Biotechnology and Soil Microbiology

James E. Struble and **Berdell R. Funke**

Microorganisms are an integral and important part of agriculture. They play a very important role in terms of plant and animal growth, production, harvest, and final use. Microorganisms are very important in plant production. Their essential role in nutrient cycling in the soil cannot be overemphasized. They are essential to the nitrogen, carbon and sulfur cycles. Nitrogen fixation in leguminous plants is also a very important aspect in agriculture.

In animal production, microorganisms are important factors in digestion of feedstuff in ruminant animals. They are also involved in animal diseases. They carry out the fermentation processes that lead to cheese, sausage, wine, and alcohol.

Naturally occurring microorganisms are being used for pest control, pesticide degradation, cleaning up toxic waste sites, leaching of ores, and enhanced oil recovery. In many of these cases, the native microorganisms are not very efficient. At this point biotechnology and engineered microorganisms come into the picture. Efforts aimed at developing new microbes for the farm, factory, mine, or toxic waste dump are simply trying to enhance the effectiveness of microbes already in use.

In one sense, biotechnology is not a new science. There has been a long history of selection, accidental or otherwise, of more efficient microbes or conditions that improve their production. The earliest deliberate attempts to select for more efficient microbes were in the brewing industry where the famous Carlsberg brewery in Denmark many years ago established a laboratory dedicated to research on brewing yeasts.

Agricultural products were also the basis for a number of very important microbial fermentations to produce chemicals such as butanol and acetone. These were extremely important during the First World War as a basis for explosives and aircraft fabric dope. Most fermentations of this type were replaced by synthesis based on petroleum, but they still are available if the price of petroleum feedstocks continues to increase. Eventually, they may become a significant outlet for agricultural production. All of us are familiar with fuel ethanol production, there is even a large plant in North Dakota. Whatever the short term prospects, in the long view the earth will have to eventually depend upon renewable fuel supplies.

Farmers are generally familiar with one application of commercial bacteria production for agriculture, the use of *Rhizobium* bacteria for inoculation of legumes such as soybeans and leguminous forage crops. These bacteria have been especially selected for their ability to effectively nodulate plants and to fix nitrogen from the atmosphere. They are commercially available in several forms. Many years ago, before these bacteria were routinely available from commercial sources, the Department of Bacteriology produced cultures for use in North Dakota. Recently, there has been intense interest in genetic manipulation of these bacteria in order to further increase their ability to infect the plant roots in competition with the rhizobia bacteria native to the soil.

Increased efforts in biotechnology and genetic manipulations are leading to the development of many novel systems/products. Examples of possible biotechnology applications include the use of microbial pesticides, sewage treatment capabilities, detoxification of chemical wastes and spills, water quality management, enhanced nitrogen fixation (symbiotic and asymbiotic), crop protection from frost, enhanced recovery of oil, microbial mining of metals, and possible insertion of bacterial material into plants (3,5). Many researchers are using biotechnological techniques in attempts to develop or alter microorganisms for the benefit of agriculture.

Some examples of biotechnology and microorganisms are (1):

- Bacteria engineered to help protect plants from frost damage.
- Bacteria engineered to enhance their nitrogen-fixing capabilities and thereby aid the growth of legumes.
- Modification of microorganisms for use in the biological control of some insects, weeds, and diseases.
- Improved strains of organisms to convert plant materials and biological wastes into better feed, or into seedstock for the manufacture of chemicals or the production of essential nutrients such as vitamins and amino acids.
- Development of improved strains of bacteria for toxic chemical degradation.

The following sections will deal with some of the specific types of research that are presently being conducted with soil microorganisms.

Much publicity has surrounded the development and release of the bacteria engineered to help protect plants from frost damage (6,7). Presently research has developed strains of ice-minus *Pseudomonas syringae* and *Erwinia herbicola* that lack the ability of parent microorganism to promote the formation of ice crystals in supercooled water. Ice

Struble is assistant professor and Funke is professor, Department of Microbiology.

nucleation activity of the parent microorganism is responsible for a substantial amount of frost damage done to crops by temperatures in the range of 24 degrees to 28 degrees Fahrenheit.

