Assessing the Risks of Microbial Release

As genetically engineered microbes move into the field, risk assessment becomes a fact of life for biotechnology researchers

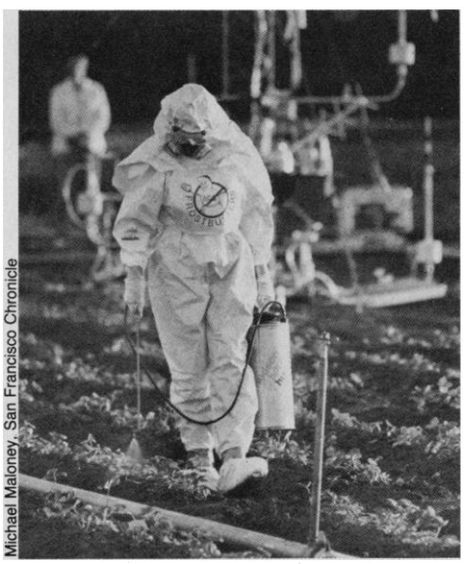
THE first authorized field trials of microbes that were produced by recombinant DNA technology began this spring, making 1987 a watershed year for biotechnology. In April, researchers in California launched the long-sought and oft-delayed field tests of genetically modified "ice-minus" bacteria that are supposed to prevent frost damage to crop plants. Additional requests for approval of studies that require the deliberate introduction of genetically engineered microorganisms into the environment are in the regulatory pipeline (see box on p. 1415). A technology once confined to the laboratory is now taking the first steps to what may eventually be large-scale application outside.

This prospect has given new spark to an issue that has dogged the 15-year history of recombinant DNA technology, especially with regard to its use in producing genetically altered microbes that contain genes from other species. Are such new microbial strains safe? Or might they prove dangerous in some way to man or other species?

No indication of hazard has emerged during the years of laboratory experiments with recombinant DNA, but the impending environmental release of recombinant microbes has raised concerns that the organisms might disrupt the ecosystem, perhaps displacing indigenous bacterial species or otherwise causing harm to plants or animals. Previous experiences with pests such as the gypsy moth and Africanized "killer" bee are frequently cited as examples of what can go wrong when new species are introduced into an environment.

As a result of these concerns, risk assessment has become a major component of the regulatory gauntlet through which a recombinant organism must pass before it can be approved for any application involving introduction into the environment. The U.S. Environmental Protection Agency (EPA) and Department of Agriculture have the primary regulatory authority over genetically engineered microbes intended for agricultural and other uses that will entail large-scale environmental release.

This responsibility is reflected by the EPA's budget for research on biotechnology risk assessment. It nearly tripled between fiscal year 1985 and 1986, going from $1.5 million to about $4.5 million. It will remain at about the $4.5-million level for fiscal year 1988. Underlying the stepped-up efforts at risk assessment, however, is the continuing controversy over the concerns about the proposed releases of genetically engineered microbes: are they justified or have they been blown out of proportion?



Michael Maloney, San Francisco Chronicle

Spraying with Frostban bacteria. *Researcher Julie Lindemann of Advanced Genetics Sciences is spraying young strawberry plants with bacteria that have been genetically altered so that they are no longer able to nucleate ice crystal formation. Although the protective garb she wears implies that the altered bacteria are hazardous to humans, they are safe. U.S. law requires that protective clothing be worn until the bacteria are approved for use as a pesticide. The apparatus in the background was installed by the EPA for monitoring weather conditions and the dispersal of the bacteria in air during administration to the small test plot.*

The current approach to determining if the introduction of a particular genetically engineered microbe into the environment constitutes a possible hazard focuses on finding the answers to five questions, largely as posed a few years ago by ecologist Martin Alexander of Cornell University.

- Will a released organism survive?
- Will it multiply?
- Will it spread beyond its original area of application?
- Can it transfer its genetic material to other organisms?
- And will the original organism or any of those that might pick up its genes prove harmful?

Although these questions may be common to risk assessment for all microbial releases, the means to answering them depend on understanding the individual characteristics and behavior of the microbe and of the engineered trait. Because both will vary according to the application in question, no sweeping generalizations can be made about the methods by which risks are assessed for genetically engineered microbes. "You know you are dealing with a broad scope and can't devise a method that will work for all. When they say 'case by case,' they mean just that," says Morris Levin of the EPA's Office of Research and Development, which has the responsibility of developing and refining the analytic techniques that will produce the data that will be used by the agency's regulatory offices to make a decision about a proposed release.

