Georgia Institute of Technology
Research News

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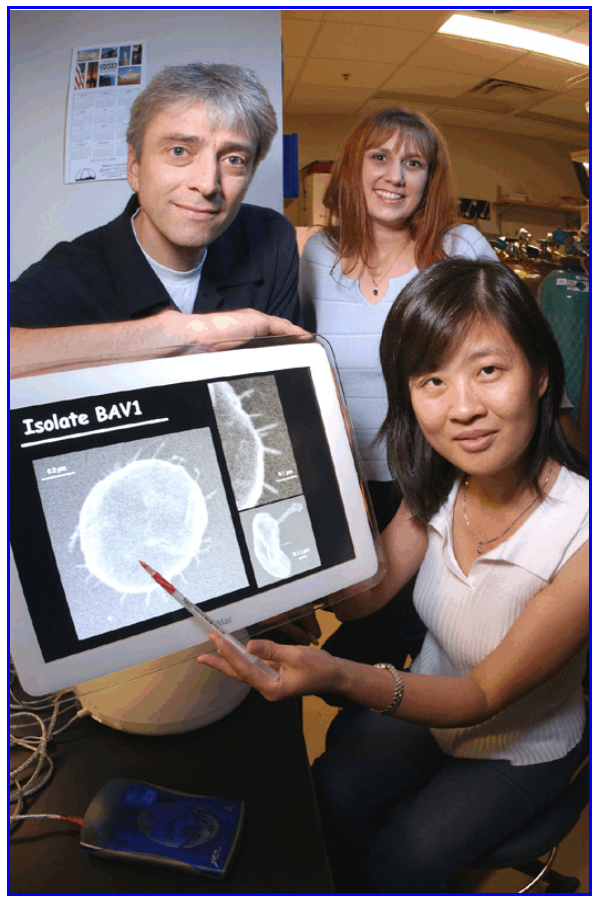
BACTERIAL DETOX: NOVEL BACTERIUM DETOXIFIES HARMFUL CHLORINATED COMPOUNDS TO PRODUCE BENIGN BYPRODUCTS

Researchers have isolated a novel bacterium that flourishes as it destroys harmful chlorinated compounds in polluted environments, leaving behind environmentally benign end products. The finding opens the door for designing more efficient and successful bioremediation strategies for thousands of contaminated sites that remain threats, despite years of expensive cleanup work.

"This organism might be useful for cleaning contaminated subsurface environments and restoring drinking-water reservoirs," Georgia Institute of Technology researchers report in the July 3, 2003 issue of the journal *Nature.*

The paper, titled "Detoxification of vinyl chloride to ethene coupled to growth of an anaerobic bacterium" is the culmination of five years of field and laboratory studies funded by the National Science Foundation and the Strategic Environmental Research and Development Program.

Scientists and engineers have struggled for years with clean up of groundwater and subsurface environments contaminated decades ago by unregulated use of the common solvents tetrachloroethene (PCE) and trichloroethene (TCE). These toxic compounds are primarily used



Georgia Tech doctoral student Jianzhong He (right) isolated the novel bacterium BAV1 in research led by Frank Loeffler. Postdoctoral fellow Kirsti Ritalahti (center) led molecular analyses for the project.

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in dry cleaning operations and degreasing of metal components. Complicating the situation are natural biotic and abiotic processes that transform these solvents to intermediate substances, such as toxic dichloroethenes, and cancer-causing agents, such as vinyl chloride.

But in a step toward engineering better bioremediation strategies, Georgia Tech researchers have isolated a naturally occurring bacterium, designated *Dehalococcoides* strain BAV1, in a pure culture – without other microbial species present in the sample. Though some progress was made in the past decade in understanding the bacteria involved in partial degradation of PCE and TCE, this study represents a significant advance, researchers said.

"Isolating this bacterium will allow us to study the organism and the dechlorination process in more detail," said lead researcher Frank Loeffler, an assistant professor in the School of Civil and Environmental Engineering. "We can get a lot more information that we can then use to engineer systems in the environment so PCE and TCE degradation would not stop at the toxic intermediate stage, but rather would continue to be dechlorinated to a non-toxic end product, such as ethene."

One site that appears likely to benefit from in-place bioremediation with this bacterium is the Bachman Road residential area contaminated with PCE by a former dry cleaning operation in Oscoda, Mich. There, researchers recently used BAV1 in a successful pilot demonstration, which they briefly reference in the *Nature* paper. Loeffler and his colleagues described the results of the pilot study in greater detail in a paper published in February 2003 in *Environmental Science & Technology*.

