that harbor this virus with *Wolbachia pipientis*, reasoning that this new biological burden on the vector mosquito may reduce its capacity to support viral replication and transmission to additional humans.

*Wolbachia* species are found in 65% of all insect species, including 28% of mosquito species that were surveyed. In general, these intracellular, maternally inherited bacteria form symbiotic relationships with their hosts, in many cases pro-

**SUMMARY**

➤ To quell a variety of infectious diseases, scientists are engaging nonpathogenic bacteria and viruses to counteract the culprit microbial pathogens that so far resist more conventional control approaches.

➤ One approach to controlling the dengue virus involves infecting mosquitoes that serve as its vector with bacteria that impair both the insect and the virus.

➤ Bacteriophage may help to control the bacteria responsible for causing American foulbrood in honeybees, a devastating disease that can contribute to colony collapse.

➤ Phage may also help to control bacterially caused bleaching of coral reefs.
tecting their hosts against pathogens and parasites, including nematodes and viruses as well as those that cause malaria. “An important feature of *Wolbachia* is its ability to induce resistance to a variety of pathogens, including dengue virus, in its insect hosts,” says Zhiyong Xi of Michigan State University in East Lansing, who collaborates with O’Neill.

Although the mechanism remains to be determined, *Wolbachia* can inhibit dengue replication in mosquito cell lines and in live insects, according to O’Neill, Xi, and their collaborators as well as other researchers. For example, *Wolbachia* may boost the insect immune system, priming it to attack the dengue virus. Host immune genes are up-regulated after being infected with *Wolbachia*, O’Neill reports. Meanwhile, Xi and his collaborators find reactive-oxygen-species (ROS)-dependent activation of the Toll pathway and antimicrobial peptide defensin DEFD expression. Another possibility is that the *Wolbachia* bacteria may outcompete the dengue virus for insect host cell resources. Dengue replication is inversely correlated with *Wolbachia* density, and the virus is excluded from cells and tissues with very high densities of those bacteria, according to both Xi and O’Neill.

The overreplicating strain of *Wolbachia*, called wMelPop, reduces the lifespan of its native host *Drosophila melanogaster* by 50%. This feature led O’Neill to think that this strain would make a good candidate for controlling dengue, which is transmitted by mosquitoes only toward the end of their 30-day lifespan. If the wMelPop strain could kill or otherwise hamper mosquitoes during the 7–14 days in which dengue viruses replicate within this host insect, it could drastically reduce dengue virus transmission, he says.

O’Neill adapted wMelPop to infect *Aedes aegypti*, the primary mosquito vector for the dengue virus, but a species that does not naturally harbor *Wolbachia*. Once adapted to the mosquito, wMelPop retains its life-shortening phenotype vis a vis its new mosquito host, and also inhibits dengue replication within that host. However, wMelPop does not sustainably in-

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fect mosquito populations, according to O’Neill. “Wolbachia strains that provide greater disruption to dengue transmission also confer greater fitness costs to the mosquito host, and successful invasion for dengue control therefore requires a Wolbachia strain that balances these two effects,” he says.

O’Neill and his collaborators redirected their efforts to another Wolbachia strain, wMel, which lacks the overreplication phenotype and exacts a lesser toll on host fitness. Because wMel infects mosquitoes raised in the lab and inhibits dengue virus under those protected conditions, O’Neill next sought to test this dengue-control scheme in field trials.

Following regulatory approval from Australian government officials and after receiving community support, O’Neill released approximately 300,000 wMel-infected mosquitoes at two sites in north Queensland, Australia. The trials proved successful, with Wolbachia invading native populations to 80–100% penetrance within a few months and stabilizing thereafter. Individual mosquitoes from these communities exhibit antidengue effects when recaptured and tested in the laboratory. However, the impact on dengue within human populations remains to be determined.

O’Neill plans to continue monitoring these parameters in Australia, and hopes to expand field testing to sites in Vietnam, Thailand, and other countries where dengue is prevalent. “We have demonstrated that it is possible to produce Wolbachia-infected mosquito populations that can act as ‘nursery’ areas for future human-assisted collection and further dispersal of Wolbachia-infected mosquitoes, without the need to rear additional mosquitoes in an insectary,” he says. “This should provide a strategy for sustainable dengue control at low cost, with a relatively simple deployment system suitable for implementation in developing countries.”

