ASSESSING SOIL MICROBIAL POPULATIONS AND ACTIVITY FOLLOWING

THE USE OF MICROBIAL INOCULANTS: EFFECTS ON DISEASE

SUPPRESSIVENESS AND SOIL HEALTH

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ASSESSING SOIL MICROBIAL POPULATIONS AND ACTIVITY FOLLOWING THE USE OF MICROBIAL INOCULANTS: EFFECTS ON DISEASE SUPPRESSIVENESS AND SOIL HEALTH

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VITA

Marleny Cadena Cepeda, daughter of Cenen Cadena and Maria Elena Cepeda, was born January 22, 1972 in Santander, Colombia. Marleny has three sisters, Maribel, Jacqueline and Johanna, one brother, Alvaro, and a niece Paola. In 1988, she graduated from Externado Nacional Camilo Torres high school in Bogota, Colombia. She enrolled at Universidad Distrital at Bogota in 1989 and graduated with bachelors of physics. After working as a science teacher at the university level, she decided to study agronomy at Universidad Nacional de Colombia getting B.A. in this area in 2000. She entered graduate school at Auburn University and began her Masters of Science degree in Plant Pathology in fall 2004. She was married to Nathan Burkett, son of Brenda and Donald, on July 26, 2006 in Bogota, Colombia.

THESIS ABSTRACT

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In recent years, assessing use of microbial inoculants for promotion of plant growth has increased. Optimizing application frequency of PGPR is critical to achieving the maximum benefit from this technology. The study presented here, addresses the problem of developing methods for measuring soil microbial activity and population size in relation to the application of soil inoculants. Commercial formulations of PGPR, containing bacilli strains (Equity®, Soil Builder®, Ag Blend®, PGA®, Bioyield® and FZB42®) were used on tomato and strawberry in greenhouse and field experiments. Physiological activity of microbes was measured by assessing dehydrogenase activity, arylamidase activity, and fluorescein diacetate hydrolysis (FDA). Culturable microbial

populations were determined by most probable number (MPN) and direct plate counting. In strawberry field trials, hydrolysis of FDA was significantly different among treatments at one out of four sampling times. Procedures to estimate population size (MPN) did not detect any change in microbial population; however, the use of PGPR inoculants promoted growth and increased strawberry yield. In greenhouse experiments on tomato, FDA was not always effective in measuring changes in microbial activity in the rhizosphere following of inoculants application, and arylamidase and dehydrogenase procedures were not sensitive at all. Despite detecting changes in microbial activity, no changes in microbial populations, estimated by MPN, were observed. Populations of total culturable and heat heat-tolerant bacteria were measured by plate counting. FZB42 and Bioyield treatments generally resulted in significantly greater total populations. Overall, population size measured by direct plate counts could be a useful procedure to study root colonization and persistence of introduced microorganisms in the rhizosphere. Knowing that introduced microorganisms are surviving, and their patterns of growth will help to determine when and how PGPR products should be applied. However, because of the lack of consistency the FDA procedure should not be used to decide frequency of application of PGPR products. Induction soil suppressiveness by PGPR and the relation to microbial activity and population size were also studied. The plant parasitic nematode, *Meloidogyne incognita,* and tomato were used as a model. Results showed significant reductions in number of nematode eggs per gram of root, number of juveniles per ml and number of galls in FZB42 and Bioyield treatments. Additionally, increases in population size were detected for those treatments by direct plate counting, although there was not a correlation between microbial activity and population size.

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LITERATURE REVIEW

1. Soil Quality and Health

The terms soil quality and soil health have both been used to describe agriculturally productive soils. However various authors use the terms differently. Clarifying the distinction between soil quality and soil health is essential for developing a clear understanding of soil and rhizosphere systems. Soil health refers to the soil as an ecosystem, involving biodiversity and sustainable production; therefore, it may be defined as the capacity of a soil ecosystem to function as a living and dynamic system to uphold biological productivity over time (Harris et al., 1996). Implied in this definition of soil health are the key concepts of diversity, structure, function and performance of soil. Accordingly, soil health focuses primarily on the soil's continued capacity to sustain plant growth as indicated by various essential physical, chemical and biological processes in the soil ecosystem. Because soil health depends upon these processes, specific indicators may be used to quantify or assess soil health (Doran, 2002).

In contrast to soil health, soil quality is a term used to indicate a specific purpose of the soil. Hence, soil quality can vary depending upon land use, such as forest plantation, or production of agricultural or horticultural crops. Also, it focuses on the capacity to meet defined needs such as the growth of a particular crop (Doran, 1994a; Doran, 1994b).

Thus, it is not possible to generalize by saying that good soil health always results in high quality or vice versa. For example, a rain-forest soil is healthy based upon indicators of biological diversity but it has low quality for growing crops. However, soil health typically considers soil quality because soil health includes the long-term sustainability of soil for crop production.

In summary, soil health involves the care of plants and animals, water and air quality. Soil viewed as an ecosystem will be used under sustainable conditions of plant productivity, while soil quality considers optimizing soil conditions for one particular desired purpose or use. Although the two terms are conceptually different yet related, I will use soil health instead of soil quality, as a descriptive term for soils since maintaining and enhancing soil health is imperative to sustaining agricultural productivity.

2. Evaluating Soil Health

A framework is needed for evaluating soil health. Such a framework would help to identify problems in production areas and to monitor changes in the environment related to agricultural management. Larson and Pierce (1994) proposed that a minimum data set of soil parameters should be adopted for assessing soil health and that standardized methods and procedures should be established to assess changes in those factors.

A set of basic indicators has not previously been defined, mainly because researchers have not reached agreement on which indicators should be measured to fully assess soil health. It is generally accepted that such indicators should be easy to calculate and responsive to variations in management (Visser and Parkinson, 1992).

Establishing standardized indicators is problematic because they are typically related to a specific management system and do not generally function or exist in all soils. The intricate link among biological, physical and chemical properties of soil requires more than a single factor to assess soil health. For example, it is not sufficient to use only organic matter content, which is generally related to soil quality, for specifying the overall health of a soil (Bending et al, 2003). Thus, parameters independent from soil type and environment are needed.

In the past, several methods have been employed to assess the complexity of the relationships among the physical, chemical and biological properties of soil. Karlen and Stott (1994) used a systematic method to associate soil function with soil quality. They defined a soil of high quality in terms of water entrance, transfer and absorption, resistance to surface degradation and support of plant growth. Glover et al, (2000) applied this method to evaluate the effect of conventional, organic, and integrated apple production systems on the soil's physical, chemical, and biological properties by using a soil quality index and found it effective for the purpose of that study.

As previously stated, soil health considers the chemical, physical and biological processes of the living soil ecosystem. In searching for indicators of soil health, while chemical and physical properties make an important contribution and may determine the quality of a specific soil, one could consider that biological indicators would also be key. Biological indicators monitor the living portion of the ecosystem and are most susceptible to change and therefore to degradation by agricultural practices. Although, microorganisms occupy only 5 % of the soil, they constitute a large microbial biomass and diversity, and together with plants, make up the major biological portion of soils and

rhizospheres (Brady and Weil, 2002). The rhizosphere is of special interest since the soil-root interface also influences ecological conditions, and the metabolic activity in the rhizosphere is higher than in the surrounding soil (Schinner, et al., 1996). It has been suggested consequently, that measurements of soil and rizhosphere microorganisms could be useful indicators of soil health.

2.1 Microbial Biomass

Microbial indicators that have been suggested to monitor soil health are microbial biomass, microbial activity and microbial diversity. Soil microbial biomass is the total microbiological component of soil and it is generally expressed in milligrams of carbon per gram dry weight of soil. Methods to estimate soil microbial biomass have been studied and well developed over the last two decades due to of the increasing demand for precise quantitative measurements of soil microbial processes.

Microbial biomass can be a sensitive indicator of changes in soil processes because it has a much faster rate of turnover than total organic matter (Jenkinson et al., 2004). Microbial biomass and activity measures have been used to monitor soil recovery and to show effects of toxic residues on soil. They have also given information about the effects of cropping, rotation and cultivation practices on soil health (Limon-Ortega et al., 2006). Some authors have suggested a strong link between soil microbial biomass, fertility and health (Vanlauwe et al., 1999; Ladd, et al., 2004; Villar et al., 2004).

Biochemical methods for biomass measurements are based on microbial membrane components (phospholipids, lipopolysaccharides, and ergosterol) and constituents of microbial cell wall (peptidoglican and chitin). Such methods have been

used to estimate microbial biomass in soils. (Zelles et al. 1992; Joergensen, 1996; Vanlauwe et al. 1999; Jenkinson et al., 2004; Villar, et al, 2004).

2.2 Microbial Diversity

In addition to studying microbial biomass, one can examine soil microbial diversity and community structure in relation to soil health. Soil microbial diversity and community structure are the different fungal and bacterial species and their relative abundance in the soil community (Schinner et al, 1996). Microbial diversity is measured by various techniques such as traditional plate counting and direct counts, fatty acid analysis, and the newer molecular-based procedures.

Direct counting is a method used to quantify bacteria. The plate count technique, fluorescence microscopy, and most probable number method are among the most utilized. The disadvantage of direct counting is that there is no discrimination between living and dead microbial cells and it does not enable the count of specific microbial species (Nannipieri et al, 2003).

Molecular techniques generally involve extraction of nucleic acid, directly or indirectly, from soil. They are independent of culture and, according to their sensitivity, can detect species, genera, families or even higher taxonomic groups (Nannipieri et al, 2003). The denaturizing gradient gel electrophoresis (DGGE) method has been utilized to investigate distribution, diversity, and changes of microbial communities in forest and mineral soils (Agnelli et al. 2004; Villar et al, 2004). The community structure in soil can also be assessed by measuring lipids and phospholipids (Zelles et al. 1992). These molecular techniques give an overall indication of microbial diversity and can be used to

assess soil health and to monitor changes in the composition of a microbial community after stress or changes in management.

Some research studies have found correlations among microbial biomass, activity and, diversity. Leckie, et al. (2004) evaluated the relationships between CFE (chloroform fumigation-extraction), PLFA (phospholipid fatty acids), and DNA methods in forest humus, and found a good relationship between PLFA and CFE. Agneli et al, (2004) studied the composition of fungal and bacterial communities in a forest soil by soil microbial biomass (SMB), DGGE, SIR (substrate induced respiration), and DNA. They found high bacterial diversity in the upper layer of the soil, decreasing with depth, but a low fungal diversity. Bailey et al (2002) studied the relationship between CFE, SIR, and PLFA to assess SMB and they found PLFA was best predicted by CFE, and they developed a conversion factor. Marstorp and Witter (1999) measured microbial growth in soil after the addition of glucose. Their results suggested that increases in respiration accompanied increases in the amount of dsDNA from soil, and therefore, the technique could be an alternative to measure CFE. Vanlauwe et al (1999) measured SMB as total LP (lipopolysaccharides) in soils with different organic contents.

2.3 Microbial Activity

Soil microbial activity is different from soil microbial biomass. Soil microbial biomass is used to quantify populations and also can be used to assess the dynamics of nutrients in soil. For example, the determination of microbial C, N, P and S contents by fumigation techniques have allowed a better quantification of nutrient dynamics in soil.

While soil microbial biomass describes the total microbial population size, soil microbial activity indicates the vast range of physiological activities carried out by soil

microorganisms. The term soil enzyme activity is related to microbial activity and reflects the physiological work of all living organisms in the soil, including plant roots (Ladd, 1978). In this review, a distinction will be made between enzymatic activity and microbial activity. Soil enzymes are not only related to physiological activity of microorganisms but also to such activity of animals and plants. Further, enzymes in soil are not always bound to active cells, as they can be adsorbed to clays or humic colloids (Skujins, 1978). Nevertheless, soil enzymes are relevant for assessing soil health because they are essential for organic matter turnover and the metabolic activity of soil microorganisms (Nannipieri et al., 2002).

Several methods have been used to determine soil microbial activity. Some methods measure the rate of entire metabolic processes such as evolution of CO_2 (respiration), nitrification activity, DNA synthesis in bacteria, fluorescein diacetate (FDA), and activity of dehydrogenase (Nannipieri et al., 1990). Other methods measure specific activities of either a particular enzyme or a set of enzymes that are involved in a particular metabolic pathway of interest, such as chitinases, cellulases, and trehalases (Tabatabai and Deng, 1994; Anderson et al., 2004; Pavel, et al., 2004).

a. CO_2 Evolution

Soil respiration is defined as oxygen uptake or carbon dioxide evolution by bacteria, fungi, algae, nematodes and protozoan, and includes the gas exchange of aerobic and anaerobic metabolism (Anderson, 1982). Soil respiration results from the degradation of organic matter, with the formation of CO_2 occurring in the last step of carbon mineralization. When soil is disturbed, a change in soil respiration can be observed due to more rapid growth and higher mineralization of the microorganisms

(Singh and Gupta, 1977). This respiration is characterized by several phases including an increase, exponential acceleration, delay, stationary and a decline phase. Respiration is called basal respiration when there is a balance between microorganism and their activities in undisturbed soils. CO_2 evolution from a soil is thus a measure of the total soil biological activity, including microbial activity (Alef and Nannipieri, 1995).

Substrate induced respiration (SIR) is a method used to measure respiration, after the addition of glucose as a substrate to the soil sample, with the change in respiration being measured after 8 h. SIR is a measure of soil microbial activity, but it can also be used to estimate soil microbial biomass. The maximum initial respiratory response is proportional to the amount of microbial carbon present in the soil sample. Therefore, using a conversion factor, the respiration value can be converted to mg of soil biomass carbon; however, SIR is most accepted as a measure of microbial activity (Anderson, 1973).

b. Soil Enzyme Procedures

Soil enzyme activity may serve as an indicator of soil health. As showed by Dick (1997), soil enzymes are mediators of innumerable processes in cells and catalysts of functions that include transformation of organic matter, release of organic nutrients for plant growth, nitrogen fixation, detoxification, nitrification, and de-nitrification. Assessing soil enzymes is a potential indicator of the biological status or the capacity of soil to carry out the enzyme-catalyst processes.

Soil enzymes are present inside microbial cells (intracellular) and outside of the cell (abiontic). One difficulty in studying enzymes is that only a small proportion of the total enzyme pool can be extracted from soils. Also, substrates must be added to quantify

enzymes in various assays. These assays can underestimate concentration because they are done in optimum media, and under conditions of controlled pH, temperature, and moisture. Another difficulty is that separating living cells from abiontic enzymes is not always possible. Despite the difficulties listed above, soil enzymes have been effectively used to monitor soil quality, investigate soil microbial activity (Anderson et al, 2004), estimate soil resilience to wastes (Benitez et al., 2004), and evaluate soil after fumigation with methyl bromide (Klose and Ajwa, 2004).