The first efforts at assessing the risks of releasing a genetically engineered microbe begin in the laboratory and also in the library, with what has already been reported in the literature about the microbe and the gene with which it has been engineered. Some observers think that genetically engineered organisms may even have a safety advantage over those acquired by more traditional methods precisely because the gene that will confer the new trait on the microbe has already been characterized. "When evaluating the engineered trait, you always know what it is," says James Tiedje of Michigan State University in East Lansing.

Often an organism with the same trait can be obtained simply by screening naturally occurring strains or by conventional mutagenesis in which the parent organism is submitted to a mutation-inducing agent, such as ultraviolet light, and the progeny are subsequently surveyed for the desired characteristic. The "ice-minus" bacteria, which are genetically engineered forms of *Pseudomonas syringae* or *P. fluorescens* bacteria, provide a case in point.

The parent organisms contribute to frost damage of plants by secreting a protein that acts as a nucleating center for ice crystal formation on leaves. The genetically engineered "ice-minus" strains were produced by using recombinant DNA methods to remove the gene that encodes the ice-nucle-

ating protein in hopes of producing bacteria that could grow on leaves in place of the parent strain and thereby prevent ice formation.

Ice-minus strains of the bacteria are also common in nature, however. According to a survey conducted by Susan Hirano of the Univeristy of Wisconsin in Madison, roughly half of the *P. syringae* strains collected from around the world lack the ability to nucleate ice formation. The genetic basis for this lack in the naturally occurring strains is unknown whereas it is crystal clear for those produced by recombinant DNA technology.

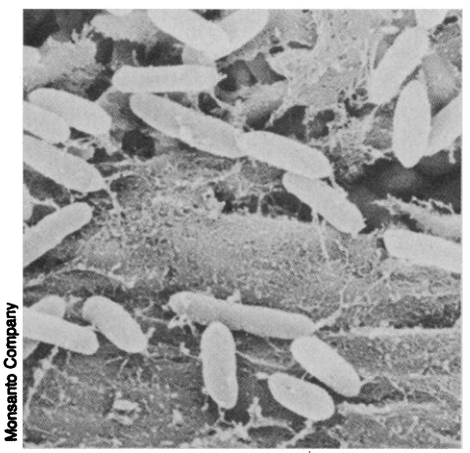
In an analogous vein, strains of *Rhizobium meliloti* with nitrogen-fixing capabilities similar to those of a genetically engineered microbe produced by Biotechnica International, Inc., of Cambridge, Massachusetts, might be obtainable by more conventional means but again would be less well characterized genetically than the recombinant organism. "If anything, the newer techniques should permit us to do things with greater safety," says Val Giddings of the Office of Technology Assessment, who describes himself as a "rabid environmentalist."

Not everyone would agree with this assessment. For example, Patrick Flanagan of the National Science Foundation points out that naturally occurring microbes occupy particular ecological niches where they are subject to natural controls.

The genes the organisms contain are also subject to careful regulation. In contrast, genes introduced by recombinant DNA technology are in effect out of their normal context, both in terms of their own control and the effects they might possibly have on the expression of other genes. "They [recombinant organisms] will only be as safe as the care taken in their preparation," Flanagan says. This care includes minimizing the possibilities of transfer of the newly introduced genes to other species and maintaining normal regulation of the gene.

Almost all genetically engineered microbes are designed to survive in the environment at least until they have done their job. For ice-minus or nitrogen-fixing bacteria, this would be for a growing season. A more complex, and perhaps more important, question concerns whether they can out-compete indigenous species, including their own parent strains, thus allowing the genetically engineered variety to persist and even displace other microorganisms. This issue can be addressed partly through laboratory or greenhouse experiments. Researchers are devising "microcosms," which aim to reproduce environmental conditions on a small scale indoors, to assess the interactions between a genetically engineered organism and other microbes. In the final analysis, however, Giddings notes, "The only way to resolve this [safety] issue is by field-testing."

Current field tests therefore require careful monitoring in and around the test sites to determine whether the released microbe multiplies or spreads. This depends on the availability of accurate methods for detecting and quantifying the organism in the environment, which is not necessarily an easy task, because the genetically engineered bacteria will usually have to be picked out from a high and varied microbial background that includes the parent organism.