Wolbachia may also be useful in curbing malaria, according to Thomas Walker of the University of Queensland, Australia, who collaborates with O’Neill. The wMelPop strains of Wolbachia can inhibit replication of various Plasmodium species—the parasites that cause malaria—within their mosquito hosts, which do not naturally harbor that bacterial species. Immune activation and competition for resources may contribute to this effect. “Plasmodium is dependent on host lipids, suggesting cholesterol could be a critical nutrient required by both Wolbachia and the mosquito-borne pathogen,” Walker says.

However, application of this approach requires stable transfection of the Anopheles species of mosquitoes, which are responsible for harboring and transmitting Plasmodium parasites. While Wolbachia strains can be maintained in Anopheles mosquito cell lines, all attempts to stably transfact these species have failed. Ultimately, the successful use of Wolbachia against malaria and other vector-borne diseases will rest on the ability to transfact the bacteria into mosquito species that serve as vectors for pathogens and parasites, and the subsequent integration of those bacteria into wild mosquito populations.

**Viruses Help to Control Bacterial American Foulbrood Disease in Honeybees**

American foulbrood disease (AFD) is another instance in which microorganisms are being evaluated as a means for treating an infectious disease. However, in this case, viruses are being used to combat bacterial infections, according to Penny Amy and Diane Yost of the University of Nevada, Las Vegas, and their collaborators. Their aim is to identify and then harness viruses—bacteriophages—to rid honeybees of the bacterial infection that leads to AFD, which may also contribute to colony collapse disorder. While phage treatment of honeybee larvae is yet to be fully tested, this approach appears promising in the ongoing battle against AFD, which has continued for nearly a century. Amy and Yost presented their findings during the poster session, “Morphogenesis, Evolution and Ecology,” convened as part of the ASM General Meeting in San Francisco last June.

American foulbrood disease is caused by the gram-positive bacterium *Paenibacillus larvae*. These highly contagious bacteria infect and kill honeybee larvae, leading ultimately to collapse of entire hives. “Honeybee health is of great concern due to the importance of honeybees as pollinators in the agriculture of the United States and therefore the current and future food supply,” Yost says. Moreover, despite its name, AFD is widespread and a highly destructive brood disease affecting honeybees worldwide, and second only to the parasitic mite *Varroa destructor* as the most economically important disease of honeybees globally, according to bee expert Elke Genersch.
of the Institute for Bee Research in Neuendorf, Germany.

Conventional antibiotics pose numerous problems when used in treating American foulbrood, according to Genersch. They disrupt the vitality and longevity of bees, partly by disrupting beneficial gut microbiota, and compromise the quality and safety of honey that the bees produce. More importantly, antibiotics are not particularly effective in controlling the disease, particularly as *P. larvae* continue to develop resistance to such treatments. “Antibiotics only suppress clinical symptoms and mask the disease, but cannot cure it,” she says.

Incineration is the most effective treatment against AFD because it kills growing *P. larvae* and spores. Although local authorities may insist that bee keepers burn infected hives to prevent the spread of AFD, this approach is extremely costly and threatens agricultural productivity in general because honeybees pollinate crops. Indeed, fruits, nuts, and vegetables are among the crops that honeybees pollinate, accounting for about one-third of the U.S. food supply. “Honeybees are the most economically valuable pollinators of crop and fruit monocultures worldwide,” Genersch says. Within the United States, honeybee pollination was valued at $19 billion per year in 2010, according to a study conducted by agronomists at Cornell University in Ithaca, N.Y.

In their search for an alternative and more sustainable way of treating AFD, Yost and her collaborators identified dozens of bacteriophages that infect and kill *P. larvae* bacteria. They isolated these viruses from environmental sources, including desert and garden soil, hive wax, honey, flowers, plants, compost, dead bees, and cosmetics containing beeswax. These phages are lytic to various strains of *P. larvae*, but not other bacteria such as *Escherichia coli*. Of particular importance for the bees, the phages apparently do not affect the microbiota of the honeybee gut or other bacteria that are found in hives. Yost and her collaborators plan to characterize the phage further, including through DNA profiling and electron microscopy.

