Meet the Superbug

Radiation-resistant bacteria may clean up the nation's worst waste sites

By JOHN TRAVIS

Hanford Nuclear Reservation in Richland, Wash., is one of many sites that contain radioactive waste. C. Anderson/*Spokesman-Review*

If a massive nuclear war ever blanketed the planet with radioactive fallout, cockroaches, despite all the jokes, would be goners. A bacterium known as *Deinococcus radiodurans* might survive, however. Its name, which means "strange berry that withstands radiation," indicates the reason that the microbe fascinates scientists. Labeled the world's toughest bacterium by *The Guinness Book of World Records*, *D. radiodurans* shrugs off doses of radiation many thousands of times stronger than those that would kill a person.

"I had difficulty believing anything like this could exist," says John R. Battista of Louisiana State University in Baton Rouge, recalling his introduction to the microbe in 1988.

Believe it or not, *D. radiodurans* does exist—and in some unusual places. Pinkish in color and giving off the smell of rotten cabbages, the bacteria were originally isolated in the 1950s from tins of meat that had spoiled despite supposedly sterilizing irradiation. Since then, they've showed up in elephant and llama feces, in irradiated haddock and duck, and in granite from Antarctica's Dry Valleys, the place on Earth thought to most closely resemble the surface of Mars.

Reviving the idea that comets seeded planets with the precursors of life, or even life itself, scientists have had fun speculating that only a radiation-tolerant bacterium such as *D. radiodurans* could survive interstellar journeys. This is the kind of organism that could do something like that," muses Marvin Frazier, director of the Department of Energy's (DOE) Microbial Genome Program in Germantown, Md., which funded the just-completed effort to sequence all of *D. radiodurans*' genes.

In more earthly matters, investigators have crafted an explanation for why the bacterium evolved its immunity to radiation. They propose that it's the byproduct of skills needed to survive a lack of water. Scientists have also established that the microbe doesn't simply shield its DNA from the radiation. It instead has an unprecedented ability to repair genetic damage.

While the basic biology of *D. radiodurans* has proved fascinating, DOE's interest in the bacterium stems from a very practical issue. The agency hopes that the microbe, after appropriate genetic manipulation, might help detoxify the thousands of toxic-waste sites nationwide that include radioactive materials. Using microbes as a cleanup crew is a strategy known as bioremediation. A recent study, in which *D. radiodurans* was engineered to degrade an organic toxin common to such waste sites, offers encouraging results, says Frazier.

Scientists usually measure radiation in units called rads, and a dose of 500 to 1000 rads is lethal to the average person. In contrast, depending upon conditions for its growth, *D. radiodurans* thrives after exposures of up to 1.5 million rads. Cool or freeze the microbe, and it may survive 3.0 million rads.

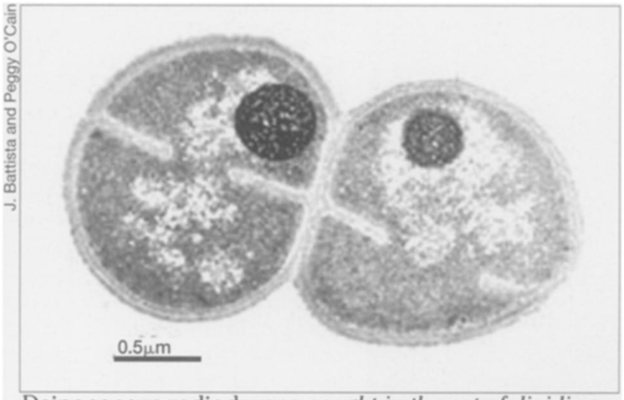
Bacteria that form hard capsules called spores can withstand large amounts of radiation but not as much as can *D. radiodurans*, which doesn't form a spore. "It is supreme in its radiation resistance," says Michael J. Daly of the Uniformed Services University of the Health Sciences in Bethesda, Md.

Working with his colleague Kenneth W. Minton, Daly has for many years sought to explain how the bacterium's DNA-repair system accounts for its exceptional hardiness. "I was interested in DNA repair, so why not study it in an organism that does it better than anything else?" he asks.

Indeed, *D. radiodurans* faces quite a challenge when it is hit with millions of rads. Literally shattering the bacterium's genetic material into hundreds of fragments, the radiation creates complete breaks in the microbe's double-stranded molecules of DNA.

A double-strand break is the most difficult kind of DNA damage to repair. The well-studied bacterium *Escherichia coli*, for example, usually can't survive more than two or three double-strand breaks. Yet within a few hours, *D. radiodurans* begins to stitch its thoroughly fractured DNA together, and it eventually resurrects a genome free of mutations.

