The Enemy of my Enemy is my Friend

A new hope in the battle against WNS?

COURTESY OF KYLE GABRIEL

Graduate student Chris Cornelison, a microbiologist at Georgia State University, works in the lab during his search for biological agents that might combat White-nose Syndrome.

by Chris Cornelison

For the past six years, the "silver bullet" sought by scientists battling White-nose Syndrome has been an ecologically acceptable tool for destroying or disabling the *Geomyces destructans* fungus, which causes this scourge that is killing millions of bats. But the search has been frustrating. While some chemical fungicides will kill the fungus, their use would likely devastate complex cave ecosystems and could contaminate water supplies.

Several teams, including ours, are exploring another, potentially more benign, option: biological agents. Now initial results from our research at Georgia State University suggest we have found a very promising candidate: a natural bacterium that in the lab is able to inhibit the fungus without actually touching the bats or the cave. More research is required to confirm this approach, but the evidence suggests we may be able to save bats and spare the caves.

My microbiology colleagues and I decided to tackle this problem after the national WNS Response Plan, published in May 2011, gave significant attention to developing biological and chemical control options. We soon noticed some intriguing activities exhibited by the bacterium *Rhodococcus rhodochrous* strain DAP 96253.

Our initial investigation found that the bacterium, when cultivated under very specific conditions (U.S.P. 7,943,549), could inhibit the growth of two *Geomyces* species that are closely related to *G. destructans*. So we obtained samples of the *G. destructans* fungus from Kevin Keel (then at the University of Georgia and now at the University of California, Davis) to explore our bacterium's anti-*Geomyces* abilities.

The initial test results were astonishing.

The cold-loving fungus attacks by sending out branching structures called hyphae that invade the bats' tissue, especially the wings. *G. destructans* grows best at about 41 to 50 degrees Fahrenheit (5° to 10° Celsius) and essentially stops growing, becoming dormant, at 68° F (20° C). We demonstrated that *R. rhodochrous* bacteria completely blocked germination of *G. destructans* spores at 59° F (15° C) and strongly inhibited growth and reproduction at 39° F (5° C).

This was the first demonstration of biological antagonism to *G. destructans*, and we were eager to explore this potentially revolutionary tool for combating WNS. I nearly gave up, however, when I was unable to secure funding for the project, part of my work toward a Ph.D. Luckily, I applied for and received a grant from Bat Conservation International's WNS Program.

So I initiated an experiment with Kevin Keel to determine whether *Rhodococcus* can prevent the WNS fungus from colonizing bat tissue. Rather than sacrificing a number of bats by exposing living animals to the fungus and bacterium, we used a groundbreaking technique developed by Keel to maintain disembodied bat-wing tissue culture in the laboratory.

We demonstrated the ability of *Rhodococcus* to prevent the colonization of bat-wing tissue by *G. destructans* spores for more than 40 days. The *Rhodococcus* was placed in close proximity to, but was not touching, the tissue and the fungus.

Our initial inquiry into the mechanism of the antagonism began with a simple question: does the contact-independent antagonism affect spore germination, mycelial elongation or both?

Next, a few simple experiments and some basic microscopy indicated that *Rhodococcus* completely and permanently inhibits spore germination (i.e., prevents it from sprouting) and significantly slows the growth of the tentacle-like hyphae. Since previous studies have shown the spores to be the primary infectious agent of *G. destructans*, these results suggest that *Rhodococcus* can prevent the initial colonization of healthy bats, and also slow progression of the disease in already-infected bats – and increase their chance of survival.