Soil enzymes include oxidoreductases, transferases, hydrolases and lyases. The enzymes most often reported to be active in soil include protease, urease, phosphatase, cellulase, b-glucosidase, saccharase, xylanase, catalase, dehydrogenase, amylase, and pectinase (Alef and Nannipieri., 1995). Proteases catalyze the hydrolysis of proteins to polypeptides and oligopeptides to amino acids; they are found in living cells, dead cells, as free enzymes, and adsorbed to organic and inorganic particles. Protease activity is significantly correlated with SIR, dehydrogenase, and aggregate stability (Kandeler and Murer, 1993). Ureases catalyze the hydrolysis of urea to CO_2 and NH_3 with a reaction mechanism based on the formation of carbamate as intermediator. In soil, ureases are tightly bound to soil organic matter and minerals. Urease activity is not correlated with microbial biomass but is affected by heavy metals, oxygen concentration, and nitrogen availability in different types of soils (Tabatabai and Bremner, 1972; McCarty and Bremner, 1991; McCarty et al, 1992).

Phosphatases catalyze the hydrolysis of a variety of organic phosphomonoesters and are important in mineralization of soil P. Benitez, et al. (2004), measured enzymatic activity (Hydrolytic- phosphatase, b-glucosidase, oxidoreductase-dehydrogenase,

diphenol oxidase-IAA production) to estimate soil resilience to a toxic organic wastes. However, the results of this research indicate that only dehydrogenase, diphenol and ß-glucosidase can be used to monitor changes.

Cellulases are a group of enzymes that catalyze the hydrolysis of cellulose to glucose, cellobiose, and higher oligosaccarides; fungi mainly produce this enzyme. Anderson et al, (2004) studied microbial enzyme activity in leaf litter, humus and mineral soil layers. They have reported no correlation among cellulase activity, basal respiration, and microbial biomass carbon, while there was a positive correlation with chitinase activity. Soil management related to the activity of eleven soil enzymes including cellulase was investigated. Results showed a significant positive impact by the cultivation system on each enzyme tested, especially where cover crops or organic residues were added (Bandick and Dick, 1999).

Fluorescein Diacetate Hydrolysis

Fluorescein diacetate is a colorless compound hydrolyzed by both free and membrane-bound enzymes, resulting in the release of fluorescein. This end product absorbs strongly in the visible wavelength and can be measured by spectrophotometry. Several enzymes, such as non-specific esterases, proteases, and lipases, are responsible for FDA hydrolysis and are plentiful in the soil environment (Schnurer and Rosswall, 1982).

Adam and Duncan (2000) adapted the method from the original for the measurement of the total microbial activity in soils. A chloroform/methanol (2:1 v/v) solution is added immediately to terminate the reaction, instead of acetone. However, Green et al. (2006) have recently optimized the FDA method with the advantage of using

static incubation and less solvent to terminate the hydrolysis, and also covering a large range of activity. The FDA method is recommended for its sensitivity, simplicity and precision to be used for studies of soil microbial activity.

FDA hydrolysis has been correlated with some of the most accurate measures of microbial biomass, ATP content and density studies (Federle and Ventullo, 1990). In addition FDA hydrolysis was found to be effective in determining how alternatives to methyl bromide affect microbial activity (Fernandez, et al. 2001).

Dehydrogenase Activity

Dehydrogenase reflects the total oxidative activities of soil microorganisms (Alef and Nannipieri, 1995). The biochemical properties of dehydrogenases are such that free, abiontic quantities are not expected to be present in soil. Dehydrogenase activity is considered to be an indicator of biological oxidation/ reduction reactions and therefore can be used as a measure of the intensity of microbial metabolism in soil (Skujins, 1978).

Dehydrogenase activity is assayed on 1 g oven-dry equivalents of buffered soil solution incubated for 48 h at 27°C after addition of a specific substrate 2, 3, 5-triphenyltetrazolium chloride (TTC) (Lenhard, 1956). The product of the reaction, triphenyl formazan (TPF), is measured colorimetrically. Benitez, et al, (2000) studied enzyme activities in the rhizosphere of pepper. Dehydrogenase, as well as urease and phosphatase, activity were evaluated. Their results showed an increase of rhizosphere dehydrogenase activity after the addition of mulches and important effects of the incorporation of organic materials on urease and phosphatase activity. Klose and Ajwa, (2004) studied enzyme activities in agricultural soils after fumigation with methyl bromide alternatives. Their results showed a decrease of the activities of β-glucosidase,

dehydrogenase, and acid phosphatase; however, alternative fumigants had no significant effect on enzyme activity.

Arylamidase Activity

The enzyme arylamidase catalyzes the release of an N-terminal nitrogen amino acid from peptides, amides or arylamides. This enzyme is also probably involved in N mineralization. The method to evaluate arylamidase activity is based on the colorimetric determination of the B-naphthylamine produced when soil samples are incubated with L-leucine B-naphthylamide in 0.1 M THAM buffer solution at 37° C for 1 h. The B-naphthylamine produced is extracted with ethanol and converted into an azo compound, and the absorbance of the color is measured at 540 nm (Acosta-Martinez and Tabatabai ., 2000a). The importance of this enzyme in soil is related to its role in the release of amino acids from soil organic matter. The amino acids released by this enzyme are substrates for amidohydrolases. Therefore, arylamidase enzyme activity has been significantly correlated with soil organic C content and with the activity of L-asparaginase, L-aspartase, urease and amidase (Acosta-Martinez and Tabatabai, 2000b).

In general, FDA hydrolysis, dehydrogenase and arylamidase enzymatic procedures may have potential to be used as indicators of microbial activity since they reflect a wide range of physiological activities carried out by soil microorganisms. It is important to highlight the relation between enzymatic activity and soil health. In this respect, soil enzymes have a unique and appropriate role in assessing soil health because soil can be thought of as a living biological entity where several biochemical reactions, mediated by enzymes, are taking place.

3. Microbial Inoculants (PGPR)

The rhizosphere, the zone of soil under the influence of the root (Uren, 2000), is characterized by high microbial diversity, activity, number of organisms, and complex interactions between microorganisms and the roots (Cheng et al, 1996; Oger et al, 2004). To measure the effect of the rhizosphere on a particular population, the number of microorganisms in the rhizosphere (R) and the number of microorganism in the bulk soil (S) are compared. This R/S ratio provides an estimate of how strongly the rhizosphere affects a particular organism. This ratio also determines the rhizosphere competence and if an organism has a good rhizosphere competence, it could be used as a microbial inoculant (Pinton et al, 2000).

Bacteria are the most numerous inhabitants in the rhizosphere, typically numbering 10^6 to 10^8 organism g^{-1} of rhizosphere soil, although they account for only a small portion of the total biomass. The concentration of bacteria in the rhizosphere is higher than in bulk soil due to the production of root exudates that can support bacterial growth and metabolism (Bais et al, 2006).

The interaction between bacteria and plant roots may be beneficial, harmful, or neutral for the plant. The bacteria that provide benefits to the plant are of two general types, those that form a symbiotic relationship, which involves the formation of specialized structures as in the genus *Rhizobia*, and those that are free-living in the soil.

Beneficial free-living bacteria, referred to as plant growth-promoting rizhobacteria (PGPR) have been found in association of many different plants. PGPR can impact plant growth directly and indirectly (Whipps, 2001). The direct promotion of

plant growth by PGPR occurs when bacteria provide the plant with a compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment.

On the other hand, the indirect growth promotion is related with antagonism and induced systemic resistance (Glick, 1995). Antagonism, one of the major groups of mechanisms of biocontrol, is defined by Cook and Baker (1983) as the actively expressed opposition, which includes antibiosis, competition and parasitism. While induced resistance refers to the activation of the host plant's chemical or physical defenses by an inducing agent and may be systemic or localized within the plant. Thus, induced systemic resistance is used to describe the process whereby treatment of plants with PGPR elicits a host defense as indicated by reduction in the severity or incidence of diseases caused by pathogens that are spatially separated from the inducing agent (Kloepper et al, 2004). In some reports induced systemic resistance has been associated with enhancement of lignification and increases in peroxidase and superoxide dismutase activity (koike et al, 1997).

PGPR have been widely used in agriculture. The results of numerous studies on various crops conducted over the past two decades generally showed beneficial effects for increasing yield, germination rate, tolerance to drought, and shoot and root weights (Lucy et al, 2003). Another major benefit is their use as biological control agents of plant disease organisms (Zehnder et al, 2001).

Several PGPR inoculants are currently commercialized to promote growth using one of several mechanisms; suppression of plant disease, improvement of nutrient acquisition, or phyto-hormone production. Inoculants suppress plant diseases through the induction of systemic resistance, and production of siderophores or

antibiotics (Zahir et al., 1998). Examples of microbial inoculants are Bioyield®, which contains a mixture of two strains *(Bacillus subtilis* and *B. amyloliquefaciens*) and Soil Builder®, Ag Blend®, and Equity®, which contain complex mixtures of over 10 strain of *Bacillus*.

Since the rhizosphere is considered the most intense ecological habitat in soil, it is of interest to study the effects that PGPR may have on the total microbial activity and bacterial population in the zone where rhizobacteria exerted a direct influence on plants. The objectives of this study were to develop a set of sensitive methods to detect increases in microbial activity following additions of microbial inoculants and to determine the relationship among soil microbial activity, microbial population and disease suppressiveness after the addition of soil inoculants PGPR.

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ASSESSING SOIL MICROBIAL ACTIVITY FOLLOWING THE USE OF MICROBIAL INOCULANTS

1. Introduction

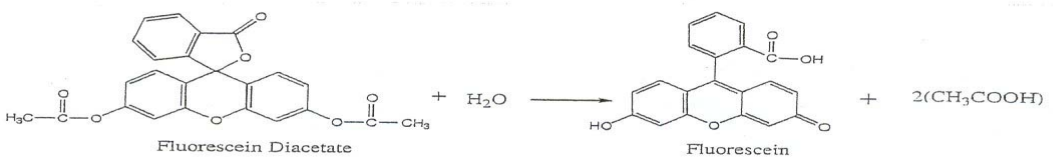
Soil is a highly complex system characterized by a variety of biological, chemical and physical processes, which are markedly influenced by environmental factors (Alef et al., 1995). Microorganisms influence soil productivity by recycling carbon, nitrogen and other minerals. Therefore, numerical abundance, fast reproductive rates, diversity of type and metabolic activity, and tolerance to a wide range of environmental conditions are key characteristics of soil microbial populations (Doran, 2002). In order to understand heterogeneity of soil, there is a need for suitable methods for studying interactions between environmental factors and microbial populations and activity in soil.

Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that colonize the rhizosphere, the surface of the root or even superficial intercellular spaces (McCulley, 2001). Applications of PGPR are usually accompanied by enhancement of plant growth or protection against certain pathogens. Several products containing PGPR strains are commercially available. However, very little is known about the influence of these inoculants on the soil ecosystem.

The term microbial activity comprises all biochemical reactions catalyzed by microorganisms in soil. Some reactions, such as respiration and heat output, can be

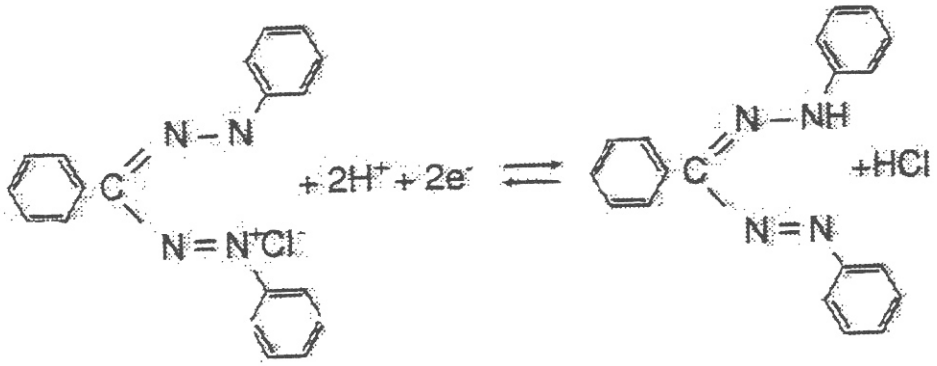
conducted by most soil microorganisms while others, such as nitrification and nitrogen fixation, can only be conducted by a restricted number of microbial species (Alef et al., 1995). In addition, differences in rhizo-deposition, plant species, age and stage of development may have directly influence microbial activity and population. Consequently, the approach of measuring overall microbial activity after application of PGPR may distinguish between treated and untreated rhizosphere soil.

Total microbial activity provides a general measure of organic matter turnover in natural habitats as about 90% of the energy in the soil environment flows through microbial decomposers (Heal and MaClean, 1975). There are several enzymatic methods for measuring total microbial activity. One method is fluorescein diacetate (3 ‘ 6’-diacetiylfluorscein (FDA) hydrolysis (Schnurer and Rosswall, 1982). Green et al. (2006) have optimized the method to assay FDA in soils by using a static incubation, reducing solvent to terminate the hydrolysis, and covering a large range of activity. As a result, the optimized method for FDA in soil samples can be used as a biochemical and biological indicator of soil quality. FDA is hydrolyzed by a number of different enzymes such as proteases, lipases and esterases. The product of this enzyme conversion is fluorescein, which can be seen within the cells by fluorescence microscopy or quantified by spectrophotometry (Schnurer and Rosswall, 1982). The equation of the reaction follows.



Fluorescein Diacetate Fluorescein

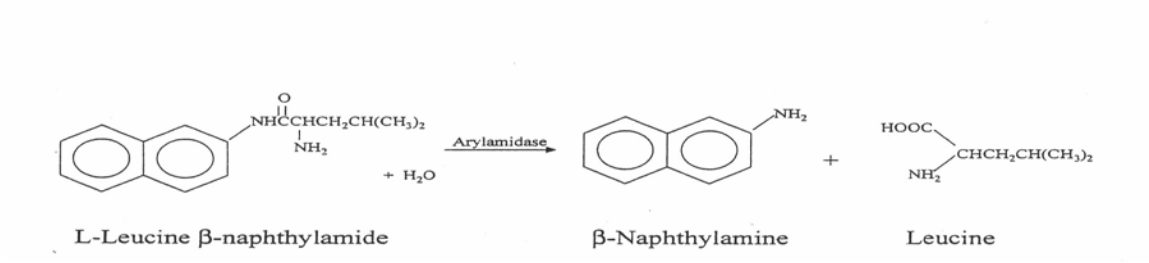
Another enzymatic assay to measure total microbial activity is through dehydrogenase activity. Dehydrogenase activity reflects the total oxidative activities of soil microorganisms, which is important in oxidizing soil organic matter. Dehydrogenase activity has been used as an indicator of microbial activity in response to successive addition of toxic organic wastes (Benitez et al, 2004). One of the most frequently used methods to estimate dehydrogenase activity in soil is based on the use of thiphenyltetrazolium chloride (TTC) as an artificial electron acceptor (Lenhard, 1956). The TTC is reduced to triphenil formazan (TPF) (Smith and Pugh, 1979). Nearly all microorganisms reduce TTC to TPF, which can be spectrophotometrically measured. The equation for the reaction follows (Alef and Nanniperi, 1995).