At this contaminated site, PCE penetrated the water table and contaminated drinking-water wells in the area. The contaminants also migrated through the groundwater into nearby Lake Huron, which attracts sunbathers and swimmers to its beaches and water.

In 14-feet by 16-feet, 20-feet-deep test plots at the Bachman Road site, researchers compared a non-treated control section to two bioremediation approaches using BAV1, which already occurs at this site in low numbers. One strategy, called biostimulation, added lactate and nutrients to the contaminated plot. In another section, researchers injected a mixed culture containing high numbers of BAV1 along with nutrients in a strategy called bioaugmentation. This technique resulted in complete dechlorination of PCE to ethene within six weeks. Biostimulation, on the other hand, worked but took more time to accomplish detoxification.

"Bioaugmentation had a relatively poor reputation," Loeffler said. "In cases targeting petroleum candidates, it didn't help any more than less expensive strategies. Now, we have a good example of bioaugmentation at work…. It is a viable option, especially at sites with this type (chlorinated solvents) of contamination. So there's a lot of excitement about this. People have spent a lot of money to clean up those sites without success. Now there's a new hope."

There are thousands of similar contaminated sites, including military installations where PCE and TCE were once used as degreasing agents.

Both laboratory and field work reported in *Nature* revealed that the growth of BAV1 depends strictly on the reduction of these chlorinated compounds (e.g., dichloroethenes and vinyl chloride) to ethene and the presence of hydrogen as an electron donor. Also, genetic analyses, analytical chemistry techniques and high-resolution scanning electron microscopy yielded information about the organism's appearance, makeup and behavior. One peculiarity is filament-like appendages extending from BAV1 cells. Loeffler speculates that these appendages may allow the organisms to colonize contaminated subsurface environments.

Also, phylogenetic analysis described in *Nature* revealed that BAV1 belongs to the only-recently discovered *Dehalococcoides* group, which comprises other organisms useful in bioremediation. The findings highlight the largely untapped reservoir of bacterial diversity, Loeffler added.

BAV1's origin is unknown, though Loeffler believes it evolved long ago, deriving energy from naturally occurring chlorinated compounds, including chlorinated solvents, in the environment. He suggested that BAV1 in some natural areas survives by eating low concentrations of chlorinated compounds formed from volcanic, biologic and possibly ultraviolet light processes. Other scientists assert that BAV1 occurred in the environment long ago, but only developed its chlorinated compound-based metabolism in response to PCE and TCE pollution.

Georgia Tech researchers will continue to learn more about BAV1 as they conduct larger-scale studies, in which Loeffler admits researchers will have a more difficult job in monitoring the dechlorination process. Also, that process may not happen as quickly as it did in the smaller, pilot demonstration. In addition to future studies at the Michigan site, Loeffler is proposing similar research to the U.S. Department of Defense at a TCE-contaminated military installation near Atlanta. There, he expects bioremediation to be complicated by a fractured rock layer beneath the water table.

In addition to opening the doors for further research on BAV1, the Georgia Tech study yielded a molecular technique that likely will be useful to scientists and engineers conducting similar research. The technique allows researchers to quantify the number of BAV1 organisms present at a contaminated site. An increasing number of organisms indicates a positive response to bioremediation efforts. This technique uses a real-time polymerase chain reaction (PCR) device to test field sites.

Loeffler is hopeful about the use of BAV1 and related organisms in bioremediation. He speculates that this group of bacteria might also be useful to treat sites contaminated with polychlorinated dibenzo-dioxins (PCDDs) and polychlorinated biphenyls (PCBs).

"Organisms like BAV1 have an enormous potential to help detoxify chlorinated pollutants," Loeffler said. "But we're just at the beginning of understanding their function, distribution and ecology in the environment."

The lead author on the *Nature* paper is Loeffler's Ph.D. student Jianzhong He, who isolated BAV1 in her professor's lab. Other authors are postdoctoral fellow Kirsti Ritalahti, who led molecular analyses, graduate student Kun-Lin Yang, who contributed scanning electron microscopy studies, and Stephen Koenigsberg of California-based Regenesis Bioremediation Products, which contributed equipment and materials.

Georgia Tech has filed two patent applications related to Loeffler's research, and Regenesis is marketing the research team's culture, called Bio-Dechlor Inoculum™, to the bioremediation community. For more information, see (www.regenesis.com/products/bd_inoculum/).

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