USE OF MICROORGANISMS FOR CROP AGRICULTURE

Since the turn of the century there have been many research programs, worldwide, attempting to develop cultures of microorganisms useful for crop agriculture. However, there are relatively few examples of such inoculants being used on the farm. In comparison, pure cultures of many types of microorganisms have been very important to the pharmaceutical and food industries, which continually genetically alter strains to improve them. Of course, these latter industries grow microorganisms under controlled conditions such as a fermenter for antibiotic production or temperature-controlled milk for yogurt or cheese. In contrast, microbial inoculants to be used by a farmer have to exert their positive effect under tremendously variable field conditions, such as weather, soil type, plant variety and field history.

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In the first decade of the 1900s, farmers in Europe and U.S. became very interested in a recently discovered bacterium, called *Rhizobium,* that dramatically increased yields of legumes such as soybean, bean, pea and alfalfa. These bacteria form nodules on legume roots and convert nitrogen gas from the air to ammonia, which is used by the plant. Thus, such inoculated plants no longer require addition of nitrogenous fertilizers, such as nitrate, to obtain high yields. Through this practice, nitrogen was added to the soil in a manner that prevented it from polluting bodies of water, through run-off. Many commercial *Rhizobium* inoculants have been marketed since then. Thousands of field tests have been performed worldwide in order to determine which *Rhizobium* strains are the best for a particular plant variety in a specific growing region. These tests were performed in university, government and commercial settings.

Success with *Rhizobium* stimulated laboratories to search for other types of microorganisms with the potential to aid agriculture. In the 1920s

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and 1930s, the literature had hundreds of examples, from a wide variety of microbial species, that seemed to stimulate growth of a plant or protect the plant from pests such as insects or fungi. This type of work has continued to the present. However, only a few strains are currently marketed. *Bacillus thuringiensis* is the most commonly used example.

Many results describing potentially useful microorganisms were either inconsistent or they were not reproduced by other researchers. In the U.S.S.R. during the 1950s about 25 million acres were inoculated with bacteria such as *Azotobacter chroococcum* and *Bacillus megaterium* for a wide range of crops, including potato and wheat. While the popularity of these inoculants has substantially decreased, papers continue to be published on limited demonstrations of efficacy. Many of these reports come from credible and sophisticated laboratories. So it seems that certain microorganisms actually do stimulate crop yield and/or pest antagonism. However, parameters that influence the effectiveness of the microorganism are not understood, or are not controllable, and yield increases usually are quite sporadic.

A major hurdle to overcome for developing useful inoculants is that the microorganism usually does not persist in high concentrations for a sufficient length of time to affect the plant in a positive manner. That is why much of the research focuses on the germination stage of the plant. To influence germinating seeds, (e.g., through microbe-produced plant-growth hormones, or microbes which inhibit fungi that cause seedling damping-off diseases), it is relatively easy to apply high numbers of the inoculant to the seed. High populations of the microorganism can be added directly to the seed coat at the time of planting. However, as the plant develops, the number of inoculant microorganisms in contact with the plant dramatically decreases, and the inoculant rapidly loses its effectiveness. To overcome this problem, researchers are looking for strains that bind to the plant (e.g., to roots) and may, therefore, multiply during plant development. So far, this has not been successful; thus, beneficial effects are transitory—usually occurring shortly after plant or soil inoculation.

With the excitement about biotechnology in the late 1970s and early 1980s, interest in microbial inoculants was stimulated. There seemed to be tremendous potential to develop new types of agriculturally useful products through these modern technologies. It is relatively easy to isolate

genes of interest, such as those that code for pest antagonists, those that produce plant growth hormones or those that degrade unwanted organic chemicals. It is quite easy to add genes to most microorganisms. Also, it seemed that it should be relatively easy to continually improve products through genetic alterations—as was the experience in the food and pharmaceutical industries. Microorganisms have the potential to be more environmentally compatible than many chemicals used in agriculture. Inoculant practices may play an important role for sustainable agriculture. These incentives induced some large chemical, agricultural and pharmaceutical companies to initiate inoculant research programs. A number of small start-up companies also focused on this area. In the past 15 years, there have been many examples of significant and reproducible plant growth stimulation, yield increase or pest inhibition in greenhouse and growth-chamber studies. However, most of these companies have now completely eliminated these programs. What happened? Promising results were not observed from initial field trials.

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Many of these projects were terminated prematurely. Most of the scientists working on these programs did not keep the complexity of the field in mind during all stages of the project. Thus, excitement from greenhouse or growth-chamber results was frequently dampened when the organism was field tested. Agronomists with extensive field experience know that greenhouse and growth-chamber data most commonly do not relate to what occurs in the field, with all of its variability and complexity.

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Field tests are essential from the earliest stages of a program to develop microbial inoculants. If this is ignored, then there is a great chance that laboratory work will be a waste of effort. Steps in a research project, such as optimizing the growth medium, genetically altering the strains and formulating the microorganism, all should be analyzed in the field. The simple activity of isolating strains can induce unwanted mutations that could keep the microorganism from being effective. The organism may behave like the parent strain in the laboratory, but in the soil, for example, it may be hypersensitive to dry conditions that may be faced in the field. Mere scale-up of a growth medium from small flasks to larger vessels may render the microorganism physiologically inactive for its beneficial property. Experience in the pharmaceutical industry has demonstrated that problem on many occasions. Thus, extensive field tests are essential at each

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step in developing a microbial inoculant. In fact, data usually become meaningful only when field tests are performed at several sites and sufficient replicates permit useful statistical analyses.