TTC TPF

Another enzyme with potential to measure total microbial activity in soil is arylamidase {α-aminoacyl-peptide hydrolase}, which is normally found in animal tissues and fluids (Hiwada et al, 1980) and has been recently detected in soil. An accurate method has also been developed for its assay (Acosta-Martinez and Tabatabai, 2000).

Arylamidase is capable of hydrolyzing the neutral amino acid β-naphthylamides or p-nitroanilides. The equation for the reaction using the amino acid L-leucine, as an example, is shown bellow.



The importance of studying the arylamidase activity in soil is related to its role in the N-cycling process (Stevenson, 1994).

In this study, FDA hydrolysis, dehydrogenase, and arylamidase activity were used as indicators/detectors of increases in microbial activity following the addition of PGPR microbial inoculants. Criteria for choosing enzyme assays were based on previous experiments with their sensitivity to detect soil microbial activity, importance in nutrient cycling and organic matter decomposition, and simplicity of the assay. Fluorescein diacetate hydrolysis (FDA) can be hydrolyzed by many enzymes (lipases, esterases and proteases) and organisms. Thus this assay provides a broad –spectrum indicator of soil biological activity. Dehydrogenases were chosen for their critical role in oxidative activities of soil microorganisms. Arylamidases were included because of their role in releasing inorganic N in the N cycle.

2. Materials and Methods

A. Field Studies

Field experiments were conducted at the Sand Mountain Research and Extension Center in spring 2005 to evaluate the effect of the application of microbial inoculants on

soil microbial activity and strawberry root architecture. Strawberry transplants were produced by Lewis Nursery (North Carolina); transplants were planted in the field in October 2004 and harvested in spring 2005. Plants were fertilized monthly with a solution of 20-20-20 fertilizer. The treatments consisted of three different inoculants and a water control. All of the microbial treatments were applied at the time of transplanting by using 34 g per liter at 1% aqueous suspension of the product and all subsequent times at 0.74 liters per hectare through the drip irrigation system.

Experimental Design:

The experimental design was a randomized complete block (RCB) with four treatments and six replications. Each plot contained 40 transplants in double rows. Treatments were (1) Ag Blend®, (2) Soil Builder®, (3) Equity® and (4) nontreated control (Table 1). Soil samples were taken once a month 10 days after the monthly microbial inoculation.

Soil Sampling

Soil was collected from the experimental plots 10 days after microbial inoculation. For each replication, five sub-samples were taken with a soil core borer to a depth of 20 cm in the strawberry root zone. Soil samples were then thoroughly mixed, placed in plastic bags, sealed, and deposited in a container with ice and brought to the laboratory. Soil samples were stored at 4ºC for four days and then sieved before measuring microbial activity.

Table 1. Commercial PGPR Products

Microbial Inoculants (PGPR)	Description	Dose
Naturize Equity®	Contains 47 strains of bacilli in a liquid formulation.	0.48g/L water
Organica Plant Growth Activator **(**PGA**)** ®	Contains 54 strains of bacilli, Pseudomonads, Actinomycetes, and Trichoderma in a powder carrier.	1 tablespoon/Gal water
Super bio Soil Builder®	Contains bacteria (Bacillus spp., actinomycetes, cyanobacteria, and others), algae, and protozoa in a liquid carrier.	118.4ml/Gal water
Super bio Ag Blend®	Contains multi-trophic community (anaerobic and aerobic, culturable and nonculturable Gram +, Gram – bacteria , actinomycetes, cyanobaceria, protozoa) in a liquid carrier.	33ml/Lwater

Total Microbial Activity

Microbial activity was assayed by measuring the hydrolysis of fluorescein diacetate (FDA). Two grams of soil (fresh weight, sieved <2 mm) were placed in a 50 ml conical flask, and 10 ml of 60 mM potassium phosphate buffer (pH 7.6) were added. One ml of the FDA stock solution (1mg FDA ml^{-1} acetone) was added to start the reaction. Flasks were stoppered and contents shaken by hand. Flasks were then placed in

an incubator at 37°C for 45 min. Once removed from the incubator, 10 ml of acetone were added immediately to terminate the reaction. Contents of the conical flasks were then transferred to 50 ml centrifuge tubes and centrifuged at 5000 RPM for approximately 5 min (MSE Scientific Instruments, Coolspin 2 centrifuge). The supernatant from each sample was poured into a vial to measure the optical density at 490 nm on a spectrophotometer (Hitachi U-1100 spectrophotometer). The concentration of fluorescein released during the assay was calculated using a calibration graph produced with standards from 0 to 5 µg fluorescein ml^{-1} which were prepared from a 20 µg fluorescein ml^{-1} standard solution. The 0 µg ml^{-1} fluorescein standard was used to calibrate the spectrophotometer to zero before each set of controls and samples were read (Schnurer and Rosswall, 1982).

Plant Growth Measurements

Plant growth measurements were performed at the second sampling 28 weeks after transplanting. One strawberry plant was harvested from each of the six replications, and analyzed. Plant material was stored over-night at 4ºC, and fresh and dry shoot and root weights (g), chlorophyll content, and growth index (cm^2) were recorded the following day. Root architecture was quantified by scanning on custom-made 20 x 20 glass tray using a HP Scan Jet 5370C (Hewlett Packard, Palo Alto, CA) and WhinRHIZO 5.0 (Regent Instruments, Quebec, Canada) computer program. Average root diameter (mm), total root length (cm), surface area (cm^2), total root volume (cm^3), and number of root tips were obtained using this software. Yield was also recorded from May until June, 2005.

Statistical Analysis

Data were statistically analyzed according to standard procedures for analysis of variance (GLM) and means separation using least significant difference (LSD) procedures (SAS Institute, Cary, NC). All differences referred to were significant at the 95% confidence level.

B. Greenhouse Studies:

Greenhouse experiments were conducted at Auburn University in fall 2005, to develop a set of sensitive methods to detect increases in microbial activity following additions of microbial inoculants (PGPR).

Seedlings and Soil:

Tomato (*Lycopersicon esculentum*) transplants were produced in the greenhouse at Auburn University. Seeds of tomato hybrid "Juliet" were planted into 32 cell trays and grown for three weeks using overhead irrigation. Transplants were fertilized weekly with 15-30-15 soluble fertilizer. Soil, collected from the E. V. Smith Agricultural Experimental Station, was autoclaved and mixed with autoclaved sand (3:1 soil: sand) before being placed in 10-inch pots. Seedlings were planted and each pot was inoculated with microorganisms the following day by drenching soil with 100 ml of a stock solution. The stock solution was prepared by diluting the commercial dose of the microbial inoculants in water as shown in Table 1.

Experimental Design:

The experimental design was a randomized complete block (RCB) with four treatments and three replicates for each sampling time. Treatments were (1) Equity, (2) PGA, (3) AG blend and (4) non-treated control. Samples were taken at 1, 3, 5, 7 and 10

days after the first inoculation. Pots were re-inoculated fifteen days later with the stock solution and sampled at the same time intervals.

Sampling:

At each sampling time three replicates of each treatment were harvested. Roots were shaken to remove loosely attached soil, and the corresponding rizhosphere soil was placed in plastic bags, sealed and brought into the lab for measurement of microbial activity and microbial populations. Fresh and dry shoot and root weights (g), height (cm), number of leaves, chlorophyll content, stem diameter (mm), and growth index (cm^2) were also measured as well as the root architecture for every plant. The root architecture was quantified using winRHIZO algorithms.

Microbial Activity

Total microbial activity was assessed by measuring FDA hydrolysis, dehydrogenase, and arylamidase activity. From each soil sample, procedures were run in triplicate. FDA activity was measured using the procedure suggested by Schnurer and Rosswall (1982). Dehydrogenase activity was assayed on 1g samples of soil. The soil was weighed and placed into test tubes and mixed with 1 ml of TTC (Triphenyl Tetrazolium Chloride) solution. Test tubes were sealed and incubated for 96 hours at 27° C. After incubation, 10 ml of methanol were added to each tube, and the tubes were shaken thoroughly for 30 seconds and then further incubated at room temperature for 3 hours. The soil suspension was then transferred into vials, and the optical density was measured at 485 nm. Standards were made by pouring 0.01 g of TPF (triphenylformazan) into a 100 ml flask with ethanol to obtain concentrations between 0-50 μg of TPF ml^{-1} (Runion, personal communication).

To evaluate arylamidase activity, one gram of soil from each sample was placed in a 25 ml test tube and treated with 3mL of 0.1 M THAM buffer solution (pH 8.0) and 1mL of 8.0 mM L- leucine β-naphthylamide hydrochloride. The test tube was swirled for a few seconds to mix the contents and was stoppered and placed on a shaker in an incubator at 37°C for 1 hour. After incubation the reaction was stopped by adding 6 ml of ethanol (95%). The soil suspension was immediately mixed and transferred into a centrifuge tube and centrifuged for 5 minutes at 8000 rpm. The supernatant was transferred to a test tube and a 1 ml aliquot of this supernatant was treated with 1 ml of ethanol, 2 ml of acidified ethanol and 2 ml of ρ-dimethylaminocinnamaldehyde. The solution was mixed in a vortex and the intensity of the resulting solution was measured using a spectrophotometer at 540 nm (Hiwada et al, 1977).

Microbial Population

The Most Probable Number (MPN) technique was used for quantifying microbial populations. One gram of rhizosphere soil was added to 50 ml sterile water in 125 ml Erlenmeyer flasks and shaken for 20 minutes. One ml of the supernatant was placed in a test tube containing Tryptic Soy Broth (TSB) and serial dilutions were made. Test tubes were incubated for 48 hours at 27ºC. After incubation, the broth tubes were observed for the presence or absence of growth and the log cfu/g of all treatments were calculated (Garthright, 2001).

Statistical Analysis

Microbial activity and microbial population values were statistically analyzed according to standard procedures for analysis of variance (GLM), comparison methods (Contrast), correlation, and regression procedures. Root and shoot measurements, as well

as root architecture data were analyzed by mean separation (least significant difference) (SAS Institute, Cary, NC). All differences referred to were significant at the 95% confidence level.

3. Results

A. Field Studies

Total Microbial Activity

Soil in the strawberry field test was sampled four times: in December 2004; in April, May and June 2005. Hydrolysis of FDA was significantly different among treatments at the third sampling, but not at other sampling time. At this time microbial activity in AG Blend and Soil Builder treatments were significantly greater than the activity in the nontreated control (Table 2.1). In the last sampling there was a reduction in total microbial activity estimated by FDA; however, the differences among treatments were not significant (Table 2.2).

Plant Growth Measurements and Root Architecture

The root architecture and other growth parameters assessed from plants in the strawberry field test also showed differences among treatments in the third sampling. Treatments with AG Blend, Soil builder, and Equity had greater growth index values compared to the control (Table 3.1). Relative to the nontreated control treatment with AG Blend significantly increased the average root diameter, surface area, total root volume (cm^3), and shoot dry weight. Treatment with Equity significantly increased surface area and dry shoot weight compared to the nontreated control (Tables 3.1 and 3.2). The control had the largest root length value compared to the treatments in which microbial inoculants were applied (Table 3.2). In the last sampling (June 2005),

treatments with Equity showed a greater mean root length (246.08 cm) and number of tips (402.83) than did Soil Builder (Table 3.3). During this field test, yield was recorded weekly from May to June. The cumulative yield, expressed as total fruit weight per plot, was significantly greater for Ag Blend than the control (Table 3.4). Treatments with Soil Builder and Equity had no significant difference in yield compared to the control.

B. Greenhouse Studies

Total Microbial Activity

The three procedures used to detect microbial activity after the application of microbial inoculants were measured in both bulk and rhizosphere soil. Bulk soil was considered that which was not attached to the roots. FDA hydrolysis, dehydrogenase and arylamidase were assessed. Results in the bulk soil indicated no difference among treatments at any sampling time.

In the rhizosphere soil, FDA hydrolysis consistently was significantly greater than the control at five, seven and ten days after inoculation (DAI) with the PGPR products (Fig. 1). There was no significant difference among the treatments and the control at 1 and 3 DAI. This was true even though the FDA procedure reflected activity due to the fact that values were different than zero. Treatments with Equity (1.33) and PGA (1.40) showed significantly greater activity than the control (0.83) five DAI. All three PGPR products showed an increase in total microbial activity as follows, Equity (0.78), PGA (0.99) AG Blend (0.78) compared to the control (0.29) 7 DAI. FDA procedure also detected changes in the microbial activity due to the application of inoculants at 10 days; however, the magnitude of activity was decreasing, following the same trend across the time from the 3-10 DAI for all the treatments (Fig 1). The commercial products were

reapplied at 15 DAI using the previous dose. However, FDA hydrolysis was not sensitive enough to discriminate between treatments (Table 4.1). Overall, FDA hydrolysis was an indicator of microbial activity in the rhizosphere soil, although the procedure was not sensitive enough to detect changes at every sampling time.

In the rhizosphere soil, dehydrogenase activity (DHA) detected changes in the microbial activity at 5 DAI only for treatments with PGA (0.62) and AG Blend (0.76), although activity in these treatments was lower than the control (1.04) (Table 4.2). During the test the DHA values were increasing and decreasing without a recognizable pattern for all of the treatments after the first PGPR application. After treatments were re-applied, activity increased 3 DAR-I (days after re-inoculation), decreased 5 days after re-inoculation (DAR-I) and remained steady through the end, regardless of the procedure's inability to detect a major variation suitable to the treatments (Table 4.3).

In the rhizosphere soil, arylamidase activity was significantly higher than the control at 1 DAI (45.38) and 3 DAR-I (42.70) for Equity treatment (Table 4.3). Microbial activity decreased to 16 at 7 DAI and increased at 10 DAI and 3 DAR-I for all treatments (Fig 3). As for FDA hydrolysis and dehydrogenase activity, values for this procedure varied considerably in a short interval. The fact that even the control was following the same deviation trend as the treatments could be an indicator of the existence of environmental factors that were affecting the response other than PGPR products.

Microbial Population

Microbial inoculants (PGPR) did not show any significant effect on total population estimated by MPN after the first or second PGPR application (Fig 2). Total population was not different in the rhizosphere compared to the bulk soil.

