A primer on microbial products, bacteria and their relationship with nitrogen

By Monica L. Elliott, Ph.D.

An increasing number of microbial-based products claim to increase plant growth or protect plants from various pests. Products that claim to directly control plant pests are referred to as biological pesticides or biopesticides. These products are regulated by the Biopesticides and Pollution Prevention Division of the U.S. Environmental Protection Agency (EPA).

There are three types of biopesticides — biochemical, plant and microbial. The latter contain a naturally occurring or genetically altered microorganism or its product as the active ingredient. For more detailed information about biopesticides in general or about specific products, please refer to the EPA Web site at *www.epa.gov/pesticides/biopesticides*. Every registered "active ingredient" is listed on this site. As with chemical pesticides, biopesticides may be formulated in a number of different ways, and so any single registered "active ingredient" may have numerous product trade names.

If microbial products only claim to improve plant health in general, without mentioning direct control of specific pests, the product does not have to be registered by EPA. This group of products is often referred to as "inoculants." Root-associated (rhizosphere) bacteria that benefit plant growth are called plant-growth-promoting rhizobacteria (PGPR). However, that term can be misleading. For example, PGPR that promote the growth of one plant species may be detrimental to another plant species. In addition, the PGPR are only beneficial to plants under specific environmental situations, such as high disease pressure or low nutrient levels.

Some products contain only a single microorganism, whereas others contain a mixture of microorganisms. The latter approach is probably more useful because at least one of the microbes in the mixture may benefit the targeted host plant. Many microbial products claim to reduce fertilizer use because they include bacteria that fix nitrogen nonsymbiotically or liberate phosphates and micronutrients from the soil.

As with chemical products, microbial products are formulated with various carrier compounds, both inorganic and organic. Carrier compounds may include small amounts of plant nutrients (N, P, K), sugars, amino acids, plant hormones — compounds that may also affect plant growth. Because evaluation of microbial-inoculant products normally includes only plant growth responses, without examining the microbial responses, it is difficult to determine if the plant responses observed may result from the microbes added.

Why are there so many microbial products available today? First, in most cases, the only data that is published in refereed scientific journals is positive data — experiments in which a positive response was observed. Even then, the data is associated with only one microbe or a mixture containing less than five microbes. Unfortunately, negative data is seldom published. Thus, it does appear that wondrous things happen when specific bacteria are applied in a specific manner in a specific environmental situation.

Furthermore, studies have primarily been conducted on field crops, where yield (and not aesthetics) is the measure of success or failure. The bottom line is these are living microbes. We do not know how adaptable they are to different crops, different environments, different formulations, different application methods and so on.

Second, turfgrass managers, especially golf course superintendents, are looking for anything that will give them an edge in the stressful situations in which they grow grass. What do they have to lose but a couple thousand dollars when buying micro-

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bial products? In most cases, the products are harmless. They may not help, but at least they will not hurt the grass.

I do have a word of caution, however: Never apply a product to an entire golf course, lawn, athletic field without first trying it on a practice green, sideline or small side yard. While the microbes in a product probably will not hurt the turfgrass, the product the microbes are mixed with may damage grass.

This brings me to my old stand-by statement of "do your own experiments." All it takes is a piece of plywood. Put the plywood down in the middle of the area to be treated. Apply the product over the area and remove the plywood. Then wait to see what happens. That plywood-covered area is your control that received no product. Remember, as long as you are willing to buy a microbial product, someone will make it available to you.

But what are microbes? What do all the terms used in the product literature and labels mean? In this article, I will discuss only one group of microbes, bacteria, as they are the primary components found in non-EPA registered microbial products. I will follow with a discussion on bacteria associated with the driving force of turfgrass growth — nitrogen.

Bacteria background

Biologists divide the world into two groups of organisms based on the types of living cells that compose each organism. Humans, and the turfgrass we manage, are eukaryotes, composed of cells that have a nucleus bound by a membrane. These cells divide through mitosis. The nucleus is where the chromosomes that contain DNA are located. Other eukaryotes in the turfgrass world are fungi, nematodes, insects — virtually everything else.

However, two important groups of organisms, bacteria and archaea, are not eukaryotes; they are prokaryotes. This means their cells have no nucleus. Instead, they have a single, circular DNA molecule (chromosome) that is not bound by a membrane. Prokaryote cells divide by binary division instead of mitosis.

Archaea microbes are not likely to be found in turfgrass because they prefer extreme environments, such as high temperatures (thermal springs) or high salts (ocean).

Bacteria can be divided into three basic groups, based on differences in cell walls. One group has no cell wall (mycoplasmas and phytoplasmas) and are not normally found living freely in the soil.

The remaining two groups are separated based on the composition of their cell wall, which is reflected in a simple test called the Gram stain. Bacteria are either Gram-positive or Gram-negative. It is simply one method used to classify bacteria into groups.