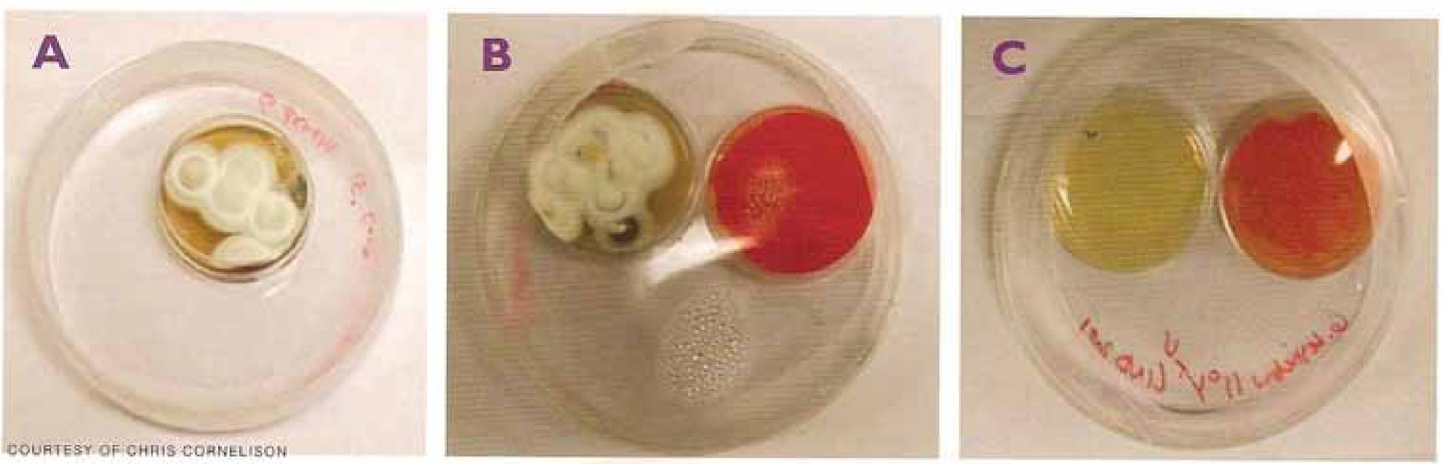
Using protocols developed by previous Georgia State University researchers, we grew *Rhodococcus* bacteria in 8-gallon (30-liter) fermentation vessels under conditions that activated its anti-*Geomyces* activity. Bacteria in the resulting "cell paste" no longer grow, but they nonetheless prevented germination of *G. destructans* spores for more than 80 days (at the time this article was written).

This was a vital step toward practical application, since it shows that we may have a control agent that can be mass produced, applied without any form of growth medium and provide long-term inhibition of *G. destructans* spores at 39° F (4° C). And it works without being in direct contact with the bats, the cave environment or the fungus.

The simplicity and efficacy of this microbial antagonism are not really surprising. The co-evolution of soil-associated fungi, such as *Geomyces*, and bacteria, such as *Rhodococcus*, lends itself to these natural antagonisms: these organisms have been waging war in a complex environment for billions of years. Humans are unlikely to devise a more effective weapon than what the natural competitors of *Geomyces* have evolved over eons of open hostilities.

Other biocontrol approaches would require microbes to be applied directly to the bats or the cave walls or soil. Our research indicates that the fermented *Rhodococcus* paste requires no direct contact with the bats or the cave. It could, for example, be introduced on plastic sheets placed near hibernating bats. When bats are no longer at risk (typically in spring and summer), the bacteria could simply be removed.

Despite the exciting possibilities demonstrated by our research results, several important questions must still be answered



COURTESY OF CHRIS CORNELISON

This experiment demonstrates the potential of the *R. rhodochrous* bacterium to inhibit the growth of spores of the *Geomyces destructans* fungus that causes WNS. The first dish (A) shows spore growth after 21 days without the bacteria. Spores grew in Dish B with bacteria that were not activated ("induced") through a fermentation process. But in Dish C, induced bacteria completely inhibited spore growth after 21 days.

before we can put this potential tool to work. We must, of course, assess any impacts to the bats and to cave ecosystems and organisms, then conduct small field trials before wide-scale application is feasible or ecologically responsible.

We are currently working on a simple model of the mycoflora of North American caves and are investigating their resilience to our biological control agent. We are also collaborating with federal wildlife biologists and toxicologists to determine any potential impacts to bats before testing our control agent in the field.

Early results are promising and provide optimism that the *Rhodococcus* control agent will give wildlife-management agencies a potent new tool to prevent the spread of WNS and begin the re-colonization of hibernacula that have been devastated by this disease.

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