HAZARDOUS WASTE CLEANUP

A Tentative Comeback for Bioremediation

After years of relative obscurity, research on pollution-eating bugs is coming of age. But DOE is not about to field test any genetically modified organisms soon.

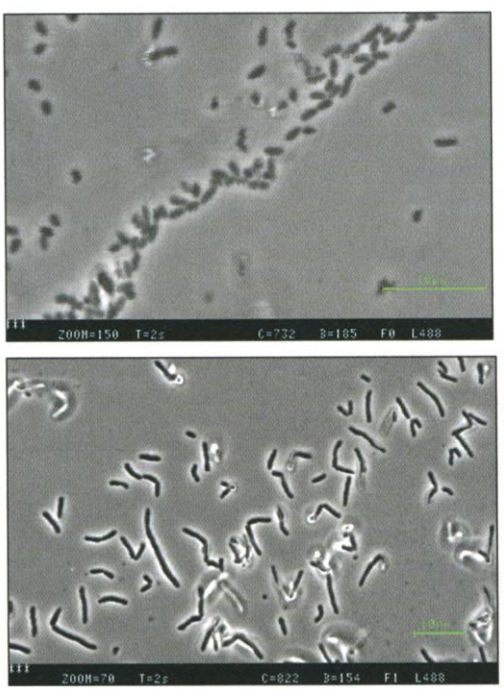
At the dawn of the age of bioengineering, in 1972, General Electric researcher Ananda Chakrabarty applied for a patent on a genetically modified bacterium that could partially degrade crude oil—sparking visions of a brave new world in which toxic wastes would be cleaned up by pollution-gobbling bugs. Researchers quickly jumped on the bandwagon, transferring genes between microbes in the hope of engineering hybrids with a taste for pollution, while a host of "bioremediation" companies sprang up to cash in on the trend. But those hopes were soon dashed. Immobilized by the high costs and technical difficulties of this research, the companies soon went bankrupt. And experimentation retreated from biotech start-ups to government and academic laboratories, where it has remained in relative obscurity.

Now, some 30 years later, bioremediation is slowly and gingerly staging a comeback. Naturally occurring microbes have been tried at a few sites with some limited success. Since 1998, for example, one group has been successfully cleaning up a carbon tetrachloride spill in Michigan using natural bacteria imported from California. Elsewhere, strains of *Pseudomonas* bacteria have succeeded in remediating halogenated hydrocarbons like trichloroethylene. And in October, the Department of Energy (DOE) will perform its first-ever field test of bioremediation to clean up one of its heavily polluted sites.

With one exception, however, none of the pollution-gobbling bugs released to date has been genetically altered—and DOE is not going to risk it, either. Public resistance to unleashing recombinant microbes—even in field tests—is too great, says Aristedes Patrinos, associate director of DOE's Office of Biological and Environmental Research. Even so, many scientists in this reemerging field, including some at DOE, believe that genetically modified microbes must eventually be employed if bioremediation is ever to succeed.

For the new efforts, researchers are taking what William Suk, who directs bioremediation funding for the National Institute of Environmental Health Sciences (NIEHS), calls "a more measured approach" than in the past. Then, he notes, microbiologists keen on engineering bacteria to metabolize pollutants quickly learned in lab tests that their bugs had trouble competing with native microbes in their target soil. And those that were effective did their jobs much more slowly than expected.

Now researchers are trying to avoid these problems by taking into account the chemical properties of the soil and the geological characteristics of polluted areas as well as the properties of the pollution-eating microbes. The DOE effort, for example, will use microbes that emerged naturally from the site they will treat, which is contaminated with heavy metals and radionuclides left over from decades of nuclear weapons programs.



Strength in numbers. When teamed up with a natural bacterium, these two strains of genetically altered bacteria, *Burkholderia* LB400 *(above)* and *Rhodococcus* RHA1, can clean up most PCBs in the lab.