Plant Growth Measurements and Root Architecture

PGPR products had effects on plant growth and root architecture; however, they were not consistent through time. As early as 1 DAI, fresh root weight and root tips were significantly higher with PGPR treatments compared to the control (Table 6.1). This increase in number of tips was also found at 3 and 5 DAI and at 5 DAR-I. At 3 DAI, treatment with Equity had an increase in root length and root surface area, while Ag Blend treatment had an increase in dry shoot weight (Table 6.2). Plants and roots sampled at 5 and 7 DAI had higher height, number of leaves, root stem diameter, and dry root weight with Ag Blend (Table 6.3, Table 6.4). Ag Blend maintained this positive response on fresh and dry root weight and root surface area at 10 DAI. Chlorophyll content and growth index were also greater (Table 6.5). At 1 DAR-I, Ag Blend had a positive effect on root stem diameter and root surface area (Table 6.6). The same positive effect of Ag Blend was also observed at 3 DAR-I on number of leaves, chlorophyll content, root stem diameter, and root length (Table 6.7). Roots sampled at 5 DAR-I had higher fresh and dry root weight for Equity and Ag Blend treatments compared to the control (Table 6.8). As shown in the previous sampling at 7 and 10 DAR-I, plants in the Ag Blend treatment also had an increase in root weight and growth index (Table 6.9, Table 6.10). In the last sampling at 15 DAR-I, height of plants was significantly greater for all the PGPR treatments when compared to the control, while chlorophyll content, growth index, and fresh shoot weight were only greater for the Ag Blend treatment (Table 6.11). Analyses of the variables associated with plant growth indicated that the commercial PGPR products were related to important changes in the tomato root architecture differentially throughout the test.

4. Discussion

Rhizosphere microbial activity involves several interactions among soil, plant and microorganisms. Microbial metabolites (plant growth regulators, phytotoxins, antibiotics, root exudates) and other compounds (enzymes, siderophores, and molecular signals) produced by both microorganisms and plants can also affect the microbial activity in soil (Pinton et al, 2000). Beneficial bacteria that promote plant growth (PGPR) have been widely studied in recent years (Zehnder et al, 2001) showing positive effects on both plant growth and health. These beneficial bacteria, when applied onto the seed or root, colonize and multiply, thereby contributing to the complex ecological interactions in the rhizosphere. Different methodologies related to microbial activity, population and diversity have been suggested to quantify introduced bacteria.

In these experiments FDA hydrolysis, dehydrogenase, and arylamidase assays which measure microbial activity were assessed in tomato rhizosphere soil. The results showed that these procedures measured the overall microbial activity, but they did not detect increases in activity consistently across time after PGPR application.

FDA hydrolysis has been reported as a promising tool for estimation of biofilms metabolic and physiological activities (Battin, 1997), and for determination of the overall microbial activity in soil (Shnurer and Roswall, 1982). FDA hydrolysis was also sensitive enough to detect increases in activity in the rhizosphere after the application of a nematicide (Fernandez et al, 2001) and for predicting suppressiveness to damping-off in sphagnum peat container media (Inbar et al, 1991). The procedure has also been standardized for use in a wide range of soils (Adam and Duncan, 2000; Green et al, 2006). According to these previous studies, FDA hydrolysis can detect microbial activity

in a wide range of environments. However it was not consistently sensitive enough for the purpose of this study. The fluorescein diacetate (FDA) assay depends on the hydrolysis of FDA to yellow-green fluorescent compound by non-specific esterases present in actively metabolizing microbes (Chand et al, 1994; Tsuji et al, 1995). It is generally assumed that FDA diffuses freely into intact cells (Rotman and Papermaster, 1966), where esterases hydrolyze it and the intracellular accumulation of fluorescein results in a useful indicator of cell activity. FDA hydrolysis is a test used only on living cells that convert FDA to fluorescein. Clark et al (2001) concluded that there are other extracts capable of the same conversion in the absence of live cells. It may be possible to find rhizosphere soil compounds that exhibit the same conversion. If that were the case, the FDA procedure would estimate not only the microbial activity but also the contribution from other sources, which could explain the lack of consistency in detecting changes in activity after PGPR application.

The microbial activity for the nontreated soil was similar to that where treatments were reapplied. This may indicate several diverse interactions among indigenous and introduced bacterial communities. Soil microbial communities are affected by plant species, soil pH, aeration, and physico-chemical characteristics (Miethling et al 2000; Marschner et al, 2001). Knowing all these key parameters could be relevant in determining correlations among physicochemical and biological indicators and their effect on total microbial activity for treated and nontreated rhizosphere soil. Regarding these interactions, Ownley et al (2003) reported an improvement in the biological control performance of phenazine-producing *Pseudomonas fluorescens* after the identification of key soil factors. In the same way these key factors also may affect the performance of

other PGPR genera, such as *Bacillus*, and could give us a better understanding of their interaction in the rhizosphere, and how they are influenced by introduced bacteria.

Total bacterial populations, estimated by MPN, did not change and did not increase when the procedures used to measure microbial activity were showing changes. A common statistic for indicating the strength of linear relationship existing between microbial activity and bacterial population was used. A negative correlation (-0.7, p=0.03) was only found 7 DAI. This indicates that inoculated microorganisms maintain in general steady levels of total culturable population in the rhizosphere and consequently there is not a correlation with total microbial activity.

In this study, with some exceptions, PGPR products exhibited a positive influence on plant growth parameters, root architecture, and yield. Consequently we determined an important correlation in changes in specific root morphological variables due to PGPR. The most consistent response obtained was with Ag Blend. This product contains metabolites of multitrophic bacterial communities, while Equity, Soil Builder, and PGA only contain the bacterial strains.

We hypothesized that microbial activity in the rhizosphere increases after the addition of PGPR and it was related to culturable bacterial population. However, this increase was not detected by the procedures used, and based on the results there was not a correlation between activity and population. In conclusion, FDA hydrolysis, dehydrogenase, and arylamidase may serve as indicators of microbial activity, but they were not sensitive enough to detect changes consistently due to PGPR application. FDA was consistent at least in three consecutive samplings after the first inoculation, while neither Arylamidase nor DGH were different than the control in almost all cases.

The questions that remain unanswered are the following. First, assuming that total culturable rhizosphere bacteria populations do not change, what changes are caused in the rhizosphere after the application of PGPR? Second, are PGPR really contributing to changes in the overall microbial activity? Although the methods used in this study have advantages and limitations, it may be possible to improve them for monitoring bacterial dynamics in the rhizosphere.

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Table 2.1 Effect of commercial PGPR products on fluorescein diacetate hydrolysis (FDA) as a measure of total microbial activity in strawberry rhizosphere soil (May 2005)

Treatment Commercial PGPR products	FDA*
1. AG Blend®	2.46[a]
2. Soil Builder®	2.50[a]
3. Equity®	2.40 [ab]
4. Control	1.96[b]
$LSD_{0.05}$	0.4942

Means followed by the same letter are not significantly different.

***Represents µg fluorescein/g dry soil per hour**

Table 2.2 Effect of commercial PGPR products on fluorescein diacetate hydrolysis (FDA) as a measure of total microbial activity in strawberry rhizosphere soil (June 2005)

Treatment Commercial PGPR products	FDA*
1. AG Blend®	0.8276[a]
2. Soil Builder®	0.8856[a]
3. Equity®	0.7583 [a]
4.Control	0.7676[a]
$LSD_{0.05}$	0.361

Means followed by the same letter are not significantly different.

*** Represents µg fluorescein/g dry soil per hour**

Table 3.1 Effect of Commercial PGPR products on strawberry plant growth (May 2005)

Treatment	Root fresh weight (g)	Shoot dry weight (g)	Growth index* (cm^2)
1. AG Blend®	11.318 [a]	53.350 [a]	1074.64 [a]
2.Soil Builder®	11.140 [a]	46.817 [ab]	1058.55 [a]
3.Equity®	12.294 [a]	60.549 [a]	1024.39 [a]
4.Control	10.179 [a]	37.517 [b]	894.65 [b]
$LSD_{0.05}$	4.743	13.744	108.03

Means followed by the same letter within a column are not significantly different.

*** Growth index was calculated as height (cm) x width (cm).**

Table 3.2 Effect of Commercial PGPR products on strawberry root architecture* (May 2005)

Treatment	Total root length (cm)	Root surface area (cm^2)	Root volume (cm^3)	Mean root diameter (mm)	Number of root tips
1.AG Blend®	139.13[b]	304.74[a]	80.12[a]	9.292[a]	296.50[a]
2.Soil Builder®	139.72[b]	269.68[ab]	51.01[ab]	7.380[ab]	302[a]
3. Equity®	137.342[ab]	323.87[a]	61.02[ab]	7.713[ab]	365.50[a]
4.Control	218.06[a]	233.78[b]	20.45[b]	3.278[b]	310.17[a]
$LSD_{0.05}$	76.983	67.803	53.732	5.7612	120.02

Means followed by the same letter are not significantly different.

*** Root architecture parameters were assessed with Whinrhizo 5.0 computer**

Table 3.3 Effect of Commercial PGPR products on strawberry root architecture* (June 2005)

Treatment	Total root length (cm)	Root surface area (cm^2)	Root volume (cm^3)	Mean root diameter (mm)	Number of root tips
1.AG Blend®	222.24[ab]	343.68 [a]	45.47 [a]	5.03 [a]	361.17[ab]
2.Soil Builder®	175.62[b]	306.63 [a]	45.42 [a]	5.50 [a]	311.02[b]
3. Equity®	246.08[a]	297.14 [a]	33.27 [a]	4.12 [a]	402.83[a]
4.Control	193.44[ab]	344.75 [b]	60.52 [a]	6.33[a]	326.00[a]
$LSD_{0.05}$	63.034	105.31	36.59	2.67	120.02

Means followed by the same letter are not significantly different.

*** Root architecture parameters were assessed with Whinrhizo 5.0 computer**

Table 3.4 Effect of Commercial PGPR products on strawberry yield

Treatment	Yield (g)
1. AG Blend®	11275 [a]
2.Soil Builder®	8814 [b]
3.Equity®	9580 [b]
4.Control	8940 [b]
$LSD_{0.05}$	2415

Means followed by the same letter are not significantly different.

Table 4.1 Effects of commercial (PGPR) products on fluorescein diacetate hydrolysis as a measure of total microbial activity in tomato rhizosphere soil

Treatment	FDA					
	Days After Re-Inoculation					
	1	3	5	7	10	15
1. Equity®	0.85	1.30	0.73	0.47	1.64	0.65
2. PGA®	1.17	0.99	0.69	0.50	0.96	0.90
3. Ag Blend®	0.94	0.98	0.70	0.68	1.12	0.75
4. Control	1.10	0.97	0.69	0.61	0.78	0.80

*** Indicates significant difference from the control**

Fluorescein diacetate (FDA) hydrolysis in µg fluorescein /h g of dry soil

Table 4.2 Effect of commercial PGPR products on total microbial activity measured by dehydrogenase activity in tomato rhizosphere soil

Treatment	Dehydrogenase Activity				
	Days After Inoculation				
	1	**3**	**5**	**7**	**10**
1. Equity®	1.18	1.20	0.98	1.04	1.39
2. PGA®	0.75	1.22	0.62*	0.98	1.48
3. Ag Blend®	0.70	0.62	0.76*	1.61	1.83
4. Control	1.27	0.95	1.04	2.53	1.57

*** within a column indicates significant difference from the control**

Dehydrogenase nmol triphenilformazan-TPF/ g dry soil h

Table 4.3 Effect of commercial PGPR products on total microbial activity measured by dehydrogenase activity in the tomato rhizosphere soil

Treatment	Days After Inoculation				
	1	3	5	7	10
1. Equity®	1.22	1.79	0.25	0.23	0.29
2. PGA®	0.41	0.66	0.32	0.29	0.04
3. Ag Blend®	0.39	0.76	0.34	0.29	0.04
4. Control	0.36	0.65	0.24	0.23	0.12

*** Indicates significant difference from the control**

Dehydrogenase nmol triphenilformazan-TPF/ g dry soil h

Table 4.4 Effect of commercial PGPR products on microbial activity measured by Arylamidase activity in the tomato rhizosphere soil

Treatment	Days After Inoculation					Days After Re-Inoculation				
	1	3	5	7	10	1	3	5	7	10
1. Equity	**45.38***	33.02	25.73	16.75	32.29	19.25	**42.7***	14.82	14.47	12.90
2. PGA	23.28	25.39	22.05	17.07	40.85	19.83	33.65	19.25	17.67	12.48
3. Ag Blend	23.73	23.16	18.93	14.95	39.67	21.80	30.69	16.60	17.47	16.63
4. Control	20.93	27.32	23.24	16.03	36.01	24.59	34.57	18.66	23.40	16.05

*** Indicates significant difference from the control**

Arylamidase activity in nmol x 10^2 β-naphthylamine /g dry soil h

Table 5.1 Effect of Microbial Inoculants (PGPR) on root architecture related variables and plant growth measurements one day after inoculation

Treatment	SHOOT MEASUREMENTS						ROOT MEASUREMENTS						
	H (cm)	NL	CH	SD (mm)	SFW (g)	SFW (g)	RFW (g)	RFW (g)	L (cm)	SA (cm^2)	V (cm^3)	D (mm)	NT
1. Equity	12.33a	5.30a	27.96a	3.37a	2.38a	0.363a	4.67a	0.051a	99.02a	60.85a	2.97a	1.93a	432.0a
2. PGA	11.67a	4.67a	28.76a	3.55a	2.67a	0.294a	4.95a	0.061a	96.74a	58.14a	2.81a	1.92a	361.3a
3. Ag Blend	13.00a	5.00a	28.93a	3.53a	3.18a	0.344a	4.71a	0.240a	90.31a	53.76a	2.57a	1.90a	355.6a
4. Control	12.13a	4.67a	28.73a	3.37a	2.30a	0.345a	4.37b	0.046a	84.28a	50.75a	2.47a	1.89a	263.0b
$LSD_{0.05}$	2.06	0.94	2.3	0.623	1.02	0.182	0.214	0.24	20.65	18.2	1.35	0.45	79.9

Means followed by the same letter within a column are not significantly different.