There may also be opportunities to increase crop yield by specifically breeding plants for enhanced effectiveness of the inoculant. This has been demonstrated with the legume-*Rhizobium* partnership as well as other experimental inoculants. Modern breeding may have removed genes important for maximizing the plant-microorganism association. Of course, extensive field trials are the only way to optimize plants for inoculants.

Unfortunately, the current regulatory situation, for field tests with genetically altered organisms, has resulted in a disincentive for university, government and industrial researchers to pursue microbial inoculants. With these new regulations and guidelines, organisms modified by traditional genetic methods are to be included with organisms modified by recombinant DNA methods, since it seems to be agreed by both researchers and regulators that a recombinant organism *per se* should be no more dangerous than the same organism modified by older, less precise, methods. An outdoor test of even one square foot must be scrutinized in enormous detail before permission is granted. How can such a research project be pursued if efforts to secure required (or recommended) data and documentation to satisfy regulatory agencies, for even the smallest field test, costs several hundred thousand dollars? Note that almost all microbial field tests, so far, have been sponsored by corporations. These companies now have become more wary of greenhouse or growth-chamber results. So, it will be even more difficult for university scientists to find a sponsor for an early field test to try out an idea. Meager federal research grants cannot support work to satisfy regulators.

Many investigators now realize the importance of field tests at early stages of an inoculant program, but very few can handle the regulatory burden of a research program that allows, for example, an interesting microorganism developed through modern or traditional genetic techniques, to be field tested in different types of fields at the earliest stages of the program. Thus, a research area with high potential to help agriculture and the environment has been considerably slowed.

Certainly, research that has a reasonable chance to damage health or the environment should be tightly regulated. It seems that current regulations

and guidelines assume that genetically altered microorganisms have reasonable potential to be harmful. This does not make sense—based on a century's worth of extensive experience with field tests of wild-type and genetically altered microorganisms. The most sophisticated technique, genetic engineering, adds a characterized gene to the microorganism. Other techniques, such as mutation or plasmid transfer are less predictable than genetic engineering, as far as the properties of the microorganism are concerned. It is well known that mere isolation of a microorganism from the soil will add uncharacterized mutations; thus, each wild-type microorganism (possibly thousands of strains) field tested since the turn of the century had "uncharacterized genetic mutations." We have yet to hear of a single health or environmental problem resulting from this type of research. Previously, many microorganisms with laboratory-directed mutations or with genes added by natural plasmid transfer have been field tested without any reported untoward effects—or regulatory concerns.

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Compare the difference between adding a genetically altered microorganism to a field versus adding an experimental chemical to a field. When a chemical pesticide or fertilizer is added to soils, it is known that certain mutations and gene transfers by indigenous microorganisms are greatly enriched. Most of these microorganisms and/or their genetic alterations are "uncharacterized." Ecological experiments continually demonstrate natural gene transfers between different genera in soils and bodies of water. So, microbes with genetic changes in chemically treated fields most probably transfer their altered genes to different genera and species. We know of no health or environmental problem that has occurred from these uncharacterized organisms with uncharacterized genetic changes.

Evolutionary principles govern microbial populations and persistence. While microbes added to a field rapidly decrease in numbers, some chemicals persist for a long time. In comparison with agricultural microbial research, agricultural chemical research routinely involves small field tests without regulatory scrutiny. As a chemical exhibits applied promise, after analysis of many small field tests, then regulatory approval is necessary to advance to large field tests and possible commercialization. So, the chemist's initial field experiments are unhampered by regulators, while the microbiologist's initial field experiments require extensive regulatory

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scrutiny. Both types of tests cause "new" microorganisms to develop. It is assured that these microorganisms will be transported out of the field by such factors as the wind and insect movement.

Some commercial inoculants may be ineffective with certain future crop varieties. Also, there may be circumstances in which an inoculant product actually decreases yield. Such situations do not cause environmental or health problems. The commercial value of the product merely decreases. These types of problems have been, and continue to be, found with some commercial agricultural chemicals.

The chance of experiments, aimed to help agriculture, unintentionally converting a harmless microorganism to one that damages health or the environment seems to be exceedingly small. The chance that current regulations and guidelines will detect this very rare event also seems to be exceedingly small.

Microbial inoculants have potential to increase crop yield without damaging the environment. If regulations would be based on scientific knowledge and would consider our extensive experience with genetically altered microorganisms, we may be able to advance the microbial inoculant research area and make concerted efforts to solve some important agricultural and environmental problems. However, current regulations and guidelines strongly inhibit advancement, while not really protecting our health and environment. Hopefully, agencies will eventually design regulations appropriate for research and commercialization. A balance must be made between protecting the public from problems, and helping the public benefit from potentially desirable agricultural practices. Unfortunately, we have seen little progress towards that balance.