This enviable talent long puzzled researchers. What evolutionary pressure could have forced *D. radiodurans* to develop such repair skills? There are rare cases in which radioactive elements such as uranium or thorium concentrate underground in large amounts, but the radiation fluxes near those sites are still small compared with what the microbe can withstand. Moreover, *D. radiodurans*' oxygen use and other aspects of its metabolism suggest that the bacterium evolved on the surface of the planet, not underground in radiation hot spots.



J. Battista and Peggy O'Cain

Deinococcus radiodurans *caught in the act of dividing.*

"There are no natural environments that have fluxes of radiation that could have selected for this organism," says Daly.

A few years ago, Battista's group tested an alternative hypothesis. Noting that *D. radiodurans* can also withstand extended periods without water, Robert G. Murray of the University of Western Ontario in London, Ontario, many years ago put forth the theory that its DNA-repair system evolved to combat desiccation. A much more common problem for bacteria, dehydration helps explain the popularity of spores: The capsules hold in the last available drops of water.

Battista and his colleagues found that dehydration does indeed generate the same double-strand breaks in DNA as irradiation does. Moreover, using chemicals that create mutations that the microbe is unable to fix, the scientists produced strains of *D. radiodurans* that had lost their resistance to radiation. Each mutant strain proved vulnerable to dehydration.

"Our conclusion was that *D. radiodurans* was an organism built upon the ability to survive prolonged periods of desiccation," says Battista.

The researchers are still struggling to prove that case. They unsuccessfully searched a Chilean desert for the bacterium, for example. Meanwhile, relatives of the microbe have recently been found in areas unlikely to suffer a lack of water, such as hot springs. Since heat induces double-strand DNA breaks, the springs might have also forced the bacteria to develop their survival skills, notes Battista.

While the evolutionary origin of *D. radiodurans*' repair system remains unresolved, so does the system's secret to success. Daly and Minton have studied two DNA-repair strategies employed by the bacterium.

Initially, it uses a process called single-strand annealing to reconnect some chromosome fragments. Its more crucial method, known as homologous recombination, uses a protein called RecA to patch double-strand breaks. After searching through the multiple copies of the genome that exist in each bacterium, RecA and associated proteins identify an intact copy of a DNA sequence that needs repair and uses that copy to mend and rejoin the broken strand.

Yet neither single-strand annealing nor homologous recombination is unique to *D. radiodurans*. "Those systems alone can't account for its radiation resistance," says Daly.

Nor does redundancy in the microbe's genome explain the phenomenon. Four to 10 copies of the *D. radiodurans* genome exist in each bacterium. These backup copies are crucial because they increase the odds that a mutated gene will have an undamaged counterpart. Battista's group has shown that when *D. radiodurans* grows under conditions that reduce its number of genome copies, the bacterium becomes more vulnerable to radiation. Nevertheless, other bacteria also keep extra copies of their genome.

"The fact that there are multiple copies of the genome is not in and of itself a sufficient explanation for why they're so radiation resistant. They have to have the capacity to use that redundant genetic information in a way that most organisms cannot," says Battista.

Daly and Minton have proposed that, to speed homologous recombination, the bacterium aligns copies of its genome so that identical DNA sequences are near each other. Since bacterial chromosomes usually come in circles, this theory invokes pictures of stacked loops of DNA, resembling a roll of hard candies, and so has earned the name the Life Saver hypothesis.

Battista finds this concept attractive and even has microscope images of the microbe's chromosomes that suggest there's some unusual organization among them. "The hypothesis is probably going to hold up. The Life Savers are there. We've got really pretty pictures," he says.

Battista also notes that when he irradiates normal strains of *D. radiodurans*, the Life Saver arrangement remains. Yet when he zaps mutant strains that can't repair DNA, the Life Savers vanish.

Workers in protective clothing tackle dangerous waste, some of it radioactive, at the Department of Energy's Hanford site.

C. Anderson/Spokesman-Review

Attempts to understand *D. radiodurans*' unusual talents should receive a huge boost early next year, when scientists plan to publish the organism's full DNA sequence. "Knowledge of the genome will drive a whole bunch of additional experiments to nail down the mechanism of radiation resistance," says Owen White of The Institute for Genomic Research in Rockville, Md., who led the sequencing effort. "After the paper comes out, I think a lot of people will be racing to the [laboratory] bench to test their pet theory."

White, for example, remains skeptical of the Life Saver hypothesis. He plans to test the importance of the one clear oddity of the genome revealed by the sequencing: a large number of repetitive DNA sequences strewn throughout the bacterium's genetic material. White and his colleagues have also discovered that the microbe's genes come packaged in four distinct circular chromosomes. Most bacteria have a single chromosome, he notes.