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Bacteria are also classified according to cell shape — rods (bacilli), spheres (cocci), spiral-shaped rods (spirilla) or branching filaments (actinomycetes). We further classify bacteria based on their need for oxygen for growth — aerobic (need oxygen), anaerobic (don't need oxygen), facultative anaerobic (sometimes they do and sometimes they don't need oxygen).

Just like humans or turfgrass, bacteria also need nutrients. Carbon, nitrogen, phosphorus and sulfur are the elements needed in the greatest quantities and obtained from the environment in which the bacteria are growing.

Bacteria also need hydrogen and oxygen, but obtain those elements from water. Bacteria take these elements and then either assimilate them into cellular components or transform them into energy.

The source of the carbon — and how a bacterium obtains its energy — are other means of classifying bacteria.

The majority of soil and plant-associated bacteria are chemoheterotrophs. They use organic carbon compounds to obtain carbon and energy. The energy is obtained by the biodegradation of organic compounds, including carbohydrates and proteins. Saprophytic bacteria feed on dead organic compounds (plant or animal residues). Pathogenic bacteria feed on living organic compounds and thus harm their host.

Fortunately, plant pathogenic bacteria in the turfgrass system are rare. Symbiotic bacteria feed on living organic compounds, but do so in a manner that benefits the host. Turfgrass species are not known to have symbiotic relationships with bacteria.

The remaining soil bacteria are autotrophs, either chemoautotrophs or photoautotrophs. Autotrophic bacteria obtain carbon from inorganic carbon compounds, such as carbon dioxide or methane. Chemoautotrophs obtain their energy from light-independent chemical reactions. These bacteria include nitrifying and sulfur-oxidizing bacteria. Photoautotrophs obtain their energy from light-dependent chemical reactions. These bacteria include cyanobacteria, often referred to as blue-green algae.

Bacteria and nitrogen

Now I will examine these bacteria relative to one element in the turfgrass system — nitrogen. Many different sources of nitrogen are used on turfgrass. These range from inorganic sources, such as ammonium nitrate, to organic sources that include synthetic organic materials formulated from urea and natural organic materials.

For inorganic sources, the nitrogen is in a form already available to the plant. No microbes are required. For organic sources, microbes are required to turn the nitrogen into a form that can be used by the plant. The biological process that transforms organic, synthetic or natural nitrogen to ammonium is called ammonification.

Synthetic organic nitrogen fertilizers include ureaformaldehydes (UF), sulfur-coated urea (SCU), isobutylidenediurea (IBDU) and resin-coated urea (RCU). IBDU and RCU are not dependent on microbes for urea release. Urea from SCU can be released by microbial decomposition of the sulfur coating or by water entering through cracks in the coating.

Ureaformaldehydes (also referred to as methyleneureas) are dependent on biodegradation by microbes (bacteria and fungi) for release of the nitrogen. Some bacteria release nitrogen from UF as ammonia and urea; the formaldehyde released is immediately oxidized to carbon dioxide. Once urea, which is not normally taken up by plant roots, is released by any of these processes, it is hydrolyzed in the presence of the enzyme urease to carbon dioxide and ammonia/ammonium. The urease enzyme itself probably comes from soil microbes.

There is no single bacterial species that is capable of oxidizing ammonia directly to nitrate.

Examples of components in natural organic fertilizers include proteinaceous materials, such as animal manures, poultry litter, crop residues, sewage sludge, hoof and horn materials, and blood meal. These fertilizers are completely dependent on microbial decomposition for release of ammonia/ammonium from the proteinaceous materials. The microbes associated with the processes that release ammonia from synthetic and natural organic fertilizers are not well-defined, but certainly will include a range of chemoheterotrophic bacteria that produce, for example, extracellular enzymes required to break down urea, proteins, amino acids (building blocks of proteins), or aminopolysaccharides (sugars combined with amino acids).

No matter whether you apply a synthetic organic or natural organic nitrogen fertilizer, ammonia/ammonium is the nitrogen compound released from either the urea or proteinaceous materials. Ammonia can volatilize because it is a gas.

Ammonium can be taken up by the plant, but more often the nitrogen is made available to the plant as nitrate. The ammonia is oxidized to form nitrate, a process referred to as nitrification. This process cannot occur without microbes. Nitrification is usually discussed in terms of autotrophic nitrification. Chemoheterotrophic bacteria and fungi can also oxidize ammonium to nitrate, however. The importance of this type of nitrification is still unknown. In pure cultures, it is of minor importance, but the verdict is still out in regard to its importance in soils.

The bacteria associated with autotrophic nitrification are well defined and are all members of the bacterial family, Nitrobacteraceae. Since this process produces energy for these bacteria, the bacteria would be classified as chemoautotrophs.

Two steps are involved in autrotrophic nitrification: 1) ammonia is oxidized to nitrite by ammonia-oxidizing bacteria, which is the rate-determining step; and 2) nitrite is oxidized to nitrate by nitrite-oxidizing bacteria.

