Terragen BIOTECH

AGRICULTURAL BIOLOGICALS

The global agricultural biologicals market was estimated to be valued at US$5 billion in 2015. With a projected annual growth rate of 12%, the market will be worth more than US$11 billion by 2022.

Biological products can be segmented as follows:

- **Biostimulants:** organic compounds and live microbials used to stimulate biological processes for productivity or stress management.
- **Biofertilisers:** microbials enhancing nutrient mobilisation and/or uptake. E.g., rhizobia, free living PGPB and fungi. Symbiotic N-fixing bacteria by far the largest group.
- **Bio-control products:** biopesticides containing natural compounds derived from extracts or products of microbial digestion; and, live microbial formulations with biocidal properties.
- **Eubiotics:** feed additives targeted to enhance functionality of feed utilisation, gut micro-flora and digestive health. Includes microbes and non-microbe products.

Terragen Biotech products fit into a number of these market segments.

- Great Land is a soil conditioner which can fit into either of the first two segments, biostimulant or biofertiliser.
- Further research and development is underway for new formulations targeting bio-control applications.
- Mylo is a microbial livestock feed supplement which fits within the Eubiotic group of animal products.
- Animal studies are being conducted towards registration of directly administered products for prevention and treatment of ailments, such as mastitis and inflammation.

SOIL AND PLANT MICROBIOLOGY

Research over the last decade has sharpened our awareness of the soil microbiome in agricultural. It is now more broadly accepted that the composition of microbial biomass in the soil will strongly influence plant health. Productivity gains conferred by beneficial microbes in agricultural systems are becoming more widely accepted but we have only just begun to understand the potential scope of benefits.

Substantial investment by the agricultural industry into microbiome research is a testament to the limitations of a chemically driven approach to soil and plant nutrition and biocontrol.

Characterising the microbial community into functional groups such as bacteria and fungi provides a basis on which to measure responses to management practices as indicators of soil health, enabling informed changes in the management of nutritional or biological inputs.

Following is a summary of functional groups of microbes associated with the soil. *Source: University of Missouri, USA.*

GREAT LAND®

Gram-negative bacteria

Gram-negative bacteria are smaller than gram-positive bacteria. Gram-negative bacteria are involved in all phases of the nitrogen cycle, including those such as Rhizobium, that form mutually beneficial relationships with plants (legumes) and fix atmospheric nitrogen to produce ammonium, a nitrogen form plants can use. Gram-negative bacteria predominate in the rooting zone and breakdown newly added organic matter. Gram-negative bacteria are more sensitive to water stress than gram-positive bacteria partly because they cannot form endospores as many gram-positive bacteria can.

Gram-positive Bacteria

Gram-positive bacteria are larger than gram-negative bacteria. Gram-positive bacteria are widely dispersed in soil and tend to decompose complex organic material that has been partially decomposed by fungi or gram-negative bacteria. They are not as closely tied to the rooting zone as are gram-negative bacteria. Gram-positive bacteria are resistant to water stress because some such as Bacillus and Clostridium form endospores. Endospores are stress resistant structures of these bacteria that can remain dormant for years during unfavorable environmental conditions and reproduce new cells when conditions become favorable.

Actinomycetes or Actinobacteria

Actinobacteria produce the earthy smell of soil. The cells grow as branching, thread-like structures similar to fungal hyphae. They reproduce by means of spores and fragmentation similar to fungi, but are actually single-cell, grampositive bacteria. They get their energy by breaking down soil organic matter including complex compounds such as starch, cellulose, and chitin. They continue the decomposition of organic matter started by saprophytic fungi and other bacteria to produce dark-colored humus. Actinobacteria grow deep into the soil, but are sensitive to acidic, low-oxygen, and wet or saturated conditions. Some species are heat-resistant and are common in compost. Many actinobacteria participate in chemical warfare against other soil organisms including plant pathogens. Their antibiotics such as streptomycin have been discovered and produced commercially.