Monsanto Company

A close-up of genetically altered *P. fluorescens*. *This scanning electron micrograph shows the rod-shaped bacterial cells on corn roots. A toxin gene from* B. thuringiensis, *an organism widely used as a biological insecticide, was introduced into the* P. fluorescens *cells in hopes of producing a new strain that could be used to combat insect pests that feed on plant roots.*

A classic way of doing this is to use a selective culture medium that only allows the growth of bacteria with a particular characteristic. This is the method used, for example, by Steven Lindow and his colleagues at the University of California at Berkeley to monitor the fate of the ice-minus strain of *P. syringae* that they are field-testing near Tulelake, California.

Potato plants were planted at the site at the end of April and sprayed with the bacteria about a month later. Since then, the Berkeley group, in collaboration with researchers from the EPA laboratory in Corvallis, Oregon, have performed monitoring operations that include assaying for the bacteria in soil, foliage, and insect samples taken from the site and the surrounding area.

According to Lindow, the modified bacteria can be identified both because of their nutritional requirements, which are characteristic of *P. syringae*, and because they carry a spontaneous mutation that makes them resistant to the antibiotic rifampicin. Naturally occurring strains do not grow in the presence of rifampicin.

So far there is no indication that the ice-minus bacteria have spread. "We have monitored the site for 14 weeks," Lindow says, "and there are no [ice-minus] bacteria outside the immediate area of the test plot." The treatment with the modified *P. syringae* apparently did reduce frost damage, however. The treated plants showed 80% less injury during two early-season frosts than the control plants.

Advanced Genetics Sciences, Inc. (AGS), of Oakland, California, has conducted a similar field-test of ice-minus bacteria on a small strawberry plot located in Contra Costa County, California. In this case the species modified included *P. fluorescens* as well as *P. syringae*, but the detection methods and the results were comparable to those at Tulelake. "We found throughout the experiment that the bacteria were not detectable beyond the test area," says Trevor Suslow of AGS. The strawberry plants were removed from the test plot at the beginning of July, and the bacteria, which largely associate with the plant matter, are no longer detectable even at the test site.

Although there were no frosts at the Contra Costa site, chilling experiments in the laboratory indicated that the plants treated with the modified bacteria were more resistant to frost damage than the controls. The difference was somewhat less than expected, Suslow notes, apparently because the foliage of the controls carried less than the usual amounts of the parental ice-nucleating bacteria and were therefore themselves less susceptible to frost damage.

The Berkeley and AGS groups relied on natural properties of the ice-minus bacteria as their selection targets, but another approach is to engineer selectable traits into the microbe. *Pseudomonas fluorescens*, for example, cannot normally grow on the sugar lactose. Peter Drahos, Bruce Hemming, and their colleagues at the Monsanto Company in St. Louis have introduced genes from the bacterium *Escherichia coli* into *P. fluorescens* that give it the ability to grow on lactose. The same genes also permit the bacteria to split a chemical analog of lactose called "X-gal," thereby producing a compound with a bright blue color.

The lactose-metabolizing genes make the altered *P. fluorescens* bacteria very easy to detect in soil samples. Soil bacteria are simply cultured in a selective medium that contains lactose as the sole energy source and X-gal as an indicator. Only bacteria that can grow on lactose will form colonies, which will be easily detected because of their

blue color. *Pseudomonas fluorescens* bacteria have a natural fluorescence under ultraviolet light that allows them to be readily distinguished from bacteria of other species that might have lactose-metabolizing capabilities. "The method is so efficient," Drahos says, "that you can find one in a gram of soil, which is several hundred times better than any other technique we tried."

The Monsanto workers plan to collaborate with researchers at Clemson University in Clemson, South Carolina, in a field test in which the genetically altered *P. fluorescens* strain will be released and then tracked. However, because the organism was produced by recombinant DNA technology, it too must undergo regulatory review by the EPA and the U.S. Department of Agriculture before it can be introduced into the environment. The scientific panel convened by the EPA to review the field trial proposal concluded on 28 August that the trial poses little risk and should be permitted to begin on schedule in November of this year.