H: Height
NL: Number of leaves
CH: Chlorophyll
SD: Stem diameter
SFW: Shoot fresh weight
SDW: Shoot dry weight
RDW: Root dry weight
L: Length
SA: Surface area
V: Volume
D: Root diameter
NT: Number of tips
RFW: Root fresh weight

Table 5.2 Effect of Microbial Inoculants (PGPR) on root architecture related variables and plant growth measurements three days after inoculation

Treatment	SHOOT MEASUREMENTS						ROOT MEASUREMENTS						
	H (cm)	NL	CH	SD (mm)	SFW (g)	SFW (g)	RFW (g)	RFW (g)	L (cm)	SA (cm^2)	V (cm^3)	D (mm)	NT
1. Equity	13.16a	6.3a	33.2a	3.81a	3.10a	0.55b	6.7ab	0.253a	146.3a	99.52a	5.46a	2.16a	732.0a
2. PGA	12.83a	6.0a	34.0a	3.72a	3.08a	0.60b	5.6b	0.077a	115.3ab	92.56ab	6.25a	2.56a	533.0a
3. Ag Blend	11.83a	6.0a	35.4a	3.95a	3.72a	0.91a	7.2a	0.058a	115.7ab	79.71ab	4.44a	2.21a	503.6a
4. Control	12.16a	6.3a	34.7a	4.42a	3.64a	0.56b	6.7ab	0.063a	97.96b	58.65b	2.47a	1.91a	361.3c
$LSD_{0.05}$	3.37	1.21	2.94	0.84	1.9	0.13	1.38	0.291	33.44	37.56	3.74	0.785	141.67

Means followed by the same letter are not significantly different.

Table 5.3 Effect of Microbial Inoculants (PGPR) on root architecture related variables and plant growth measurements five days after inoculation

Treatment	SHOOT MEASUREMENTS						ROOT MEASUREMENTS						
	H (cm)	NL	CH	SD (mm)	SFW (g)	SFW (g)	RFW (g)	RFW (g)	L (cm)	SA (cm^2)	V (cm^3)	D (mm)	NT
1. Equity	18.0b	7ab	34.47ab	4.9ab	6.73b		5.11a	0.160a	96.02a	148.15a	11.3a	3.00a	717.7a
2. PGA	15.6c	5c	33.46b	4.7b	5.46b		5.42a	0.085b	104.75a	100.80a	7.8a	2.92a	731.3a
3. Ag Blend	20.6a	7a	35.66a	5.5a	11.47a		5.09a	0.180a	126.49a	128.45a	10.5a	3.21a	534.0a
4. Control	18.0b	6bc	33.90ab	4.7b	6.30b		4.88a	0.146ab	99.57a	101.28a	8.3a	3.26a	333.3c
$LSD_{0.05}$	1.215	1.08	2.15	0.76	1.71		0.658	0.062	70.7	53.52	5.44	0.87	101

Means followed by the same letter are not significantly different.

Table 5.4 Effect of Microbial Inoculants (PGPR) on root architecture related variables and plant growth measurements seven days after inoculation

Treatment	SHOOT MEASUREMENTS						ROOT MEASUREMENTS						
	H (cm)	NL	CH	SD (mm)	SFW (g)	SFW (g)	RFW (g)	RFW (g)	L (cm)	SA (cm^2)	V (cm^3)	D (mm)	NT
1. Equity	19.16b	8.3b	38.3b	4.86a	10.31c	1.3b	2.9a	0.82a	185.48a	155.48a	11.48a	2.85a	448.67a
2. PGA	19.16b	8.3b	37.3b	5.71a	11.49b	1.4b	2.8a	0.45a	182.25a	166.87a	12.55a	3.04a	431.67a
3. Ag Blend	23.33a	9.6a	41.0a	5.67a	17.57a	1.9a	2.2a	0.42a	139.60a	160.84a	14.86a	3.66a	363.33a
4. Control	19.33b	9.0ab	37.4b	4.98a	10.23c	1.3b	2.2a	0.26a	130.90a	166.91a	18.65a	4.44a	359.00a
$LSD_{0.05}$	2.22	0.94	2.35	1.16	1	0.26	0.92	1.05	76.5	29.34	9.78	2.04	114.38

Means followed by the same letter are not significantly different.

Table 5.5 Effect of Microbial Inoculants (PGPR) on root architecture related variables and plant growth measurements ten days after inoculation

Treatment	SHOOT MEASUREMENTS							ROOT MEASUREMENTS						
	H (cm)	NL	CH	SD (mm)	GI (cm^2)	SFW (g)	SFW (g)	RFW (g)	RFW (g)	L (cm)	SA (cm^2)	V (cm^3)	D (mm)	NT
1. Equity	56a	10.3b	37.3a	6.70a	2260ab	39.67a	8.4a	4.92a	0.348a	224.57a	306.7a	34.93a	4.57a	547a
2. PGA	56a	11.3ab	37.2b	7.06a	2682a	36.48a	13.2a	2.75c	0.331a	218.28a	252.2b	26.27ab	4.11a	474a
3. Ag Blend	54a	13.3a	43.0a	7.67a	2642a	46.42a	13.7a	3.62b	0.401a	190.93a	224.4c	21.04b	3.62a	474a
4. Control	51a	11.3ab	38.1b	7.43a	1868b	40.90a	9.8a	2.40c	0.212b	174.25a	177.7c	14.37b	3.22a	395a
$LSD_{0.05}$	5.35	2.17	3.1	1.66	530.75	10.72	6	0.83	0.1	101.27	15.6	12.26	1.92	169.2

Means followed by the same letter are not significantly different.

Table 5.6 Effect of Microbial Inoculants (PGPR) on root architecture related variables and plant growth measurements one day after re-inoculation

Treatment	SHOOT MEASUREMENTS							ROOT MEASUREMENTS						
	H (cm)	NL	CH	SD (mm)	GI (cm^2)	SFW (g)	SFW (g)	RFW (g)	RFW (g)	L (cm)	SA (cm^2)	V (cm^3)	D (mm)	NT
1. Equity	42.0a	10.33a	41.4a	7.7a	2540a	39.57a	8.43a	10.55a	0.91a	191.2a	494.9ab	154.4a	12.6a	572.3a
2. PGA	43.0a	11.66a	39.9a	6.8bc	2580a	36.48a	13.16a	12.24a	0.92a	148.6a	507.5ab	145.2a	11.2a	381.3a
3. Ag Blend	47.6a	11.66a	41.5a	7.2ab	2156a	46.42a	13.76a	10.26a	1.01a	191.9a	587.4a	133.5a	9.4a	452.0a
4. Control	50.3a	10.33a	36.4a	3.5c	1974a	40.90a	9.86a	9.98a	0.69a	173.2a	441.2b	97.0a	8.9a	533.3a
$LSD_{0.05}$	8.66	1.95	9.8	0.63	913.88	10.7	6	3.36	0.56	57.96	92.6	70.55	5.2	235.93

Means followed by the same letter are not significantly different.

Table 5.7 Effect of microbial inoculants (PGPR) on root architecture related variables and plant growth measurements three days after re-inoculation

Treatment	SHOOT MEASUREMENTS							ROOT MEASUREMENTS						
	H (cm)	NL	CH	SD (mm)	GI (cm^2)	SFW (g)	SFW (g)	RFW (g)	RFW (g)	L (cm)	SA (cm^2)	V (cm^3)	D (mm)	NT
1. Equity	49.0a	11.0ab	35.5b	6.37b	1910.3a	31.8b	6.86a	11.54a	1.10a	214.7ab	480.0ab	88.6a	7.4ab	593.3a
2. PGA	53.7a	10.0b	34.8b	6.93b	2813a	44.3ab	6.13a	12.24a	1.34a	282.0a	370.3b	63.2a	5.4b	669.7a
3. Ag Blend	50.3a	12.3a	46.6a	7.85a	2674a	50.1a	7.96a	12.68a	1.37a	173.6b	449.7ab	92.8a	8.3ab	727.0a
4. Control	55.0a	10.3ab	36.7b	6.38b	2018a	44.1ab	7.03a	13.02a	1.16a	173.5b	535.4a	84.7a	10.8a	548.0a
$LSD_{0.05}$	7.74	2.24	3.61	0.88	917.33	12.84	3.11	3.57	0.6	90.8	153.01	30.5	4.2	243.55

Means followed by the same letter are not significantly different.

Table 5.8 Effect of microbial inoculants (PGPR) on root architecture related variables and plant growth measurements five days after re-inoculation

Treatment	SHOOT MEASUREMENTS							ROOT MEASUREMENTS						
	H (cm)	NL	CH	SD (mm)	GI (cm^2)	SFW (g)	SFW (g)	RFW (g)	RFW (g)	L (cm)	SA (cm^2)	V (cm^3)	D (mm)	NT
1. Equity	54.00a	11.33a	41.0ab	7.13a	2823a	46.26a	14.7a	15.93a	2.95a	259.62a	535.43a	86.45a	6.56a	925.7a
2. PGA	46.67b	10.66a	33.3b	7.08a	2373a	38.66a	11.4a	14.19ab	1.66b	257.60a	517.44a	85.80a	6.76a	760.3b
3. Ag Blend	47.67b	12.33a	43.9a	7.20a	2553a	52.66a	12.2a	16.96a	3.22a	155.69a	493.13a	125.75a	10.37a	545.0c
4. Control	55.67a	10.67a	38.3ab	6.33a	2589a	43.06a	13.9a	12.00b	1.63b	177.82a	516.97a	129.70a	10.00a	601.6c
$LSD_{0.05}$	4.21	1.95	8.36	1.5	748.5	14.08	5.41	3.6	1.2	113.96	84.84	54.67	4.37	136.55

Means followed by the same letter are not significantly different.

Table 5.9 Effect of microbial inoculants (PGPR) on root architecture related variables and plant growth measurements seven days after re-inoculation

Treatment	SHOOT MEASUREMENTS							ROOT MEASUREMENTS						
	H (cm)	NL	CH	SD (mm)	GI (cm^2)	SFW (g)	SFW (g)	RFW (g)	RFW (g)	L (cm)	SA (cm^2)	V (cm^3)	D (mm)	NT
1. Equity	48.0b	12a	33.7b	6.38c	1891a	31.11b	7.5b	7.83b	1.03b	188.23ab	473.88a	102.98a	9.0b	481.3a
2. PGA	53.3a	12a	35.9b	7.06ab	2373a	36.16ab	15.8a	9.06ab	1.13b	203.62a	481.73a	102.26b	8.2b	486.0a
3. Ag Blend	51.6ab	13a	48.9a	7.59a	2042a	46.28a	15.6a	12.67a	1.96a	73.53b	534.37a	319.06a	23.9a	464.6a
4. Control	49.3ab	11a	35.4b	6.76bc	1854a	33.17ab	10.7ab	9.56ab	1.6ab	187.37ab	494.73a	124.00b	9.6b	545.6a
$LSD_{0.05}$	4.51	1.71	5.92	0.6	860.7	11.1	7.4	4.72	0.73	124.15	114.52	110.75	8.5	222

Means followed by the same letter are not significantly different.

Table 5.10 Effect of microbial inoculants (PGPR) on root architecture related variables and plant growth measurements ten days after re-inoculation

Treatment	SHOOT MEASUREMENTS							ROOT MEASUREMENTS						
	H (cm)	NL	CH	SD (mm)	GI (cm^2)	SFW (g)	SFW (g)	RFW (g)	RFW (g)	L (cm)	SA (cm^2)	V (cm^3)	D (mm)	NT
1. Equity	56a	10.33b	37.36b	6.7a	2261ab	39.32b	11.06b	1.06b	1.53b	76.17a	470.57a	274.17a	23.26a	294.67b
2. PGA	56a	11.33ab	37.20b	7.1a	2682a	39.67b	9.06bc	10.70b	3.43ab	114.71a	470.61a	144.4ab	12.81ab	448.33ab
3. Ag Blend	54a	13.33a	43.06a	7.7a	2661a	60.51a	18.73a	17.86a	6.06a	79.78a	519.67a	200.17ab	13.59ab	430.00b
4. Control	51a	11.33ab	38.06b	7.4a	1868b	36.20b	6.46c	11.06a	1.93b	93.58a	489.51a	96.40b	7.96b	601.33a
$LSD_{0.05}$	5.35	2.17	3.06	1.67	444.7	10.38	2.66	2.4	2.87	98.8	78.07	140.87	12.91	169.94

Means followed by the same letter are not significantly different.

Table 5.11 Effect of microbial inoculants (PGPR) on root architecture related variables and plant growth measurements fifteen days after re-inoculation

Treatment	SHOOT MEASUREMENTS							ROOT MEASUREMENTS						
	H (cm)	NL	CH	SD (mm)	GI (cm^2)	SFW (g)	SFW (g)	RFW (g)	RFW (g)	L (cm)	SA (cm^2)	V (cm^3)	D (mm)	NT
1. Equity	59.33a	11.7bc	34.3bc	7.54a	2286b	51.4b	25.0a	13.03b	2.3a	154.34a	368.9a	423.5a	12.65a	517.7a
2. PGA	60.66a	11.0c	36.0b	7.55a	2118b	47.9b	16.8a	11.86b	2.7a	182.90a	496.3a	144.9a	11.53a	602.3a
3. Ag Blend	60.66a	14.7ab	40.0a	8.13a	3020a	65.4a	24.9a	17.26a	4.2a	178.25a	550.2a	148.5a	10.80a	536.0a
4. Control	51.33b	15.3a	33.2c	7.46a	2196b	39.9b	16.4a	14.36ab	1.7a	11.18a	548.7a	233.8a	16.75a	400.0a
$LSD_{0.05}$	6.67	3.52	2.4	1.42	488.19	13.93	11.24	4.23	2.7	154.42	193.43	427.9	12.34	297.37

Means followed by the same letter are not significantly different.