Anaerobic Bacteria

Anaerobic bacteria can survive in the absence of oxygen. Some can only survive in the absence of oxygen. It has been determined that water-saturated soil aggregates greater than 6 mm in diameter have no oxygen in their interior. Therefore, anaerobes can occur commonly in surface soils in these microsites. Depending on extent of moisture status, anaerobes reduce nitrogen, iron, manganese, and sulfur which may be lost as gasses or leached into the soil profile or form compounds toxic to plants.

Fungi

A wide variety of fungi occur in soil. They range from single cell yeasts to some of the largest organisms in the world. Some of them cause plant diseases while others live cooperatively with plants. Saprophytic fungi break down organic matter to help produce humus and release plant-available nutrients. Versatile groups of fungi decompose organic matter ranging from simple sugars and starches to the more complex and resistant cellulose and lignin substances. Fungi generally prefer more acidic soils than do bacteria, and, because they are aerobic, tend to prefer the surface soil horizons over greater soil depths.

AM Fungi

Arbuscular mycorrhizal fungi grow in long, thin strands called hyphae and form mutually beneficial relationships with most plants. They colonize roots and produce structures called arbuscles inside plant root cells. Plants provide the fungi with a supply of carbon from which they produce energy. Mycorrhizae provide plants with drought tolerance, and increased nutrient uptake of phosphorus, nitrogen, sulfur, zinc and copper. Additional functions of the fungal association include protection from disease organisms and pests through production of antibiotics and from heavy metals through immobilization.

Mycorrhizae also produce glomalin, a protein released through the hyphae. Glomalin contains sugar and protein and glues soil particles together promoting soil aggregate stability. Many cover crops increase mycorrhizal inoculum in soil for the succeeding crop. Be aware that some cover crops such as buckwheat, radishes and other brassicas are non-mycorrhizal. These crops provide other benefits to soil, but overuse could decrease mycorrhizal populations.

Eukaryotes

Eukaryotes have more complex cell structure than prokaryotes like bacteria. Their cells contain a membranebound nucleus and other membrane-bound organelles. Soil eukaryotes, other than fungi, include algae, protozoans, nematodes, micro-arthropods and other insects, and earthworms. Eukaryotes feed on soil organic matter, soil bacteria, plants and each other leading to release and recycling of plant-available nutrients. After new organic residues are added to a soil the initial increase in bacterial numbers usually produces an increase in protozoa that feed on the bacteria. The larger soil eukaryotes continue the food chain and also help mix the soil and create channels for water and air movement in the soil.

PLANT GROWTH PROMOTING BACTERIA

A wide group of microbes loosely referred to as plant growth promoting bacteria (PGPB) have been extensively studied and found to exert beneficial effects on plant growth and development through colonising either the soil, seed, roots, and other plant material, and impacting these environments.

Documented functions of plant growth promoting bacteria were outlined in a paper published by R. Glick in the journal Scientifica in 2012, summarised on the following two pages.

An Overview of Plant Growth Promoting Bacteria (PGPB)

A summary of the review article: *Plant Growth-Promoting Bacteria: Mechanisms and Applications*
(Bernard R. Glick, Scientifica, Volume 2012)

1. Introduction

The current global population is expected to reach 8 billion around 2020. To meet the demands of this population growth the world will need to increase agricultural productivity but in a sustainable and environmentally friendly way.

This means re-examining the use of current chemical fertilisers, fungicides and herbicides, and making greater use of new technologies, including plant growth promoting bacteria (PGPB).

2. Plant Growth Promoting Bacteria (PGPB)

Bacteria make up about 95% of all microscopic life found in soil (with up to 10^9 bacterial cells / g soil), but are often found in lower quantities in environmentally stressed soils. The number and type of bacteria are also influenced by conditions, including: temperature, moisture, salt & other chemicals, and type of plant. Bacteria are found in the greatest concentration around the roots of plants (the rhizosphere), taking advantage of nutrients secreted by the root tips.

Bacteria in general may be (a) beneficial to the plant (b) harmful to the plant or (c) neutral to the plant. However, this may change under different conditions and even beneficial bacteria may affect different plants in different ways.

Specifically, PGPB may be free living; living in a symbiotic relationship with plants; or live within the plant tissue itself (endophytic) – but they all utilise the same mechanisms. They either: (a) promote plant growth directly, by facilitating resource acquisition or modifying plant hormone levels, or (b) by acting indirectly as a bio control and decreasing the impact of pathogens on plants.