Microbes In or Near Field-Testing

The researchers and regulators who are involved in determining the potential risks of introducing genetically engineered microbes into the environment often say that the organisms must be assessed on a "case by case" basis because of their diversity. A sampling of the genetically engineered microbes that are in or near field-testing illustrates this point. The species, the habitats, and the altered traits may all differ.

■ "Ice-minus" bacteria are either of the *Pseudomonas syringae* or *P. fluorescens* species. In bacteria of both species, recombinant DNA technology was used to delete a gene that encodes a protein that nucleates ice-crystal formation on foliage. The idea is to reduce frost damage in crops such as strawberries and potatoes by spraying the modified bacteria on the young plants and replacing the naturally occurring ice-nucleating bacteria. Small-scale field trials, conducted by researchers from the University of California at Berkeley and Advanced Genetics Sciences, Inc., in Oakland began this summer in two remote areas in California. Early results suggest that the ice-minus bacteria do provide some protection against frost damage.

■ *Rhizobium meliloti* is a species of nitrogen-fixing bacteria that associate with the roots of alfalfa plants. Researchers at Biotechnica International, Inc., in Cambridge, Massachusetts, used recombinant DNA methods to amplify one of the bacterial genes that is needed for the conversion of atmospheric nitrogen to ammonia. "The net effect," says Biotechnica's David Glass, "is increased nitrogen fixation." Although bacteria of the *Rhizobium* genus are not ordinarily subject to regulation, the recombinant DNA methodology employed in making this strain brought it under the regulatory umbrella. The company expects approval by the Environmental Protection Agency (EPA) of a field trial, Glass says, but not in time for this year's growing season.

■ Researchers at the Monsanto Company in St. Louis have introduced two genes from *Escherichia coli* into *P. fluorescens* bacteria. The transferred genes, which encode proteins for obtaining energy from the sugar lactose, serve as the basis of a sensitive method for monitoring the concentrations of the altered *P. fluorescens* bacteria in the soil. Monsanto has applied to the EPA for approval of a field trial of the bacterial monitoring procedure to be conducted by researchers from Clemson University at the university's Edisto Research and Education Center near Blackville, South Carolina.

■ Monsanto researchers have produced another modified strain of *P. fluorescens* by introducing into the bacteria the delta endotoxin gene of the bacterium *Bacillus thuringiensis*. Naturally occurring strains of *B. thuringiensis* bacteria infect and kill a variety of insect pests, including mosquitos and the leaf-munching larvae of the gypsy moth, and have found wide application as biological pest control agents.

The delta endotoxin, a protein in *B. thuringiensis* spores, helps to kill insects that eat the spores by damaging their intestinal tracts. The spores can be readily applied by spraying as a suspension but can now be used only to control insects that live above ground. By introducing the gene into the soil-dwelling *P. fluorescens*, the Monsanto workers hoped to create a bacterial strain for controlling insects that damage plants by nibbling on their roots.

Although Monsanto applied to the EPA for approval of a field trial of the altered *P. fluorescens*, the request was denied. The company produced the recombinant *P. fluorescens* strain that contains the *E. coli* lactose genes partly to answer questions raised during the regulatory review of the strain carrying the delta endotoxin gene.

Genetic manipulation of *B. thuringiensis* and its genes is generally one of the more active areas of biotechnology research. Investigators are variously trying to alter the host range of the bacteria or to improve the stability of the bacterial preparations, which currently must be applied frequently because they break down very rapidly in the field. The delta endotoxin gene has even been introduced into plants so that they carry their own built-in insecticide.