Numerous microbial toxins against insects and weeds are already known; many bacterial pesticides, including several brands of *Bacillus thuringiensis*, have been marketed since the 1960s (Table 1). The limitations are that in their natural forms they are short lived and slow to kill. The use of biotechnological techniques in transferring genetic material from one species to another may be able to improve this situation. If one could insert genetic material into an indigenous microorganism it might be longer lived and prove better for control.

Several researchers have been successful in inserting the gene for toxin production from *B. thuringiensis* into other soil microorganisms. A group at Monsanto Chemical Co. had produced a strain of *Pseudomonas* that was able to produce the *B. thuringiensis* toxin. They had applied to the EPA for field testing but were denied. In response they engineered a strain of *Pseudomonas* to produce enzymes that enabled it to digest lactose and X-Gal (a dye that changes color from clear to blue when digested) (4). This alteration allowed them to track the organism, since other soil organisms do not have the ability to digest these compounds.

Biotechnology gives us the tools to speed up the process of genetic exchange or rearrangement. In some cases researchers are using conventional mutagenesis to develop microorganisms for specific purposes.

Using conventional mutagenesis selection techniques, Dr. David Sands at Montana State University is attempting to adapt the fungus *Sclerotinia sclerotiorum*, a plant pathogen, to control spotted knapweed in Montana (2).

Colletotrichum gloeosporioides is a fungus that is registered for controlling weeds in rice. However, fields treated with this fungus cannot be sprayed with fungicides to control other fungi without killing *C. gloeosporioides*. David TeBeest at the University of Arkansas has found a mutant of *C. gloeosporioides* that is resistant to the fungicide benomyl (2). The use of a mutant to benomyl would allow rice farmers to control weeds with *C. gloeosporioides* and spray the fields to control other fungi.

Table 1. Microbial Agents Registered in the United States.

Name of Microbial Agent	Used to Control
Bacillus thuringiensis **(B)***	Lepitodopterous larvae
Bacillus popilliae and *Bacillus lentimorbus* **(B)**	Japanese Beetle larvae on turf
Heliothis NPV (inclusion bodies) **(V)**	Cotton bollworm and cotton budworm
Douglas fir tussock moth NPV (inclusion bodies) **(V)**	Tussock moth on Douglas fir
Gypsy moth NPV (inclusion bodies) **(V)**	Gypsy moth
Nosema locustae **(P)**	Rangeland grasshoppers
Hirsutella thompsoni	Citrus mites
B. thuringiensis var. *israeliensis* **(B)**	Mosquitoes
B. thuringiensis var. *aizawai* **(B)**	Wax moth larvae in honeycombs
Phytophthora palmivora **(F)**	Citrus strangler vine
Colletotrichum gloeosporioides **(F)**	Northern Joint Vetch
Neodiprion sertifer NPV	Pine sawfly

* B = bacteria, V = virus, F = fungus, P = protozoa

One of the most interesting applications of bacteria to agriculture, and one with perhaps the most far-reaching potential, is the use of the crown gall pathogen, *Agrobacterium tumefaciens*. This soil microorganism infects plants, causing a tumor-like growth of plant cells on the plant stem called crown gall disease. Research has shown that this cell proliferation is due to a transfer of genetic information carried in a plasmid in the bacteria (plasmids are small pieces of DNA carried outside of the main chromosome of the bacteria). This genetic information is inserted into the genetic material of the plant. This then represents a model for a mechanism for inserting new information into plants.

The initial interest was in the possibility of converting plants such as wheat into nitrogen fixers. The plant gall is morphologically similar to a root nodule, and *A. tumefaciens* is in many ways similar to the *Rhizobium* group of bacteria that nodulate legumes. It appears now that the technology to accomplish this is still many years away. However, there have been many demonstrations that useful attributes can be transferred from bacteria to plants.

