Not All Microbes Are Created Equal
Identifying Beneficial Ones

By David J. Drahos, Ph.D.

Research has revealed that both plants and soil microbes seek to function in symbiotic relationships. In other words, they seek to live together in a way that benefits both of them. Under ideal conditions, beneficial microbes aid a plant's health and vigor, while the roots provide food to sustain the microbes. For this reason, many turf managers turn to microbial products to add 'beneficial' microbes to a soil's profile in hopes that these microscopic organisms will help their turf resist disease and stay healthy.

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Unfortunately, not all microbes are created equal. There are millions of microbes competing for the right to live in tandem with plants, and not all of them will survive the competition for a home in the root zone. This fact raises several questions for anyone using microbial products:

- How can these products guarantee that the *right* microbes are selected?
- If they are, how do they ensure those microbes survive in the root zone long enough to provide benefits for the turf?

While suppliers of many currently available microbial products can't provide solid answers to these questions, the technology does exist.

DNA Fingerprinting

DNA fingerprinting, protein fingerprinting, and enzyme analysis allow companies to identify specific microbes with proven beneficial traits and to verify their presence in soil after application. This procedure, using new genetic technology, enables comparative identification of an unknown microbial strain based on its unique fingerprint -- much like human fingerprints are used for identification purposes.

All microorganisms, including bacteria, possess a unique complex set of genes (DNA) which act as a blueprint for behavior, growth and survival. In fact, each individual bacterial strain type has a special DNA fingerprint, which has been shown to remain virtually the same over many years, through many generations, no matter what materials the microbe is growing on or where the strain has been in the environment. These DNA fingerprints are unique not only to the identification classes of Genus and Species, but below even the Subspecies level.

In the DNA fingerprinting method, a small amount of DNA is isolated and purified from an overnight laboratory culture of a particular microbe. This DNA from each bacterial cell looks like a microscopic "ribbon." Since each cell is an identical clone of its parent, all the DNA strands are also identical, with the same sequence of building block components that make up the DNA ribbon.

Next, the DNA strands are treated with special restriction enzymes, which look for very specific locations (coding sequences) on the DNA. These restriction enzymes act as molecular scissors to slice the DNA ribbon into fragments of different lengths, corresponding to the location of the cut sites on the DNA strands. Sites, which are close together, produce shorter length fragments; sites farther apart give longer fragments. The mix of fragments are then separated based on size by placing the cut DNA into a gelatin-like material (agarose), then sub-

jecting this to an electric field. Since DNA is naturally negatively charged, it will move toward the positive anode. However, the smaller fragments will migrate faster than the larger, since they move more freely in the agarose gel than larger pieces.

The DNA thus spreads out, forming a pattern or fingerprint, which appears much like an IUPC bar code seen on many product packages in stores. The DNA pattern can be stained and photographed, providing a permanent record of the fingerprint. No two patterns are alike, unless they came from the same parental microbial strain, just as no two human individuals have the same fingerprints (unless of course they are identical twins). The entire fingerprinting process can be completed in less than eight hours.

DNA fingerprinting provides a very powerful tool in the successful application of microbes for environmentally beneficial processes. It provides a means to know for certain that the specific inoculated strain is truly present where it needs to be, even though there may be hundreds of very similar look alike (but less effective) strains naturally present. This is particularly true when live microbial strains are applied in the turf foliar or soil environment where they encounter and must compete with a population of millions of well-adapted natives.

For inoculated bacteria with strong plant-beneficial effects, a low-level presence is often all that is needed to tip the balance in favor of the plant. Actually, the most effective strains are those that do not significantly alter the overall indigenous microflora population, but specifically target themselves to the root or leaf surface finding a small niche to grow. These strains directly respond to the plant's needs, providing powerful beneficial materials, such as plant hormones (phytohormones), precisely when they can be most helpful to the plant. Only very minute quantities of these phytohormones are required for full effect (about 1 millionth of a gram per plant root system), and too much at the wrong time may be quite detrimental for overall plant health. This precision injection method is far more effective than simply a chemical dump of phytohormones on the soil surface, hoping some (but not too much) gets where it needs to go. DNA fingerprinting provides a means to be certain that these pre-identified, beneficial microbes have really shown up for work, despite the crowds of native strains around them.

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Finally, the quality of products containing the chosen strains of microbes can be verified definitively for the types and amounts of bacteria present using the DNA fingerprinting method. The presence of inoculated strains in environmental samples can be confirmed to aid in determining survival, optimal dosage, and growth. Also, in the process of isolating new natural active microbes, DNA fingerprinting insures that such isolates are, in fact, unique and different from formerly isolated strains. Furthermore, legal protection of patented strains from use by competitors can also be insured using this procedure.

Protein Fingerprinting

The protein fingerprinting technique also provides a strongy indication of strain identity, though this method is more effected by the culture history (i.e. how the strain was grown in the lab, or where it was isolated in the environment). Nevertheless, since this method is more rapid than DNA fingerprinting (about 2 hours for protein prints rather than 6 hours for DNA prints), it offers an up-front check and broader assessment approach, usually as a precursor to DNA fingerprint confirmation.

For this procedure, cultures of all strains to be tested are first grown in a very defined liquid medium. Care is taken to ensure that samples of growing cultures are taken in the same growth phase for all strains to be compared. Protein is isolated from each individual culture, separate from other cell material, such as lipids, polysaccharides and nucleic acids. These proteins represent the

large number of active enzymes (over 5,000 different enzymes being made by the cell at once), which each bacterium must produce to survive and compete.

When the proteins are separated by size through a plastic-like matrix (polyacrylamide) in an electric field, a banding pattern can be observed after a protein-binding stain is applied. While similar to, but less complex than the kind of pattern seen for the DNA fingerprinting method, the banding pattern obtained with proteins still allows effective differentiation of similar microbial strains.

Fast Enzyme Analysis

The ability to accurately determine the amount of certain enzymes produced by active microbial isolates is vital in assuring an active and effective biological product. The best strains in our products will be those very rare microbes with the robust ability to make substantial amounts of vital enzymes at the right times under the toughest conditions. But how do we find them?

A new system helps evaluate microbial growth rates to gauge their competitive fitness under the adverse conditions encountered in the sand/soil environment of a typical USGA putting green.

Typically, we select strains able to grow the best on certain media in the laboratory, then assay the amount and types of enzymes they make. In the past, however, accurate enzyme analysis had often been a laborious time-consuming procedure.

The multiple assessments and simultaneous analysis of enzyme standards necessary to gain statistical confidence in the enzyme readings often made it impossible to truly follow enzyme production by a particular microbe under actual environmental conditions.

New technology has all but eliminated these constraints. The new Plate Reader System allows for 96 samples to be analyzed for enzymes simultaneously every five seconds by one technician. Coupled with an on-board computer system, full automated analysis, instant standardization with known amounts of enzymes and accurate statistical verification, definitive enzyme levels can be determined for over 25 individual microbial samples in under 103 minutes. The production and longevity of multiple enzymes can now be followed under nearly real-time conditions. In other words, it helps find those rare, robust, high performance microbes.

In addition, the Plate Reader System is also proving valuable in streamlining the microbial characterization and naming process. Further, the system also aids in our evaluating microbial growth rates under a variety of media and conditions.

For example, the ability of key strains to grow on the exudate from plant roots often indicates competitive fitness under the adverse conditions encountered in the sand/soil environment in a typical USGA putting green.

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