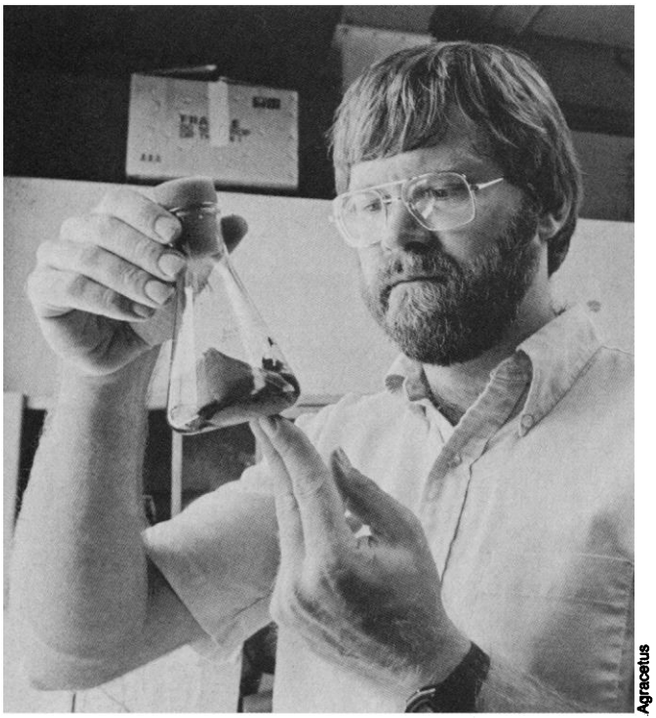
■ The development of new strains of bacteria that can degrade toxic chemicals is another very active area of biotechnology research. Although researchers are attempting to use recombinant DNA technology for producing such microbes, the conventional methods of doing this are the more highly developed (*Science*, 28 August, p. 975). No applications have yet been submitted to EPA for permits for testing detoxifying microbes in the environment.

■ Efforts at genetic manipulation are not limited to bacterial species. Researchers are also working on viruses, such as the baculoviruses, which are used for controlling insect pests, including the cotton bollworm and the Douglas fir tussock moth.

■ In addition, genetically altered fungi are under development. For example, David Sands of Montana State University in Bozeman has approval for small-scale field tests of mutants of *Sclerotinia sclerotiorum*, a plant pathogen that he hopes can be adapted for controlling spotted napweed in Montana.

And finally, David TeBeest of the University of Arkansas in Fayetteville is conducting an approved field test of a mutant of the fungus *Colletotrichum gloeosporioides* that makes it resistant to the fungicide benomyl. The parent fungus is licensed by the EPA for controlling weeds in rice fields, but treated fields cannot be sprayed with fungicides to combat other fungi that attack rice. TeBeest hopes to solve this problem by developing a fungicide-resistant strain. Both Sands and TeBeest produced the mutants they are testing by conventional mutagenesis. ■

J.L.M.



Steven Lindow

and his colleagues at the University of California at Berkeley are conducting field trials of the genetically altered "ice minus" bacteria in California.

Agracetus

Selection methods all depend on growing the target organism in laboratory conditions. Bacteria in the environment, however, may go into a dormant state in which they remain alive but lose the ability to grow in conventional laboratory culture conditions. As a result they could escape detection, even while remaining capable of multiplying under more appropriate conditions. Rita Colwell and her colleagues at the University of Maryland in College Park have found this to be the case for several species of water-borne pathogenic bacteria, including the one that causes cholera. If genetically engineered bacteria go into a similar state of dormancy when released into the environment, conventional selection methods might well underestimate their concentrations or fail to find them altogether.

Consequently, researchers are working on ways of detecting bacteria in the environment that do not depend on an organism's ability to grow. These include introducing the *lux* gene, which encodes a light-generating enzyme, into the genetically engineered bacteria. The *lux* gene comes from bacteria that associate with fish and would be a good marker for bacteria sprayed on foliage or introduced into the soil. Bacteria containing the gene can be detected because they would light up when exposed to the substrate for the enzyme.

Other methods depend on probes that react with specific cellular components of the target species. Such probes include fluorescent antibodies. Colwell's group, for example, has a fluorescent antibody that specifically binds to a *Vibrio cholerae* strain.

Because antibodies react with dead and living cells, the Maryland workers incubate the cells from a water sample in a growth medium that causes living cells to grow, but inhibits their division, before staining with the antibody. The living cells therefore have an elongated appearance that allows them to be distinguished from dead ones in a microscopic examination.

Another molecular method for detecting genetically engineered bacteria, which was developed by Tiedje's and Barry Chelm's groups at Michigan State, uses DNA probes to identify a particular gene sequence. The foreign gene carried by a genetically engineered microbe is the obvious target for such a probe. Bacterial species can also be identified on the basis of their ribosomal RNA "fingerprints," which are characteristic for each species, and researchers are working to adapt this procedure to the bacteria in environmental samples.