Many of these attributes were naturally in bacteria other than *A. tumefaciens* and were transferred to it by genetic engineering. As an example, the glyphosate group of herbicides kill plants by suppressing amino acid synthesis that the plant required for growth. Genetic information from *Salmonella* bacteria has been transferred to tobacco plants, where it stimulates the production of certain amino acids--which neutralizes the effect of the herbicide. The advantage is that a useful plant can be made immune to the effects of a herbicide that can then be applied to surrounding weeds without adversely affecting the desired crop. So far, genetic modifications of soybeans, tomato, oil seed rape, cotton, and flax have been made for one trait or another.

In the past decade, the development of techniques used in genetic engineering has advanced greatly. However, among limitations to the use of many of these discoveries are the concerns about fates of genetically modified organisms in natural ecosystems (whether it be animals, soils, or water). Little is known about the fate of genetically engineered microorganisms (GEMs) in natural ecosystems.

In agriculture, the soil ecosystem is most significant. Many of these modified 'microorganisms' are intended for release onto or into soils. Most of these "new microorganisms" are natural inhabitants of the soil that have been modified to perform some specific function, so no complications are expected. However, we do not have enough information to predict if a genetic modification might increase or decrease survivability, if genetic material might be exchanged with other microorganisms, or if other effects might occur. None of the biotechnological benefits may be realized until testing for environmental impacts regarding the release of each microorganism is completed. In several articles pertaining to the release and risk assessment of GEMs, Martin Alexander (6,7) has posed the following questions:

1. Will a released organism survive?
2. Will it multiply?
3. Will it spread beyond its original area of application?

4. Can it transfer its genetic material to other organisms?
5. And will the original organism or any of those that might pick up its genes prove harmful?

Before we can apply molecular techniques to the study of microbial ecology we need to have a better understanding of the microbial diversity in a given situation. Research is presently underway to understand differences in microbial populations as affected by cropping and tillage systems in North Dakota.

Some of the preliminary data are given in Table 2. The majority of the population data only yield numbers of microorganisms and not the diversity. It is very hard to take a large mixed population and break it down into the genus or specie level. The use of molecular techniques such as DNA-DNA hybridization may allow us to look for a specific group of microorganisms based on DNA homology.

Another technique that is gaining some use is DNA homology. This entails purifying microbial DNA from samples, then treating the DNA to form single stranded DNA. The single stranded DNA is then allowed to combine to form double stranded DNA. The rate at which the DNA anneals is an indicator of the diversity of the population.

Many of us remember the return of astronauts from the moon a decade ago or so ago was accompanied by serious concerns about introduction of strange organisms into the earth's ecosystem. Astronauts were confined in isolation chambers for a period of time. These concerns were probably exaggerated, considering what was known of conditions on the moon. Similarly, many of us have seen photographs of a technician spraying genetically engineered bacteria onto a field while garbed head to toe in a "moonsuit" equipped with a breathing apparatus. Few saw later photographs of the same technician spraying the same organisms at a later date more sensibly attired in jeans and blouse, breathing normally. It had been concluded, finally, that there was little disease-causing potential in a common soil microorganism that had been modified slightly to encourage ice formation on plant leaves.

However, this example illustrates the fact that there will always be tight controls--whether needed or not--over the release of genetically engineered organisms. The potential benefits of such engineering are so apparent that we can expect to see more and more microbial modifications and benefits from their impact on agriculture.

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Table 2. Microbial populations as affected by tillage systems.

Tillage	Fungi 10^4	Bacteria 10^6	Actinomycetes 10^6	Denitrifiers 10^6	Nitrosomonas 10^2	Nitrobacter 10^5
Minot						
NT*	9.9	34.7	4.1	15.1	7.6	7.6
SP	6.8	44.3	6.1	3.8	14.8	1.1
ST	9.2	43.7	3.4	2.4	3.5	2.7
Williston						
NT	19.0	20.7	0.9	0.4	4.6	5.6
SP	12.5	11.0	0.9	0.3	0.8	1.8
ST	16.7	7.5	0.7	0.3	8.1	0.9

Population values are for samples taken from 0-3 inch depth.
* NT = No till, SP = Spring plow, ST = Sweep till