3. Direct Mechanisms

A. Facilitating Resource Acquisition

- Nitrogen Fixation: Free living and Rhizobia spp. bacteria are able to fix nitrogen and provide it to plants. Research on maximising the efficiency of this process is ongoing.
- Phosphate solubilisation: Despite many soils having high levels of phosphorus, most of it is insoluble, therefore unavailable to plants. Moreover, soluble inorganic phosphate fertiliser often becomes immobilised soon after application, meaning much of it is wasted. Because of this, a lack of phosphorus often limits plant growth. PGPB and plant growth promoting fungi typically solubilise and mineralise phosphorus as a side effect of synthesising various organic acids, and of other biological processes.
- Sequestering Iron: While iron is a common element, the predominant form (ferric iron, or Fe^{+3}) is not highly soluble, meaning only marginal amounts are easily available to plants. In the soil, microorganisms synthesise molecules called siderophores which attract, bind and transport ferric iron - which is then easily able to be taken up by plants and increase plant iron levels.

B. Modulating plant hormone levels

Plant growth is regulated by a number of hormones (phytohormones), including cytokinins (which promote growth in plants shoots and roots), gibberellins (which stimulate stem growth, germination and flowering), ethylene (which has many effects including the promotion of plant ripening), and auxins (including Indoleacetic Acid or IAA – which affects plant growth in a wide range of ways). PGPB have the ability to produce or modulate these phytohormones, affecting plant growth, the ability of a plant to take up nutrients, and its stress response.

4. Indirect Mechanisms

Biocontrol is a method of controlling pests and pathogens using other organisms (in this instance, using PGPB). Biocontrol can be a viable commercial alternative to the use of chemical pesticides or fungicides. There are several main ways in which PGPB may act as a biocontrol, outlined in the following.

- The Production of Antibiotics: Many PGPB can synthesise antibiotics which kill pathogenic cells (especially pathogenic fungi) and prevent their proliferation. However, pathogens may develop a resistance to the antibiotics that the bacteria produce; therefore this method is most useful as a biocontrol when the bacterial strain also produces hydrogen cyanide to work in conjunction with the antibiotic.

- Siderophores: These are molecules produced by bacteria which attract, bind and transport ferric iron. PGPB siderophores tend to be more efficient at attracting iron than plant pathogens, and can prevent them from proliferating due to an iron deficiency. This generally does not affect plant growth, as most plants require a much lower iron concentration than most microorganisms, and they can also utilise the iron bound by the PGPB.
- Competition: Indirect evidence shows PGPB limiting disease incidence by outcompeting plant pathogens, rapidly colonising plant surfaces and using all available nutrients.
- Modulating Stress Hormones: (notably ethylene) When affected by pathogens, plants produce excess ethylene, which exacerbates the plant's stress. PGPB can lower the hormone response, reducing the impact of stress on the plant.
- Induced Systemic Resistance (ISR): This is similar to Systemic Acquired Resistance (SAR), which is the term given to plants activating their defence mechanisms against pathogens. PGPB have the ability to trigger ISR before a plant has been attacked - essentially priming a plant to defend against a pathogen attack.

5. Modulating the Effects of Environmental Stress

In ideal circumstances, a plant's growth would be relatively linear over time. However, plants are affected by a variety of stressors in the field which impact upon growth, resulting in periods of maximal growth, interspersed with periods of inhibited growth. These stressors include temperature extremes, flooding or drought, toxins, wounding, insect predation and infection by various pathogens (bacteria, viruses and fungi). PGPB may work to overcome stress triggered growth inhibition in a number of ways:

- By modulating stress ethylene (as discussed above). PGPB which act to modulate ethylene production have been found to be effective against temperature extremes, flooding, drought, metals, salt, and organic contaminants.
- By providing the plant hormones Indoleacetic Acid (which directly stimulates growth even in the presence of inhibitory compounds) and cytokinins (which promote cell division and therefore growth).
- In some studies, PGPB have been engineered to over produce trehalose, a useful molecule which is able to decrease damage from drought, salt and temperature extremes. When these transgenic PGPB are applied to plants the result is more nodulation, higher nitrogen fixation and greater recovery from drought stress. Although plants themselves can also be engineered to produce more trehalose, it is simpler to manipulate PGPB, with the added advantage that one modified strain can be used on a range of different crop plants.
- Some cold tolerant (psychrotropic) PGPB are able to secrete antifreeze proteins into the soil to protect themselves from damage by ice crystals. This enables them to survive and be effective in low soil temperatures, which is often when fungal plant pathogens are most destructive.