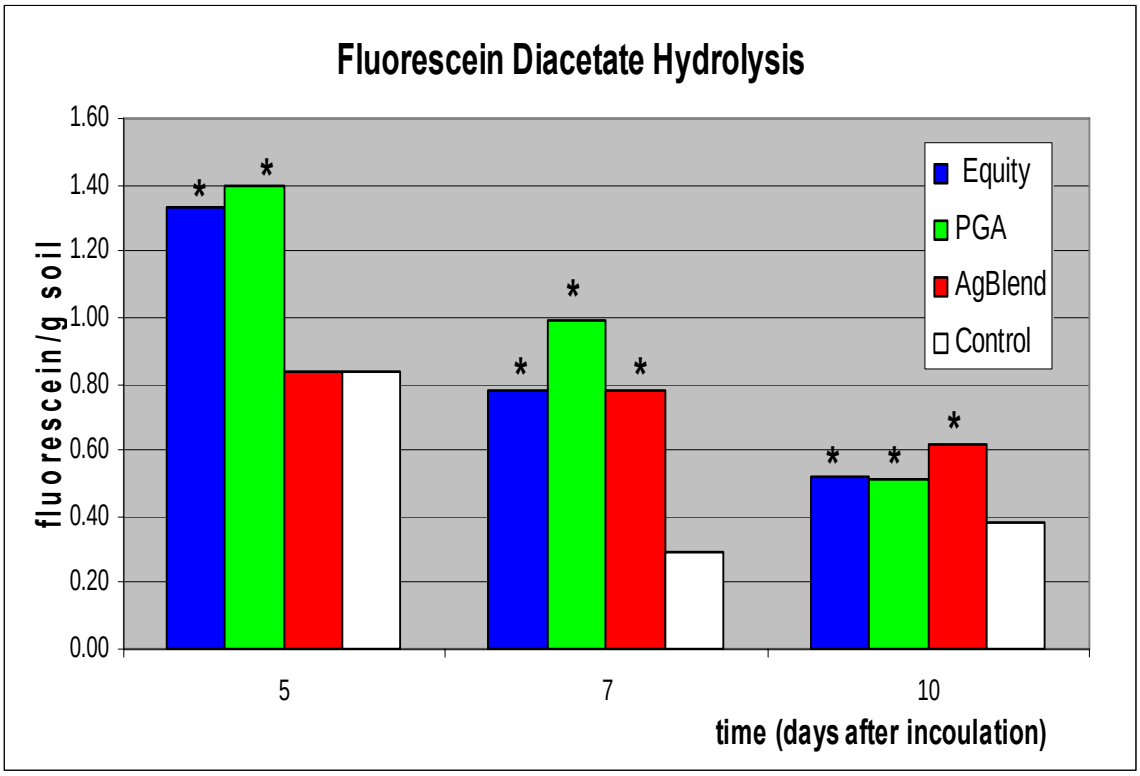

FIGURE 1 Effect of commercial PGPR products on total microbial activity measured by FDA hydrolysis in the tomato rhizosphere soil

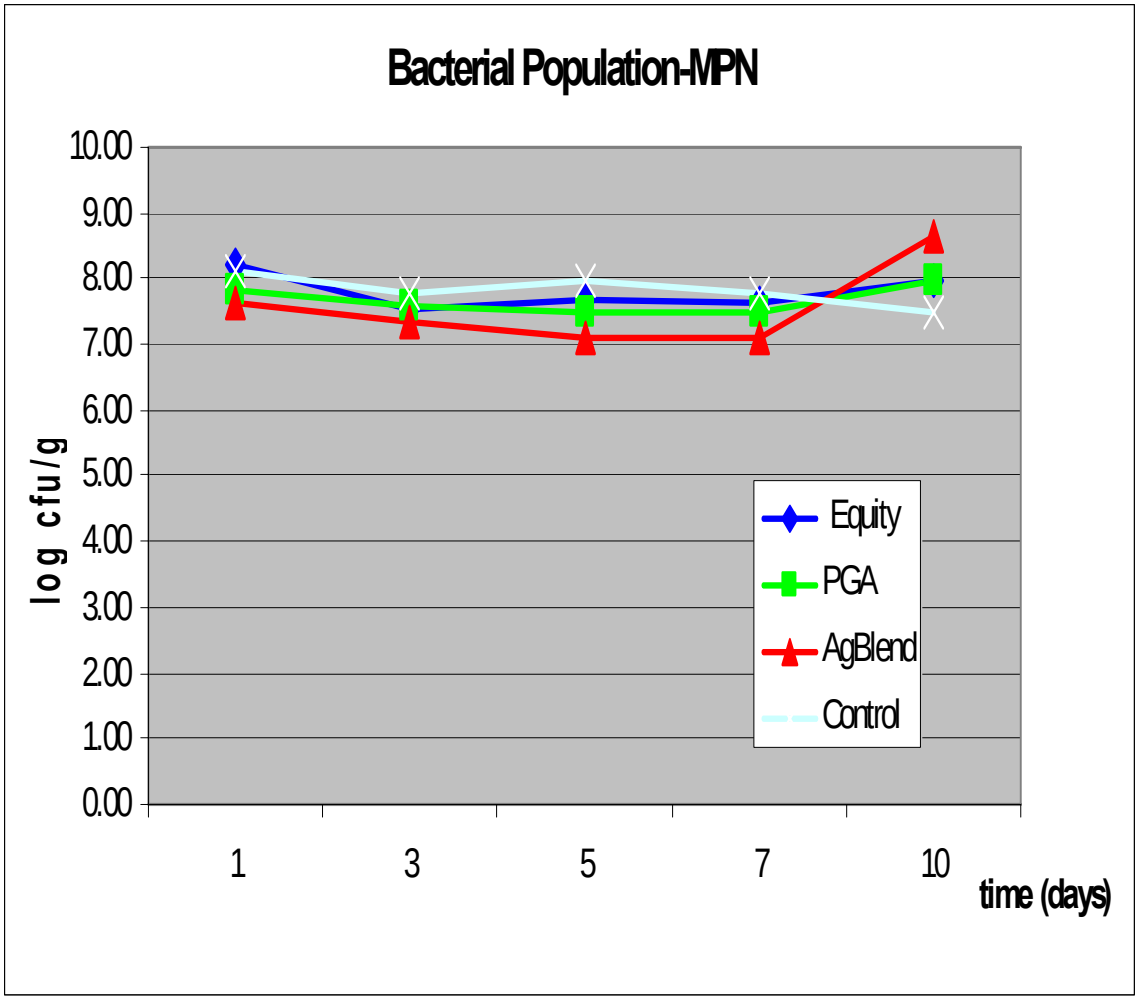

FIGURE 2 Effect of commercial PGPR products on culturable bacterial populations in the tomato rhizosphere

EFFECTS OF MICROBIAL INOCULANTS ON SOIL MICROBIAL POPULATIONS, DIISEASE SUPPRESSIVENESS, AND SOIL HEALTH

1. Introduction

Soil suppressiveness is the result of factors that constrain pathogen establishment, persistence, or increase in population (Pyrowolakis et al, 2002). Therefore, under certain conditions, the pathogenicity of fungi, nematodes, or bacteria is expressed, and the susceptible plants remain unharmed even under environmental conditions favorable to the expression of disease. Soil suppressiveness may be general or specific (Cook and Baker, 1983). General suppressiveness of a pathogen is directly related to the biological, physical, and chemical environment. Specific suppressiveness operates against a select group of microorganisms. Thus, the soil initially allows disease development, but after repeated cultivation of the host plant in the same field, the soil progressively becomes suppressive (Davet, 2004). Take-all disease, caused by *Gaeumannomyces graminis* var. *tritici* (Ggt), is common in wheat-producing areas. A phenomenon called take-all-decline, which describes a reduction in the severity of the disease after a few years of wheat monoculture, results from specific suppression (Weller and Thomashow, 2003).

General soil suppressiveness against plant parasitic nematodes can be the result of physical factors, such as soil type, moisture, and temperature. Antagonists such as egg-parasitic fungi, nematode-trapping fungi, bacteria, and polyphagous predatory nematodes

also limit nematode abundance and may be used for inducing suppressiveness in the soil to reduce nematode populations (Gray, 1995; Kerry, 1998).

Most nematodes are free-living and sustain themselves by feeding on bacteria or other microorganisms (Barker and Koenning, 1998). Other species are parasites of plants and animals. The root knot nematode (*Meloidogyne sp.*) is a biotrophic pathogen of numerous plant species (Williamson and Gleason, 2003). This organism causes important changes in the morphology and physiology of its host. Juveniles of the root-knot nematode move intercellularly after penetrating the root, migrating down towards the root tip where they enter the base of the vascular cylinder and migrate up the root (Wyss et al., 1992). The juveniles establish a permanent feeding site in the differentiation zone of the root by inducing nuclear division without cytokinesis in host cells. The plant cells around the feeding site divide and swell, causing formation of galls (Williamson and Hussey, 1996).

Root knot nematodes are economically important for losses they cause on many crops world wide (Sikora and Fernandez, 2005). There are several control alternatives that include the use of chemicals. Recently, attempts have been made to use antagonistic fungi and to identify root-knot nematode suppressive soils (Pyrowolakis, et al. 2001; Kerry and Hidalgo-Diaz, 2004; Nico et al, 2004).

Lara et al. (1996) demonstrated that *Paecilomyces lilacinus* significantly reduced *Meloidogyne incognita* soil populations and increased tomato yield. Fernandez et al. (2001) studied the effect of the nematicide Ditera on the induction of soil suppressiveness to the root-nematode. Changes in the rhizosphere bacterial community were found as well as enhancement of antagonism to the nematode.

Pyrowolakis et al. (2002) rated 12 California soils for suppressiveness to *M. incognita* by comparing the nematode population development in fumigated and re-infested soils to the equivalent non-treated soils. In three of 12 soils the population density development of the nematode was significantly suppressed compared to methyl iodide-fumigated soil. However, infestation timing or life stage of *M. incognita* did not influence soil suppressiveness against the nematode.

Kokalis-Burelle and Kloepper (2004) discussed soil ecosystem heath and its role in plant disease suppression. Practices such as crop rotation, cover crops, organic amendments, and application of a biological agent modified the rhizosphere microbial community and led to enhanced plant health (Schippers et al., 1987; Weller et al., 2002). Such changes in soil microbial communities via antagonism are related to soil suppressiveness.

Understanding soil microbial community interactions is fundamental for developing practices to manage plant diseases. The knowledge that agricultural production depends on complex biological equilibria will aid in modifying agro-ecosystems and obtaining more favorable conditions for plant growth and health.

The goal of this project was to determine the relationship among soil microbial activity, microbial population, and disease suppressiveness after the addition of soil inoculants PGPR (plant growth promoting rhizobacteria).

2. Materials and Methods

Two greenhouse studies were conducted at Auburn University in 2005 and 2006. For both studies tomato transplants “hybrid Juliet” were produced in the greenhouse at Auburn University. Transplants were fertilized weekly with Miracle Gro 15-30-15 (N-P-

K fertilizer, Scotts Miracle GRO) soluble fertilizer and transplanted 3 weeks after seeding. Soil, collected from the E. V. Smith Agricultural Experimental Station, was autoclaved twice (90 minutes each cycle at 117 ºC) before being placed in pots. A stock solution of each microbial treatment was prepared by diluting the commercial dose of the microbial inoculants in water (Table 1). Seedlings were planted and each pot was inoculated the following day by pouring 100 ml of microbial stock solution onto the soil surface.

A. Trial 1

This experiment was designed to test the hypothesis that microbial activity is related to population size. Fluorescein diacetate hydrolysis was used to quantify microbial activity. Direct plate counting was used to assess culturable bacterial population.

Experimental Design

The experimental design was completely randomized with five treatments and six replications. Treatments were (1) Equity (Naturize Inc, Jacksonville, FL) (2) PGA (Organica, Norristown, PE), (3) Ag Blend (Superbio, Pilot Point, TX), (4) FZB42 (Abitep, Berlin, Germany), and (5) nontreated control. Treatments 1 to 4 are commercial PGPR products discussed in Table 1. Samples were taken at 1, 5, 10, and 15 days after the inoculation with PGPR commercial products for microbial activity, population size and growth measurements. This experiment was conducted twice. For the second experiment only Bioyield and FZB42 were used. Sampling was done at 1, 4, 8, 12, and 16 days after inoculation for microbial activity and population size measurements.

Table 1. Commercial PGPR Products

Microbial Inoculants (PGPR)	Description	Dose
Naturize Equity®	Contains 47 strains of bacilli in a liquid formulation.	0.48g/L water
BioYield®	Contains strain *B. subtilis* GB03 and *Bacillus amyloliquefaciens* strain GB99 in a chitosan carrier.	1cc product: 40 cc soil
Organica Plant Growth Activator **(**PGA**)** ®	Contains 54 strains of bacilli, Pseudomonads, Actinomycetes, and Trichoderma in a powder carrier.	1 tablespoon/Gal water
Ag Blend®	Contains multi-trophic community (anaerobic and aerobic, culturable and nonculturable Gram +, Gram – bacteria , actinomycetes, cyanobaceria, protozoa) in a liquid carrier.	33ml/Lwater
FZB42®	Contains strain *Bacillus amyloliquefaciens* in a liquid formulation.	10^6 cfu/ml water

Microbial Activity and Population

Total microbial activity was assessed by FDA hydrolysis, using the procedure suggested by Schnurer and Rosswall (1982) for the first experiment. For the second experiment, FDA hydrolysis was determined by a modified procedure described by Green et al. (2006), where 1 g of air-dried soil and 50 ml of 60 mM sodium phosphate buffer (pH 7.6) were placed in a 125 ml Erlenmeyer flask. Then 0.50 ml of 4.9 mM FDA

lipase substrate solution was added, suspension was shaken and placed in an incubator for 3h at 37 °C. The reaction was stopped by the addition of 2 ml of acetone. About 30 ml of the suspension were transferred to a 50 ml centrifuge tube and centrifuged at 8000 rpm for 5 minutes. The supernatant was filtered through a Whatman No. 2 filter paper and the filtrate transferred to a colorimeter tube to measure the absorbance at 490 nm. The concentration of fluorescein released was expressed in mg/ dry soil x 3h.

Direct plate counts were used to quantify total culturable bacteria, heat tolerant bacteria, and fluorescent pseudomonads. One gram of rhizosphere soil was added to 50 ml sterile water in 125 ml Erlenmeyer flask and shaken at 150 rpm for 20 minutes. Serial dilutions were made, and 50 µl were plated onto 50% Tryptic Soy Agar (TSA) for total bacteria (10^{-1}, 10^{-2} and, 10^{-3} dilutions) and onto King's B media (king et al., 1954) for fluorescent pseudomonads (10^{-1}, 10^{-2} dilution). For total heat tolerant bacteria after serial dilution, cell suspensions were heat-treated for 13 minutes at 80 ºC and then 10^{-1} and 10^{-2} dilution were plated onto 50 % TSA. All plates were incubated 48 hours at 28 ºC. Numbers of colonies were counted, and population size expressed as log cfu/g of soil for all treatments.

Plant Growth Parameters and Root Architecture

Plant measurements and root architecture analysis were performed at every sampling time for the first experiment. Fresh and dry shoot and root weight (g), growth index (height x wide (cm^2)), and height (cm) were measured. WinRHIZO (Regent Instruments, Quebec Canada) algorithms were used to quantify tomato root architecture.

Statistical Analysis

Microbial activity and population data were statistically analyzed according to standard procedures for analysis of variance and by mean separation, least significant difference (LSD) (SAS Institute, Cary, NC). Root architecture and plant measurements values were also analyzed using LSD. All differences referred to were significant at the 95% confidence level.

B. Trial 2

This experiment was designed to test the hypothesis that microbial inoculants can be used to induce suppressiveness to soilborne pathogens by maintaining adequate microbial population and activity. Soil suppressiveness was studied using the root knot nematode *Meloidogyne incognita,* and tomato as a model.

Proven Nematode Pathogenicity

To prove pathogenicity on tomato, three-week-old tomato seedlings and inoculum of *M. inocgnita* race 3 were used. Inoculum of *M. inocgnita* was prepared by extracting nematode eggs from cotton roots, obtaining a solution with 1000 eggs/ml of water. Seedlings were placed in pots previously filled with autoclaved soil and 5 ml of the inoculum solution applied per pot as a drench after transplanting. Forty five days after inoculation the experiment was harvested and pathogenicity was confirmed on tomato. Nematodes eggs were extracted from tomato roots and used as inoculum for each trial.