There is no single bacterial species that is capable of oxidizing ammonia directly to nitrate. All known terrestrial ammonia-oxidizing bacteria are strict autotrophs. They are members of the "b" subdivision of the Proteobacteria class and primarily belong to the genera nitrosomonas, nitrosospira or nitrosolobus. All nitrite-oxidizing bacteria are members of the "a" subdivision of the proteobacteria, with nitrobacter normally considered the primary genus of terrestrial nitrite-oxidizing bacteria. Nitrobacter is capable of heterotrophic growth, but growth is much slower under those conditions.

All members of the nitrobacteraceae are extremely difficult to isolate from their respective environments. To obtain pure cultures requires an enrichment technique and can easily require six to 12 months. This is why these bacteria will not be found in the microbial products on the market, despite what the literature might claim.

The slow growth also eliminates using a culturable plate count method to monitor or identify Nitrobacteraceae populations in the soil. While probable number techniques have been developed for quantifying ammonia-oxidizing bacteria and nitrite-oxidizing bacteria, these techniques do not allow for identification of the members of the population and are probably biased for the strains most amenable to these techniques. Molecular techniques are the best tools for working with this group of bacteria.

Once the nitrate is released, it can be taken up directly by the plant, or another group of soil microbes can assimilate the nitrate and convert it to ammonium to make amino acids. This process is one form of denitrification and is sometimes referred to as nitrate immobilization. Another form of denitrification occurs under anaerobic conditions. The resulting products are dinitrogen (N_2) and the greenhouse gas nitrous oxide (N_20).

Another process by which plants obtain nitrogen is through biological nitrogen fixation. In this process, bacteria reduce one molecule of atmospheric nitrogen (N_2, so it is really dinitrogen) to two molecules of ammonia. Bacteria that use atmospheric dinitrogen as their only source of nitrogen for growth are called diazotrophs. All nitrogen-fixing microbes are free-living bacteria. Those which form a symbiotic association with legumes belong to a group of bacteria commonly referred to as rhizobia (e.g., rhizobium, bradyrhizobium). Another bacterium, frankia, forms symbiotic associations with non-legumes, primarily forestry plants. frankia belongs to the general group of bacteria called actinomycetes, which look like fungi but are really bacteria.

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However, bacteria do not have to form symbiotic associations with plants to biologically fix nitrogen. These nonsymbiotic diazotrophs are represented by numerous genera of bacteria. Common ones found in the soil are azotobacter, beijerinckia, acetobacter, azospirillum, xanthobacter, pseudomonas, alcaligenes, bacillus, klebsiella, enterobacter and numerous cyanobacteria genera. If these names look familiar, it is because these are the common bacteria associated with microbial products used on turfgrass.

Azospirillum is a genus of gram-negative bacteria that has been examined the most, both genetically and in laboratory or field studies, in regard to promoting plant growth by nitrogen fixation. However, the contribution of biological nitrogen fixation to the positive plant responses sometimes observed are still often questioned by scientists.

Three important factors limit biological nitrogen fixation in these nonsymbiotic diazotrophs. Except for photoautotrophs like

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the cyanobacteria, all diazotrophs require an organic or inorganic energy source — and lots of it. The biological fixing of nitrogen requires a considerable amount of energy.

It takes 16 ATPs (adenosine triphosphate, a biological compound that provides the energy to make biochemical reactions possible) to make the two molecules of ammonia from one dinitrogen. Therefore, the first limiting factor is carbon sources needed to produce the massive amounts of energy required.

The second limiting factor is the level of nitrogen (ammonium, nitrate, and organic nitrogen) already present in the environment. As with symbiotic nitrogen fixation, the enzyme complex called nitrogenase mediates the fixing process. It takes very small quantities of nitrogen (think micro, as in virtually undetectable) to limit the nitrogenase enzyme complex.

The third limiting factor is oxygen. Nitrogenase is extremely sensitive to oxygen. Some bacteria have devised unique ways in solving this problem. Rhizobia do this by having the plant produce nodules. The nitrogen fixation occurs inside these nodules, where a compound called leghemoglobin binds the oxygen, thus protecting the nitrogenase enzyme from oxygen. Most of the diazotrophs do not have such an efficient mechanism of binding oxygen.

It is important to understand that the nonsymbiotic diazotrophs probably do not function in most soils and root systems as biological nitrogen fixers. It is only when the environmental conditions meet all of the exacting criteria that the nitrogenase enzyme complex requires that atmospheric dinitrogen is converted to ammonia.

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Research regarding Klebsiella pneumoniae has shown that at least 21 genes are involved in controlling and supporting the nitrogenase complex.

Does the turfgrass benefit from adding nitrogen-fixing bacteria?

Realistically, the only turfgrass systems that will likely benefit from the addition of diazotrophs in terms of supplying nitrogen are those grown without nitrogen fertilizer inputs — low-maintenance situations or roadside vegetation. If this describes your turfgrass system, then maybe it is worthwhile to experiment with these microbial products. Otherwise, it is highly unlikely that you will receive a positive effect from these bacteria due to their fixing of nitrogen for plant growth.

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