At present, selection methods tend to have an advantage in sensitivity, while the molecular detection methods tend to have an advantage in specificity. However, this situation should soon change, according to Hap Pritchard of the EPA's laboratory in Gulf Breeze, Florida. "Eventually the molecular approaches will be the best we have because of their specificity," he predicts. "They are getting as sensitive as the selection methods."

No matter how sensitive the methods are, however, they will never be able to rule out the possibility that a few genetically engineered microbes have survived in the environment. This may not matter. At such low concentrations they would not seem to be in danger of displacing other species. "If the organism is not detectable by our current methods, most people would say there is no problem," Alexander says.

Most indigenous microbial communities are already complex, especially in the soil. "When it comes to the soil," explains Larry Moore of Oregon State University in Corvallis, "there is a tremendous array of competing microbes that impact on the target organism. If the population of genetically altered microbes declines to a low level, the possibility of its mushrooming is very low."

There is a caveat, however, in the event that environmental conditions change in some fashion to favor the growth of the genetically engineered microbe. The emergence of antibiotic-resistant strains of pathogenic bacteria, for example, resulted from an environmental change—the widespread use of antibiotics in human and veterinary medicine. No genetic engineering figured in the emergence of the resistant bacteria. Instead, the dissemination of antibiotics in the environment provided a selective pressure that favored the spread and survival of preexisting genes for antibiotic resistance.

That bacteria can exchange genetic material with one another has been known for decades. The spread of antibiotic resistance is one indication that such exchanges occur in nature, although a great deal less is known about gene transfers among bacteria in environmental conditions than in the laboratory.

Because the transfers provide a possible route by which genes from a genetically engineered microbe might persist and spread in the environment even though the organism itself fails to survive, studies of bacterial gene exchanges are an important aspect of risk assessment. The early results indicate that the transfers occur among bacteria both in soil and in water.

Robert Miller of the Stritch School of Medicine (Loyola University of Chicago) in Maywood, Illinois, Gary Sayler of the University of Tennessee in Knoxville, and their colleagues have been studying genetic exchanges between bacteria in lake water. The researchers fill watertight Teflon bags with water from Fort Loudon Lake in Tennessee that has its own indigenous microbial population.

They then introduce the donor and recipient bacteria, neither of which have been recombinant DNA products, into the bags, which are closed and suspended in the lake waters. "We tried to be as close as possible to what happens in the lake," Miller says. Although the populations of the introduced bacteria decline, probably as a result of predation or competition from indigenous

organisms, gene transfers occur.

Genes carried on chromosomes are thought to be less subject to transfer than those on plasmids, which are circular, extrachromosomal DNA molecules. For this reason, researchers usually insert foreign genes into the chromosomes of bacteria, especially when the gene comes from an unrelated organism. The Miller group finds, however, that both chromosomal and plasmid genes can be transferred between bacteria.

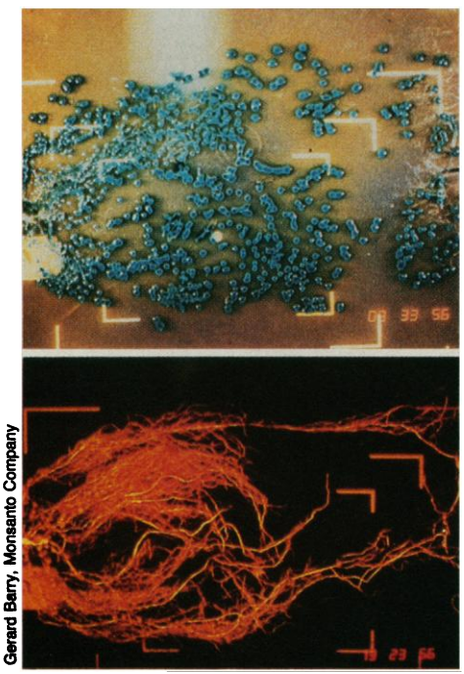
Similar results with soil bacteria come from Guenther Stotzky of New York University in Manhattan and his colleagues, who have carried out experiments in which recombinant bacteria were introduced into soil samples in a laboratory test-tube system. "The point is that they do survive and do in some cases transfer their genetic information to other species," Stotzky says.

He nonetheless notes that soil conditions, such as *p*H and nutrient supplies, have to be just right for transfer to occur. "The potential is there," Stotzky explains, "but we don't know if there is any impact."