Nematode Inoculum Preparation

This trial was conducted four times. For each time nematode eggs were extracted from tomato roots of the previous trial, and a nematode inoculum solution was prepared and applied as a drench one day after PGPR application.

Experimental Design

The experimental design was completely randomized with four treatments and 8 replicates. The trial was conducted four times. For the first and second time four treatments were used: (1) Equity (2) BioYield (3) Ag Blend (4), and Control. For the third and fourth times, Ag Blend treatment was replaced by FZB42 (Table 1).

Sampling:

The experiment lasted for 45 days. At the end of this time, a rhizosphere soil sample was taken for microbial activity and population determinations. Root systems were then rated for the nematode-induced galling on a scale of 0 to 6 as follows: 0= no galls, 1 = 1-10%, 2= 11-25%, 3= 26-50%, 4=51-75%, 5=69-90%, and 6= 91-100% roots with galls (Kathy Lawrence, personal communication). Fresh and dry shoot and root weights (g) were also recorded for each plant.

Eggs were recovered from excised roots by agitated extraction in a 10% bleach solution (1.5% sodium hypochlorite, NaOCl). The extracted solution was poured through a sieve with a pore size of 73.7 µm nested over a sieve with a pore size of 25.4 µm in which the eggs were collected. Eggs were rinsed gently with running water and transferred into a vial with 10 ml of water (Klump and Thomas, 1987). The total number of eggs were counted under a dissecting scope (4X) and expressed as number of eggs per gram of root. The extraction of juveniles from the soil was completed by direct soil screening followed by sucrose centrifuge flotation. Direct soil screening technique consisted of washing the content of every pot separately through a 40-mesh (425 µm) opening, screening with tap water to remove larges pieces of debris, and collecting liquid in a bucket. The soil suspension was then mixed by hand and allowed to settle for 45

seconds. The suspension was poured through a 325-mesh sieve to collect nematodes. The contents of the sieve were rinsed with a wash bottle into a beaker. The same procedure was repeated twice. Juveniles were extracted, counted under the dissecting scope, and expressed as juveniles per ml.

Microbial Activity and Population

Total microbial activity was assessed by measuring FDA hydrolysis, using the procedure suggested by Schnurer and Rosswall (1982). Direct plate count technique was used for measuring bacterial population as described in trial 1.

Statistical Analysis

Data collected were statistically analyzed according to standard procedures for analysis of variance (GLM) and mean separation using least significant difference LSD (SAS Institute, Cary, NC). All differences referred to were significant at the 95% confidence level.

3. Results

A. Trial 1

Total Microbial Activity and Population

In the tomato rhizosphere soil, total microbial activity measured by FDA hydrolysis was significantly higher than the control only for Equity treatment at 10 days after inoculation (DAI) (Table 2.1).

Treatment with Equity resulted in significantly higher populations of total bacteria and fluorescent pseudomonads than the control at 1 DAI. In contrast, FZB42 treatment resulted in greater populations of total bacterial and fluorescent pseudomonads at all

times, with the exception of 1 DAI (Table 2.2). FZB42 treatment also caused a significant increase in total heat tolerant bacteria at all sampling times.

This experiment was conducted a second time in which only FZB42 and Bioyield PGPR products were applied. FDA hydrolysis, determined by a modified procedure (Green et al, 2006), detected significant increases in total microbial activity for FZB42 treatment compared to the control at 4 and 12 DAI (Table 2.3). The same increase in total microbial activity was obtained with Bioyield treatment at 4, 8 and 12 DAI (Table 2.4). In the same experiment total bacterial populations were significantly greater than the control with FZB42 treatment at the first and last sampling (1 and 16 DAI); total heat-tolerant bacteria were greater at 1 and 8 DAI; and fluorescent pseudomonads were greater at 12 DAI (Table 2.5). Bioyield treatment also resulted in increases in populations of total bacteria. Thus, populations were greater than the control at 1 and 8 DAI; however, populations of fluorescent pseudomonads were not different than the control at any sampling time (Table 2.6).

Plant Growth Parameters and Root Architecture

PGPR products had positive effects on plant growth parameters and root architecture. PGA treatment increased shoot dry weight (SDW) at 1 DAI (Table 3.2). Ag Blend treatment caused significantly higher shoot fresh weight, SDW, and growth index (GI) than the control at 10 and 15 DAI (Table 3.6). Additionally, root fresh and dry weights were increased at 15 DAI (Table 3.8).

Two variables of root architecture were higher than the control for Equity and Ag Blend treatments. Increases in number of root tips were detected 10 DAI for Equity treatment (Table 3.5) and in root diameter for Ag Blend at 15 DAI (Table 3.7).

Five days after microbial inoculation significant effects on root architecture related variables were not observed for any PGPR treatment (Table 3.3). Additionally, plant growth measurements such as SFW, SDW, GI and Height were not significant different for Ag Blend treatment than the control; however, those growth parameters for PGA, Equity and FZB42 treatment were significantly lower than the control (Table 3.4). Ten DAI FZB42 treatment had also the lowest root length (Table 3.5).

B. Trial 2

Population of *M. incognita* on tomato rhizosphere soil inoculated with PGPR products and effects on root growth

Bioyield-treated tomato plants had a significantly lower mean gall rating and numbers of eggs/g and juveniles than the control (Table 4.1). No other PGPR treatment had a significant effect on all three parameters. Fresh root weight was significantly higher for Bioyield and Ag Blend treatments than the control (Table 4.1).

For the third and fourth times this experiment was conducted, Ag Blend treatment was replaced by FZB42. Bioyield treatment had similar results on the development of nematode populations (Table 4.2) as described above, as did FZB42 treatment (Table 4.3). Reductions in gall rating, egg/g, and juveniles were observed for both treatments. Equity treatment again had a significant reduction in gall rating and number of juveniles per ml (Table 4.3). Shoot dry weight was significantly higher in all PGPR treatments than the control (Table 4.2)

Total Microbial Activity and Population

Applications of microbial inoculants (PGPR) resulted in some significant effects on population size measured by direct plate counts. Populations of total bacteria and

total heat-tolerant bacteria were significantly higher in Bioyield and FZB42 treatments than the control (Table 5.1, Table 5.2). However, increases in microbial activity assessed by FDA hydrolysis were not detected (Table 5.1).

4. Discussion

Plant growth-promoting rhizobacteria (PGPR) are plant associated microorganisms that benefit plant growth and health. Stimulation of plant growth includes a variety of mechanisms that provide the plant with fixed nitrogen, phytohormones, iron and soluble phosphate (Glick et al, 1999). PGPR also compete with pathogens that inhibit plant growth and development (Glick and Bashan, 1997).

The results presented here indicate that different treatments of commercially available PGPR-based inoculants (Equity, PGA, Ag Blend, Bioyield, and FZB42) generally exerted positive effects on tomato growth. Additionally, under our experimental conditions, application of Bioyield and FZB42 at transplanting time contributed to the suppression of root-knot nematode, consistently reducing *M. incognita* population and root damage.

Under our experimental condition a phosphorous deficiency was detected in the first trial. This deficiency could explain the negative effects of the application of PGPR products on tomato growth parameters at five days after inoculation because the plants were under obvious stress conditions, their physiological state would be altered, possibly allowing PGPR to exert an unexpected negative effect.

When comparing populations of nematodes in PGPR-treated soils with nontreated soils, reductions were observed for gall rates, egg/g, and juveniles/ml. Bioyield is a product that contains *B. subtilis* strain GBO3 for control of soilborne pathogens via

production of antibiotics, chitosan for nematode control via promotion of indigenous soil predators and antagonists to root-knot nematodes, and a strain of *Bacillus amyloliquefaciens* that elicits induced systemic resistance (Kloepper et al, 2004). Consequently, results obtained in this study are another example of effectiveness of this PGPR product against root-knot nematode and also of the promotion of free-living nematodes (data not reported).

Bacillus amyloliquefaciens strain FZB42 can be distinguished in this study by several important features for rhizosphere competence and for suppression of soil-borne pathogens such as root-knot nematode. It has been reported that FZB42 is a producer of lipopeptides, surfactins, bacillomycins D, and fengycins, which are secondary metabolites with mainly antifungal activity (Chen et al, 2006). This antibiotic production not only could have antifungal and antibacterial effects but also may influence *M. incognita* population development by reducing the number of second stage juveniles.

The rhizosphere is known to be a zone of increased microbial activity and consequently enzyme activity. Because of the intensive and extensive interactions in the rhizosphere, we used microbial activity and population size to assess the positive effects of inoculated microorganisms and to determine frequency of PGPR applications.

FDA hydrolysis is an important enzymatic assay; it is simple, sensitive, and precise in measuring soil microbial activity. Green et al. (2006) optimized the FDA hydrolysis procedure first suggested by Schnurer and Rosswall (1982) to measure soil microbial activity. They found that this modified procedure provided a better quantification of microbial activity when it was tested in soils with a wide range in pH, organic C and texture (Green et al., 2006). This recently optimized FDA procedure was

also used in our studies and better detected changes in microbial activity after PGPR applications, although FZB42 and Bioyield treatments only had greater activity than the control at 4 and 12 DAI.

In general, enzymatic assays can be useful indicators of soil quality management and be related to soil health (Dick, 1997). Under field conditions, seasonal fluctuations may occur and those assays should have consistency in showing differences among treatments throughout the year. Detection of changes of microbial activity over time is the key point for an assay to be useful as indicator for PGPR application frequency in the field. Under greenhouse conditions, the FDA procedure was too variable to detect increased microbial activity, following applications of PGPR. Thus, if inconsistency occurs under greenhouse conditions, where environmental variables are under largely control, the FDA procedure cannot be recommended as a field assay for determining PGPR application frequency.

Effects of PGPR applications on population size were also studied. Total culturable bacterial populations measured by plate counting, ranged from 1.1×10^{8} to 5.2×10^{7} in FZB42 treatment to $\sim 6 \times 10^{6}$ in Equity, PGA, and the nontreated control. Results suggested that the single strain PGPR (FZB42) colonized the root system and maintained higher population levels than PGPR products containing complex microbial communities (Equity and PGA). Equity and PGA are recommended as plant growth enhancers, while FZB42 also produces antibiotics. Antibiotic production might explain FZB42 colonization pattern and the increased total bacterial carrying capacity of the rhizosphere. FZB42 was an effective and persistent rhizosphere colonizer; once it was established, it increased total heat tolerant bacteria and thereby total population. FZB42

also exhibited a very distinguishable colony morphology, which made it more recognizable on plates inoculated with rhizosphere dilutions.

Bioyield, a double-strain PGPR product, also increased total population within the same range as FZB42. Both FZB42 and Bioyield treatment markedly increased total heat tolerant bacteria population, with a few exceptions, throughout the experiment.

Kokalis-Burelle et al., (2006) studied the effects of PGPR applied in the potting media, at transplanting, and during the growing season on indigenous rhizosphere microorganisms. When PGPR were applied in the potting media, they found increases in total heat-tolerant bacteria at all sampling times throughout the field trial. Additional PGPR applications during the growing season did not increase population size, although they did increase plant growth. Even if our experiments were conducted under greenhouse conditions, similar increases in total heat-tolerant bacteria, applied only at transplant, were recorded for Bioyield.

Kokalis-Burelle et al., (2006) also obtained significant increases in total bacteria only at the end of the season, while we found those increases from the second sampling until the end of the experiment. PGPR products (single or double strains) did increase total heat-tolerant bacteria and were able to establish stable populations in the rhizosphere. Consequently, if root colonization is used as the criterion to decide when to reapply PGPR products, only applications in the potting media would be required. However, additional applications of PGPR may result in increases of plant growth and improved health, but not always in yield (Kokalis-Burelle et al., 2006).

Increases in population were not related to changes in total microbial activity using the procedure suggested by Schnurer and Rosswall (1982). However, a recently

optimized FDA method (Green et al, 2006) was also used, and it performed better in the detection of changes in microbial activity after PGPR applications. Those changes were not always related to increases of either total bacteria or total-heat tolerant bacteria, meaning that microbial activity does not increase when population does.

Overall, population size measured by direct plate counts could be a useful procedure to study root colonization and persistence of introduced microorganisms in the rhizosphere. Knowing that introduced microorganisms are surviving, and their patterns of growth will help to determine the application frequency of PGPR. In contrast, because of the lack of consistency, the FDA procedure is not useful for this purpose.

We hypothesized that microbial inoculants can be used to induce suppressiveness to soil-borne pathogens by maintaining adequate microbial population and activity. PGPR have been reported for plant growth promotion in field and greenhouse conditions and also for pathogen suppression. During this study, positive effects on tomato growth were obtained and soil suppressiveness against *M. incognita* was induced. Additionally, increases in population size were detected by the direct plate counting for a single and double strain PGPR, although there was not a correlation between total microbial activity and population size.

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Table 2.1 Effect of commercial PGPR products on fluorescein diacetate hydrolysis (FDA) using procedure suggested by Schnurer and Rosswall as a measure of total microbial activity in tomato rhizosphere soil

	FDA*			
Treatment	**Days after Inoculation**			
	1	**5**	**10**	**15**
1. Equity®	1.61ab	2.23a	1.94 a	1.03 b
2. PGA®	1.50 b	1.61b	1.34 b	1.43 a
3. Ag Blend®	1.94 a	2.03a	1.44ab	1.33 a
4. FZB42®	1.80ab	1.49b	1.49ab	1.41 a
5. Control	1.74ab	2.07a	1.83ab	1.23ab
$LSD_{0.05}$	0.425	0.383	0.593	0.217

Means followed by the same letter within a column are not significantly different.

*** FDA hydrolysis expressed as µg fluorescein / g oven dry soil x h**

Table 2.2 Effect of microbial inoculants PGPR on total bacteria, total heat-tolerant bacteria, and total fluorescent pseudomonads

Treatment	Total population (Log cfu/g)				Total heat-tolerant bacteria (Log cfu/g)				Fluorescent pseudomonads (Log cfu/g)			
	Days after inoculation											
	1	5	10	15	1	5	10	15	1	5	10	15
1. Equity®	7.34a	6.73b	6.57c	6.87b	3.05b	6.26b	6.12b	6.20b	5.29a	1.60b	2.35b	1.57b
2. PGA®	6.83b	6.63b	6.41c	6.65b	6.30a	6.31b	6.01b	6.28b	1.06b	3.01b	2.219b	1.60b
3. Ag Blend®	6.76b	6.63b	6.39c	6.71b	6.34a	6.24b	6.08b	6.28b	0.94b	1.67b	0.00c	2.40b
4. FZB42®	6.92b	8.01a	8.06a	7.75a	6.81a	7.27a	7.56a	7.22a	3.9ab	7.37a	6.36a	6.78a
5. Control	6.77b	6.70b	6.78b	6.87b	6.30a	6.33b	6.20b	6.25b	1.00b	2.83b	2.28b	3.69b
$LSD_{0.05}$	0.41	0.18	0.19	0.30	1.67	0.16	0.21	0.12	3.17	2.60	2.14	2.24

Means followed by the same letter within a column are not significantly different.