6. Conclusions

The time has come for PGPB to be an integral part of agricultural practice. These bacteria are currently being used successfully in a number of countries in the developing world and this practice is expected to grow. In the developed world, where agricultural chemicals are relatively inexpensive, the use of PGPB occupies a small but growing niche in the development of organic agriculture.

However, there are a number of issues that will need to be addressed before the use of PGPB can become more widespread, and areas which need further study. These include:

- The requirement for new practical and effective approaches for the growth, storage, shipping, formulation and application of PGPB.
- The need to educate the public about the usefulness of PGPB in agriculture, and addressing the misconception that bacteria are merely agents of disease.
- The need to prove to regulators and the public that transgenic PGPB do not present new hazards or risks.
- Determination of whether further research is most advantageous into rhizospheric or endophytic bacteria, and a greater understanding of the relationship between bacteria and mychorrhizae (root fungi).

Despite these issues, it is likely that there will be a shift in focus to the effective use of PGPB in agriculture, and the future of this technology is bright.

SOIL HEALTH – MYTH VS FACT

BUSTING THE MYTHS

Throughout history there are many cases of widely held beliefs, often driven by commercial and political interests, that turn out to be wrong. For example, smoking was considered healthy until the mid-1900s.

In agriculture, the industrial approach to food production is on an unsustainable path - failing to feed our growing demand and having an unacceptable contribution to environmental pollution, chemical toxicity, antibiotic resistance and poor nutrition. Fortunately, technological advances and experiences can dispel some notable doctrines in conventional agriculture and proven alternatives are available as exemplified below.

THEY SAY...	THE FACTS
'NPK' are the primary three limiting nutrients of significance.	• At least 16 mineral elements are required for a healthy farm system. • Reliance on soluble 'NPK' fertilisers supresses natural nutrient cycling, microbial activity and the building of soil carbon. • The 'NPK' paradigm's inherent waste of nutrients represents a significant cost - borne by farmers. • Good nutrition practices feed the soil, not the plant.
Lime/calcium is primarily used as a tool for correction of soil acidity.	• Calcium is a core foundation mineral - a base fertiliser - not a tool for correcting acidity. • The importance of calcium for nutrition and soil structure cannot be overstated. • pH is not an indication of calcium availability. • In biologically healthy soils with nutrients at appropriate levels, pH will be corrected by natural biological processes.
You can't avoid a large portion of applied phosphorous (P) being 'locked up', remaining unavailable to the plant.	• More phosphorus is made available to plants when microorganisms are active in the soil. • In healthy soils, a dynamic exchange of phosphorus occurs between significant reserves (humus, undegraded organic matter, soil particles and in solution) and plants – all of which is facilitated by active soil biology converting phosphorus to plant available form.
High rates of soluble nitrogen (N) are needed to maximize crop and pasture growth.	• High soluble nitrate levels impede the normal processes by which plants most effectively assimilate nitrogen. • Soluble nitrogen provides a short term 'sugar hit' but pastures accumulate excessive nitrate which can cause metabolic disorders in cows and affect milk quality. • Only 10-40% of applied soluble nitrogen is used by the plant. The balance is either lost to the atmosphere as gaseous nitrous oxide (N2O), formed by undesirable bacteria, or leached into waterways. Such waste is a direct cost to the farmer.
Potassium (K) is easily leached or 'locked up', therefore, plenty of soluble K is needed to feed the plant.	• Similar situation as phosphorous. In healthy soils, significant quantities of potassium are held in dynamic balance between humus, un-degraded organic matter, soil particles and in solution. • Active soil biology releases potassium from these reserves in plant available form. • Replenishment should aim for recommended levels of potassium as a % of base saturation.