Otherwise, *D. radiodurans*' genome, smaller than *E. coli*'s (SN: 2/8/97, p. 84), at first glance doesn't seem noteworthy. Its array of DNA-repair genes contains no surprises, for example. "There's nothing unique about the set of genes in *D. radiodurans* that makes it stand out from other organisms," says White. As with previously sequenced bacterial genomes, however, researchers have yet to assign functions to many of the genes. In this case, as with the other genomes, scientists have pinned down the roles of only about two-thirds of the genes.

The genome's availability may help scientists apply *D. radiodurans* to bioremediation. This strategy of using bacteria that feed on or simply degrade dangerous compounds has always sounded appealingly easy, but scientists have struggled to fulfill bioremediation's promise. They have discovered microbes with an ability to metabolize one toxic substance, only to find that other compounds in a waste mix inhibit the bacterium's growth. "It's more complex than we originally thought," acknowledges Frazier.

Radioactive substances add another layer of complexity to DOE's bioremediation challenge. To date, the agency has disclosed about 3,000 sites contaminated by weapons production and development of nuclear reactors. Many contain a dangerous mix of wastes that include organic pollutants such as trichloroethylene (TCE) and toluene, radioactive elements such as uranium and plutonium, and an array of heavy metals. The cleanup cost using traditional methods, such as digging up the waste and burning it, could run in the hundreds of billions of dollars and take many decades, say government estimates.

"It's a huge environmental problem, a disaster, in fact," says Daly. "There's increasing pressure on the DOE and the Department of Defense, given that the Cold War is over, to start dealing with the legacy of that war."

Bioremediation offers a way to deal with the pollution potentially far more cheaply than the conventional methods do. Yet DOE sites pose a stiff challenge to the microbial approach. "The problem with all bioremediation organisms, whether they degrade a toxin or immobilize a metal, is that they are sensitive to radiation. In other words, if you're dealing with radioactive waste, all the standard bioremediation organisms are killed," explains Daly.

D. radiodurans may offer a solution. The government has long had an interest in the microbe. Ever since the United States dropped atomic bombs on Japan in World War II, federal scientists have conducted pioneering studies on DNA repair in this bacterium, notes Frazier. More recently, DOE has funded Daly and Minton, as well as a research group led by Mary E. Lidstrom of the University of Washington in Seattle, to explore *D. radiodurans*' bioremediation potential.

The first fruits of this effort appear in the October NATURE BIOTECHNOLOGY, where Daly and Minton, working with Lawrence P. Wackett and Cleston C. Lange of the University of Minnesota in St. Paul, report that the bacterium can grow and partially metabolize toluene or related compounds, even while subjected to constant irradiation of 6,000 rads per hour. Because the microbe doesn't normally find those organic pollutants tasty, the researchers had to import genes into *D. radiodurans* from a bacterium already known to degrade such compounds.

The researchers still hope to furnish the radiation-resistant bacteria with the genes needed to break down toluene fully. The carbon and other molecules that result can then generate more energy for microbial growth. The addition of genes required to metabolize other compounds, such as TCE, is also planned.

As part of the attempt to create a "superbug," the investigators are designing a microbe to attack the waste sites' radioactive elements and heavy metals as well. These substances are water soluble, and since the pits in which they're contained are increasingly riddled with cracks, they're leaking into the environment. "These metals are just racing through the soil and threatening groundwater supplies," says Daly.

Although no known bacterium can actually metabolize uranium or the other metals into harmless substances, some microbes do have genes encoding proteins that immobilize metals with which they come in contact. Equipping *D. radiodurans* with such genes could help stem any spread of the radioactive elements and other metals until other cleanup strategies are available.

As with past bioremediation efforts, however, it's unclear whether scientists know how to apply bacteria to a waste site in an effective manner. Most experiments so far have taken place in the ideal environs of a laboratory. Moreover, considering the outcry over past releases of genetically engineered plants and organisms, bioremediation investigators are cautious about predicting when *D. radiodurans* will be ready for a debut in the real world.

"Even if we produced a superbug tomorrow, it might be years before we were even allowed to test it in the field," notes Daly.

"I'm not optimistic about putting a genetically engineered, radiation-resistant bacterium into the ground," agrees Lidstrom. In fact, her concern about the public acceptance of such an approach has persuaded Lidstrom to take a different tack. She hopes to develop mobile above-ground treatment systems, still using genetically engineered *D. radiodurans*, that would process excavated contaminated soil.

If *D. radiodurans* isn't up to the task, other bacteria may step forward. While screening soil samples, says Battista, he and his colleagues have recently found a family of bacteria even more resistant to radiation. Time to call *The Guinness Book of World Records*. □