This first field test will occur adjacent to a particularly nasty site at Oak Ridge National Laboratory in Tennessee known as S-3. Now capped by a parking lot, S-3 was once a series of ponds contaminated with radioactive uranium, cesium, and cobalt mixed with mercury and other toxic heavy metals. Without any human prodding, several species of bacteria have adapted to feed on components of the toxic soup that have leached out into the surrounding soil. For instance, these bacteria can transform dangerous metals into less mobile forms that don't dissolve in groundwater. But the natural metabolism of the bacteria is too slow to handle the job, so researchers will add nutrients such as lactate and acetate to the soil in an effort to stimulate the local microorganisms into a toxic feeding frenzy.

"Our ultimate goal is to harness natural processes to immobilize harmful metals," says Anna Palmisano, who manages bioremediation projects for DOE's Natural and Accelerated Bioremediation Research (NABIR) program. If the strategy works, NABIR will next transplant natural bioremediating bacteria from other areas to S-3 to see how well they operate in the new environment.

Conspicuously absent from NABIR's field-testing program are experiments with genetically altered microbes. Although NABIR funds some of this research in outside laboratories, safety concerns, regulatory hurdles, and anticipated negative public reactions are keeping NABIR from considering field-testing recombinant bioremediators "in the near future at all," says Palmisano.

But Oak Ridge microbiologist Robert Burlage and others insist that recombinant technology is exactly what is needed. The problem with naturally occurring microbes, he says, is that "some sites are so bad they will kill off a bacterium as soon as it hits." And no natural bug is equipped to deal with the "witch's brew" of pollutants present at sites like S-3, the way a specially designed microbe could. *Deinococcus radiodurans* is one example, says Burlage. This "extremophile" is able to thrive under radiation doses of 1.5 Mrads, up to 300 times the fatal dose for humans. But it can't on its own detoxify the other chemicals that often accompany radioactive contamination.

In January, geneticist Michael J. Daly and colleagues at the Uniformed Services University of the Health Sciences in Bethesda, Maryland, announced in *Nature Biotechnology* that they had transferred into *D. radiodurans* a gene from the common lab bacterium *Escherichia coli* that enables *D. radiodurans* to resist toxic mercury II. The result was a microbe that could convert mercury II to less toxic elemental mercury, while withstanding high levels of radiation. Daly and colleagues have since added other genes that code for enzymes capable of metabolizing the toxic organic chemical toluene. The researchers wound up with a microbe able to metabolize a heavy metal and an organic toxin in the presence of radiation, at least under lab conditions.

CREDITS: A. KATCHEL/MICHIGAN STATE UNIVERSITY

Test case. DOE will try out its bioremediating bugs at this site at Oak Ridge National Laboratory, contaminated with radionuclides and heavy metals.

At Stanford University, in as-yet-unpublished work, environmental engineer Craig Criddle and colleagues have also designed bioremediating microbes fit for a witch's brew. Criddle's team has taken a gene from a carbon tetrachloride–metabolizing bacterium known as *Pseudomonas stutzeri* strain KC and transferred it into a heavy-metal metabolizer called *Shewanella oneidensis*. Now, says Criddle, they have a recombinant strain that can both degrade carbon tetrachloride and immobilize heavy metals. But there's a catch: In lab tests, when the strain metabolizes carbon tetrachloride, it leaves behind chloroform—"and that can leave you worse off than you were before," says Criddle. So that's the next problem his team is tackling, with funding from NIEHS.

At Michigan State University in East Lansing, James Tiedje is trying a combination approach to degrade polychlorinated biphenyls, or PCBs. He starts with a natural bacterium that can consume PCBs. Then he adds genetically altered strains of two other bacteria, *Rhodococcus* RHA1 and *Burkholderia* LB400, both designed to remove chlorine and break the phenyl rings in PCBs. The mop-up effort by the engineered strains "can remove the majority of the remaining PCBs, but not all" in lab tests, says Tiedje about his as-yet-unpublished work.