Honeybees in a hive. American foulbrood disease is an economically devastating disease caused by the bacterium *Paenibacillus larvae*. Researchers are working on treatments with phage that could help protect bee colonies from the disease. (Photo © fatchoi/iStockphoto.)
Phage therapy is a safer solution than antibiotics because phages are already naturally present in the environment and not harmful to humans or bees,” Yost says. “We are not introducing foreign or genetically modified organisms into the environment.” Treating AFD with phage carries advantages over antibiotics. *P. larvae* are less likely to develop resistance against phages, particularly if several phage types are applied simultaneously. Also, unlike antibiotics, phages will not infect or affect nearby animals or plants.

The *P. larvae*-infecting phage remain viable for at least three months, based on experiments designed to mimic conditions in the hive and larval gut, according to Yost. “A hive could be sprayed with phage whether it is known to be infected or not, and once the bee larvae ingest the phage, they would be protected against infection for a few months,” she says. The presence of phage in and around the hive would also target bacteria not yet ingested by bee larvae, whether from cross contamination with other hives or from germinating spores.

**Phage Eyed to Combat Coral Bleaching**

Eugene Rosenberg of Tel Aviv University in Israel is also harnessing bacteriophage, but in this case as a potential means for defending coral reefs against bacterial infections. “Over the past 30 years, there has been an approximately 30% worldwide decline in the coral population, largely due to emerging diseases,” he says. “At the global scale, coral bleaching is the most serious disease threatening coral reefs.” Rosenberg presented some of his recent results on this research during the poster session “Regulation, Replication, Recombination, and Biotechnology,” during the 2012 ASM General Meeting in San Francisco.

Bacterial pathogens cause coral bleaching, according to Rosenberg. He and his colleagues have identified several species that cause bleaching in specific corals of the Mediterranean, Indian Ocean, and Red Sea. The bacteria produce a toxin that inhibits photosynthesis by endosymbiotic algae, which provide coral with oxygen and carbon-containing nutrients. Without algae to pro-
vide these essential nutrients, the growth rate of the coral is impaired, as is its reproductive capacity, making it susceptible to secondary diseases. “If the process is not reversed within a few weeks or months, depending upon the specific coral species and conditions, the coral dies, since a major portion of a coral’s nutrition comes from the photosynthetic products of algae,” Rosenberg says.

Treating infected corals is challenging. “Immunization and antibiotic treatment, two widely used methods for controlling human diseases, are not applicable to coral disease because corals have no adaptive immune system, and introduction of antibiotics in an open system such as a coral reef is not practical,” Rosenberg says. Bacteriophages overcome at least some of those disadvantages.

For example, the phage called BA3 infects and kills one of the bacterial pathogens, *Thalassomonas loyana*, that infect corals—in this case, the one responsible for white plague-like (WPL) disease in *Favia favus* coral in the Red Sea. When added to diseased coral, this phage stops disease progression and prevents its transmission to other corals. Phage-treated corals suffered only 5% tissue death, compared to 65% in untreated controls, according to Rosenberg. In these field experiments, phage-treated diseased corals transmitted the pathogen to only 1 of 19 surrounding healthy corals. In comparison, untreated-diseased corals transmitted infection to 11 out of 18 surrounding healthy corals.

However, timing is crucial; phage must be applied within 24 hours after *T. loyana* infects the coral to prevent damage, Rosenberg continues. Hence, he adds, “Phage therapy may be more valuable in preventing the spread of disease rather than curing an already infected coral.” In laboratory experiments, phage treatments effectively inhibit transmission of the bacterial pathogens independent of when the phages are applied. Another concern is that the target pathogen, *T. loyana*, is likely to develop resistance to phage. “One possibility for overcoming this potential problem is to carry out phage therapy in the field with more than one *T. loyana*-specific lytic phage,” he says.

All in all, Rosenberg is optimistic about using phage to treat coral disease, emphasizing how environmentally safe it is. For instance, BA3 treatment does not harm other species of nearby coral, and is also safe for food and aquaculture applications. Another advantage is that phage replicate to replenish the supply, he adds. “The phage multiplies rapidly at the expense of its host bacterium, increasing the phage titer for more effective control of the pathogen.” Moreover, the phage is highly specific, sparing beneficial microorganisms within the coral community.