Field trials are already being carried out in England, France, and Germany to determine whether conditions in nature will produce bacterial gene exchanges at significant frequencies. At the Rothamsted Experimental Station in Harpenden, England, for example, a test plot of pea plants has been inoculated with a nonrecombinant strain of *Rhizobium leguminosarum* that carries marker genes both on the bacterial chromosome and on a plasmid. According to John Spokes of Rothamsted, from 1 to 10% of the nitrogen-fixing nodules on the pea roots contain the test bacterial strain. Experiments to determine if it transferred genes to other bacteria are still in progress.

The last risk assessment question is currently the most difficult to answer. Will genetically engineered microbes or the genes they contain, and might transmit to other species, harm the environment? "Measuring the effects is the biggest gap in the whole area of determining whether there is a problem," Alexander says. The problem is not so much with determining whether there is injury to humans or to crops, he continues. "We know how to do that." Much less is known, however, about evaluating possible hazards to other species, especially indigenous microbial species, in a complex and changeable environment.

Although the conclusion is not uniformly accepted, many observers discount the idea that organisms produced by recombinant DNA technology pose a hazard to the environment simply because of their method of production. A recent report from the National Academy of Sciences came down firmly in favor of the proposition that the risks associated with the release of recombinant organisms are the same as those associated with releases of unmodified organisms or organisms modified by conventional techniques (*Science*, 21 August, p. 840).



Gerard Barry, Monsanto Company

Bacteria that turn blue. *The lower view shows plant roots that are covered with* P. fluorescens *bacteria that have been genetically altered by the addition of two* E. coli *genes. (The roots appear bright orange because the color photography intensified the orange color of the culture medium in which they were bathed.) The upper view shows colonies of* P. fluorescens *bacteria that were cultured from the roots. The* E. coli *genes enable the altered bacteria to use the sugar lactose as an energy source and also to break down a chemical that releases a bright blue compound. According to Monsanto researchers, the transferred genes provide the basis of a sensitive selection method for monitoring the presence of genetically engineered bacteria in soil.*

The report cites experience with nitrogen-fixing *Rhizobium* bacteria, which have been used in agriculture for nearly 100 years. "To our knowledge, their widespread use has not resulted in detectable adverse effects on the microbial balance in the diverse soils into which they have been introduced . . .," the report concludes. In a similar vein, Winston Brill of Agracetus in Middleton, Wisconsin, says, "I have been looking for examples of problems with putting microbes into the environment and have found no serious problems."

A final issue, and one that may still raise some concerns, is scale of usage. Even organisms considered safe when applied in small-scale field trials, such those currently planned or in progress, might conceivably pose an environmental risk if applied to very large areas. The ice-minus bacteria, produced as they were by deleting a gene from a bacterial species that occurs widely in nature, would be unlikely to pose a direct threat to other species.

The main environmental issue about these bacteria concerns the possibility that their widespread use could alter rainfall patterns, although Christen Upper of the University of Wisconsin in Madison notes that this is a "soft conclusion based on several assumptions, none of which have been proved."

The assumptions are that the ice-nucleating bacteria found on plants produce all or most of the highly efficient ice nuclei of clouds; that the ice-nucleating bacteria contribute to the formation of the cloud nuclei in proportion to the bacterial presence on plants; and that the ice-minus bacteria displace ice-nucleating bacteria over very large acreages of landscape. According to Upper, displacement would have to occur over 250 to 500 contiguous acres for very minimal effects lasting for perhaps seconds.

Another possibility, mentioned by Tiedje, is that genetically altered *Rhizobium* bacteria that yield increased amounts of fixed nitrogen might contribute to nitrate pollution in ground waters if their use became widespread. One noteworthy aspect of these examples is that neither case of possible risk would, if it arises, be the result of the recombinant origins of the bacteria. It would instead result from their effects in the environment, which in both cases might well be achievable in other ways, such as by selecting for naturally occurring bacteria with similar characteristics.

The controversy over the hazard potential of genetically engineered microbes is unlikely to dissipate in the near future. The need for doing risk assessment studies of organisms intended for environmental release is therefore going to remain, too. But that does not mean that the work will be wasted even if further experience proves that genetically engineered microbes are safe. "I'm convinced that the research needed to support risk assessment is the same as that needed to develop successful products," Tiedje concludes. ■ JEAN L. MARX

ADDITIONAL READING

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