In theory, says Tiedje, these PCB-eating bacteria should be ready for field-testing "by the next warm season," when they would be most effective. But strict regulations on recombinant bugs mean that these and other engineered microbes are unlikely to see the light of day anytime soon. The Environmental Protection Agency must approve any field tests of recombinant organisms. So far, out of 35 recombinant microbes approved for a variety of agricultural and other uses, only one bioremediator—a *Pseudomonas* species that fluoresces when it contacts naphthalene—has made the grade.

Suk of NIEHS and Burlage chafe at the sluggish pace with which the field is moving; in particular, they would like DOE and other funding agencies to push harder to bring recombinant bacteria to the field. "There are plenty of toxic waste sites far away from population centers that would be ideal for testing," asserts Suk. "Those are the sites to do demonstration research. We need to take some chances to restore [toxic sites] faster, better, and cheaper than we are now."

But DOE, which has some 3000 sites to clean up, is not budging. Says Patrinos: "If we rush into field-testing of recombinant microbes and it fails, we may be worse off in the long run." **–TODD ZWILLICH**

Todd Zwillich is a free-lance writer based in Washington, D.C.

INTELLECTUAL PROPERTY

Critics Say Rulings Give State U. License to Steal

U.S. Supreme Court rulings that give states more protection from patent infringement suits could be a potential windfall for research universities

The U.S. patent system is supposed to level the playing field for inventors. But recent Supreme Court decisions may have given states, including research universities, a leg up on the competition by making them immune from suits over patent infringement. Some lawmakers and biomedical executives are pushing Congress to pass legislation closing what they see as a potential multibillion-dollar loophole in the patent laws. But some academics and state officials say that Congress should wait to see if a problem develops before acting.

At stake is the ability of private software, biotech, and publishing companies—and even poets and musicians—to recover lost profits from state universities, hospitals, and other agencies that have copied or used their work without paying a fee. Critics say the rulings, issued last October, will tempt states to become intellectual property pirates, helping themselves to everything from patented genes to copyrighted textbooks, while at the same time shielding their own increasingly valuable patent portfolios from infringement claims. "It's inequitable … states are now in the enviable position of having their cake and eating it, too," says Q. Todd Dickinson, head of the U.S. Patent and Trademark Office (PTO). But law professor Peter Menell of the University of California (UC), Berkeley, predicts that the rulings "will have more of a symbolic than substantive impact." So far, he notes, states have claimed immunity in only a few cases, with mixed results.

"Bizarre" judgment

The debate centers on two highly technical constitutional rulings. In the cases, collectively known as Florida Prepaid, a private bank charged that a college savings program run by the state of Florida infringed on a financial patent it had obtained. In narrow 5–4 votes last October, however, the high court upheld the state's claim that it was immune from the federal lawsuit under the 11th Amendment to the U.S. Constitution, which shields states from many kinds of claims. The justices also declared unconstitutional the Patent Protection Act, which Congress had passed in 1990 to overturn an earlier Supreme Court ruling that questioned the long-standing policy of treating state patent holders the same as private entities.

The decisions sparked fierce criticism. "Truly bizarre," Charles Fried, President Ronald Reagan's solicitor general and now a professor at Harvard Law School in Cambridge, Massachusetts, wrote in *The New York Times*. If the decision stands, he and other critics claim, research labs and hospitals could use patented tests without paying royalties. They could also get into the manufacturing business, producing cheap knock-offs of popular biomedical products without fear of paying damages. State officials could even buy a single copy of a software program and copy it, while state university professors could do the same with chemistry textbooks—perhaps while offering the politically popular justification that the rip-off saved

Diet doctor. Patent commissioner Q. Todd Dickinson says states are wrongly "having their cake and eating it, too."

CREDITS: OAK RIDGE NATIONAL LABORATORY; U.S. PATENT AND TRADEMARK OFFICE