Table 2.3 Effect of FZB42 on fluorescein diacetate hydrolysis (FDA) using procedure suggested by Green et al. (2006) as a measure of total microbial activity in tomato rhizosphere soil

Treatment	FDA* Days after Inoculation				
	1	4	8	12	16
1. FZB42®	0.16b	0.25a	0.26a	0.29a	0.27a
2. Control	0.27a	0.20b	0.25a	0.23b	0.26a
$LSD_{0.05}$	0.058	0.055	0.053	0.04	0.096

Means followed by the same letter within a column are not significantly different.

*** FDA hydrolysis expressed as mg fluorescein / g dry soil x 3h**

Table 2.4 Effect of Bioyield® on fluorescein diacetate hydrolysis (FDA) using procedure suggested by Green et al. (2006) as a measure of total microbial activity in tomato rhizosphere soil

Treatment	FDA* Days after Inoculation				
	1	4	8	12	16
1. Bioyield®	0.16b	0.35a	0.28a	0.30a	0.32a
2. Control	0.27a	0.21b	0.25a	0.23b	0.26a
$LSD_{0.05}$	0.05	0.066	0.032	0.042	0.078

Means followed by the same letter within a column are not significantly different.

***FDA hydrolysis expressed as mg fluorescein per g dry soil x 3h**

Table 2.5 Effect of FZB42 on total bacteria, total heat-tolerant bacteria, and fluorescent pseudomonads

Treatment	Total population (Log cfu/g)					Total heat tolerant (Log cfu/g)				Fluorescent pseudomonads (Log cfu/g)				
	Days after inoculation													
	1	4	8	12	16	1	4	8	16	1	4	8	12	16
1. FZB42	7.87a	7.87a	7.92a	7.33a	7.76a	6.56a	6.02a	6.60a	5.94a	6.28a	6.89a	6.68a	6.90a	6.45a
2. Control	7.60b	7.94a	7.81a	7.34a	7.51b	5.00b	6.21a	6.00b	6.14a	6.25a	6.85a	6.29a	5.81b	6.23a
LSD 0.05	0.21	0.12	0.10	0.20	0.19	0.11	0.47	0.18	0.50	0.19	0.36	0.82	0.33	0.30

Means followed by the same letter within a column are not significantly different.

Table 2.6 Effect of Bioyield on total bacteria, total heat-tolerant bacteria, and fluorescent pseudomonads

Treatment	Total population (Log cfu/g)					Total heat tolerant (Log cfu/g)				Fluorescent pseudomonads (Log cfu/g)				
	Days after inoculation													
	1	4	8	12	16	1	4	8	16	1	4	8	12	16
1.Bioyield®	7.68a	7.95a	8.01a	7.83a	7.81a	6.67a	6.88a	6.73a	6.83a	6.04a	4.87a	6.21a	6.33a	6.00a
2. Control	7.60b	7.94a	7.81b	7.34b	7.51b	5.00b	6.21a	6.00b	6.14a	6.25a	6.85a	6.29a	5.81a	6.23a
LSD 0.05	0.18	0.19	0.17	0.21	0.19	0.07	0.45	0.21	0.38	0.27	2.22	0.74	0.54	0.32

Means followed by the same letter within a column are not significantly different.

Table 3.1 Effect of microbial inoculants (PGPR) on root architecture related variables one day after inoculation

Treatment	Length (cm)	Surface area (cm^2)	Volume (cm^3)	Root diameter (mm)	Number of tips
1. Equity®	101.8a	66.4a	3.5ab	2.1a	283.2a
2. PGA®	106.6a	73.2a	4.1a	2.2a	213.2ab
3. Ag Blend®	100.5a	62.9a	3.1bc	2.0a	206.0ab
4. FZB42®	89.6a	50.5b	2.3c	1.8a	198.8a
5. Control	106.6a	67.0a	3.5ab	2.1a	207.8ab
$LSD_{0.05}$	26.07	31.1	0.89	0.42	80.07

Means followed by the same letter within a column are not significantly different.

Table 3.2 Effect of microbial inoculants (PGPR) on plant growth measurements one day after inoculation

Treatment	Shoot Fresh Weight (g)	Shoot Dry weight (g)	Growth Index (cm^2)	Root Fresh Weight (g)	Root dry Weight (g)	Height (cm)
1. Equity®	0.87c	0.16b	50.00b	0.82ab	0.04ab	5.20a
2. PGA®	1.70a	0.24ab	109.20a	0.89a	0.05a	5.40a
3. Ag Blend®	1.82a	0.33a	107.40a	0.60c	0.04b	5.20a
4. FZB42®	1.35b	0.17b	103.40a	0.76b	0.04ab	6.20a
5. Control	1.82a	0.30a	120.20a	0.78ab	0.03b	5.60a
$LSD_{0.05}$	0.253	0.09	28.65	0.125	0.0128	1.135

Means followed by the same letter within a column are not significantly different.

Table 3.3 Effect of microbial inoculants (PGPR) on root architecture related variables five days after inoculation

Treatment	Length (cm)	Surface area (cm^2)	Volume (cm^3)	Root diameter (mm)	Number of tips
1. Equity®	163.2a	129.7a	8.3a	2.5a	358.2a
2. PGA®	193.4a	156.2a	10.2a	2.6a	407.8a
3. Ag Blend®	148.2a	117.6a	9.1a	2.8a	320.2a
4. FZB42®	194.9a	153.6a	9.7a	2.5a	411.2a
5. Control	191.6a	147.0a	9.2a	2.4a	353.4a
$LSD_{0.05}$	49.82	42.7	3.8	0.48	129.8

Means followed by the same letter within a column are not significantly different.

Table 3.4 Effect of microbial inoculants (PGPR) on plant growth measurements five days after inoculation

Treatment	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Growth Index (cm^2)	Root Fresh Weight (g)	Root dry Weight (g)	Height (cm)
1. Equity®	2.76b	0.43b	203.20ab	2.06a	0.09a	7.10b
2. PGA®	2.72b	0.44b	201.00ab	1.95a	0.01a	6.80a
3. Ag Blend®	3.62a	0.48ab	251.00a	1.92a	0.08a	7.30ab
4. FZB42®	2.05c	0.31c	164.80b	1.80a	0.10a	7.20b
5. Control	3.52a	0.52a	234.00a	2.31a	0.10a	8.10a
$LSD_{0.05}$	0.62	0.076	52.1	0.53	0.023	0.85

Means followed by the same letter within a column are not significantly different.

Table 3.5 Effect of microbial inoculants (PGPR) on root architecture related variables ten days after inoculation

Treatment	Length (cm)	Surface area (cm^2)	Volume (cm^3)	Root diameter (mm)	Number of tips
1. Equity®	340.4a	263.0a	17.5a	2.5a	798.4a
2. PGA®	336.8a	249.2a	14.8a	2.4a	750.0ab
3. Ag Blend®	267.8ab	286.5a	25.3a	3.5a	462.0c
4. FZB42®	230.9b	233.9a	22.7a	3.6a	610.0abc
5. Control	267.7ab	274.1a	23.0a	3.3a	547.0bc
$LSD_{0.05}$	85.41	78.13	12.78	1.32	222.9

Means followed by the same letter within a column are not significantly different.

Table 3.6 Effect of microbial inoculants (PGPR) on plant growth measurements ten days after inoculation

Treatment	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Growth Index (cm2)	Root Fresh Weight (g)	Root dry Weight (g)	Height (cm)
1. Equity®	5.33b	0.87b	275.40b	4.81ab	0.25a	9.20a
2. PGA®	4.98b	0.81b	255.60b	4.54ab	0.20ab	8.50a
3. Ag Blend®	8.35a	1.14a	353.60a	5.32a	0.25a	9.40a
4. FZB42®	4.42b	0.58c	259.80b	3.82b	0.14b	9.00a
5. Control	4.93b	0.78bc	229.80b	5.29a	0.22a	8.60a
LSD $_{0.05}$	1.03	0.2	65.38	1.21	0.067	1.29

Means followed by the same letter within a column are not significantly different.

Table 3.7 Effect of microbial inoculants (PGPR) on root architecture related variables fifteen days after inoculation

Treatment	Length (cm)	Surface area (cm^2)	Volume (cm^3)	Root diameter (mm)	Number of tips
1. Equity®	364.8ab	396.7ab	30.5b	3.3ab	729.0a
2. PGA®	372.7ab	355.8ab	27.4b	30.3b	779.8a
3. Ag Blend®	317.5b	407.7a	46.3a	4.1a	748.0a
4. FZB42®	329.2b	318.5b	24.5a	3.1b	778.0a
5. Control	416.4a	390.4ab	30.8a	3.1b	814.6a
$LSD_{0.05}$	66.2	79.27	14.75	0.86	172.25

Means followed by the same letter within a column are not significantly different.

Table 3.8 Effect of microbial inoculants (PGPR) on plant growth measurements fifteen days after inoculation

Treatment	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Growth Index (cm^2)	Root Fresh Weight (g)	Root dry Weight (g)	Height (cm)
1. Equity®	8.10ab	1.32b	264.00ab	7.32ab	0.40ab	10.60ab
2. PGA®	6.87b	1.46b	271.00ab	7.58ab	0.36ab	9.60b
3. Ag Blend®	10.60a	2.06a	353.60a	9.40a	0.46a	10.80ab
4. FZB42®	8.40ab	1.24b	347.00ab	5.89b	0.35b	11.60a
5. Control	6.48b	1.22b	243.60b	6.20b	0.32b	10.40ab
LSD $_{0.05}$	3.03	0.59	104.68	2.38	0.1	1.8

Means followed by the same letter with a column are not significantly different.

Table 4.1 Effect of three PGPR microbial inoculants on nematodes and plant growth*

Treatment	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Gall Rating	Egg/g	Juveniles
1. Equity®	25.5a	3.5a	23.4ab	3.5b	5798a	1226bc
2. Bioyield®	27.0a	3.5a	30.7a	1.7c	1940b	815c
3. Ag Blend®	25.2a	3.5a	26.9a	3.3b	4368a	1708ab
4. Control	24.1a	3.3a	22.0b	4.5a	4244a	1896a
LSD 0.05	3.64	0.51	7.64	0.68	2412	436.5

Different letters within a column indicate statistically significant difference (P=0.05) among treatments.

***This table corresponds to the first and second time the experiment was conducted.**

Table 4.2 Effect of three PGPR microbial inoculants on nematodes and plant growth*

Treatment	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Gall Rating	Egg/g	Juveniles
1. Equity®	18.1a	2.6a	22.4a	1.1a	481.5ab	405.6a
2. Bioyield®	10.0b	2.6a	9.1c	0.0b	195.9b	97.0b
3. FZB42®	18.8a	2.8a	19.0ab	1.1a	361.3ab	39.0b
4. Control	16.4a	1.0b	16.3b	1.1a	807.7a	405.6a
LSD 0.05	4	0.483	4.87	0.443	558.42	264.3

Different letters within a column indicate statistically significant difference (P=0.05) among treatments.

***This table corresponds to the third time the experiment was conducted.**

Table 4.3 Effect of three PGPR microbial inoculants on nematodes and plant growth

Treatment	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Gall Rating	Egg/g	Juveniles
1. Equity®	24.4a	3.1a	13.5b	3.4b	6032ab	536.0b
2. Bioyield®	22.5a	3.2a	22.8a	2.9b	4679b	902.9ab
3. FZB42®	21.7a	3.3a	13.6b	2.9b	4753b	671.3b
4. Control	21.9a	3.3a	13.9b	4.0a	11045a	1376.1a
LSD 0.05	11.815	0.79	4.48	0.6	5714	564.5

Different letters within a column indicate statistically significant difference (P=0.05) among treatments.

***This table corresponds to the fourth time the experiment was conducted.**

Table 5.1 Effect of three PGPR microbial inoculants on total bacteria, total heat-tolerant bacteria, and FDA hydrolysis in tomato rhizosphere soil

Treatment	Total bacteria (log cfu/g)	Total heat-tolerant bacteria (log cfu/g)	FDA
1. Equity®	7.24b	6.33bc	1.69a
2. Bioyield®	7.68a	6.90a	2.42a
3. Ag Blend®	7.10b	6.27bc	1.51a
4. Control	7.16b	6.04c	1.88a
LSD 0.05	0.371	0.41	0.967

Different letters within a column indicate statistically significant difference (P=0.05) among treatments.

***This table corresponds to the first time the experiment was conducted.**

Table 5.2 Effect of three PGPR microbial inoculants on total bacteria and total heat-tolerant bacteria in tomato rhizosphere soil*

Treatment	Total bacteria (log cfu/g)	Total heat-tolerant bacteria (log cfu/g)
1. Equity®	7.00b	6.29b
2. Bioyield®	7.34a	6.88a
3. FZB42®	7.42a	6.43a
4. Control	7.06	6.08b
LSD 0.05	0.239	0.26

Different letters within a column indicate statistically significant difference (P=0.05) among treatments.

***This table corresponds to the third and fourth time the experiment was conducted.**

SUMMARY

In recent years, use of microbial inoculants for plant growth promotion has increased. Examples of microbial inoculants are Bioyield®, which contains a mixture of two strains *(Bacillus subtilis* and *B. amyloliquefaciens*), FZB42, a single strain of *B. amyloliquefaciens* and Soil Builder®, Ag Blend®, and Equity®, which contain complex mixtures of over 10 strains of *Bacillus spp*.

Optimizing application frequency of PGPR is critical to achieving the maximum benefit from this technology. Since the rhizosphere is considered the most intense ecological habitat in soil, it is of interest to study the effects that PGPR may have on total microbial activity and bacterial population in the zone where rhizobacteria exerted a direct influence on plants. The objectives of this study were to evaluate a set of sensitive methods to detect increases in microbial activity following additions of microbial inoculants and to determine the relationship among soil microbial activity, microbial population and disease suppressiveness after the addition of soil inoculants PGPR.

Commercial formulations of PGPR, containing bacilli strains (Equity®, Soil Builder®, Ag Blend®, PGA®, Bioyield® and FZB42®) were used on tomato and strawberry in greenhouse and field experiments. Physiological activity of microbes was measured by assessing dehydrogenase activity, arylamidase activity, and fluorescein diacetate hydrolysis (FDA). Culturable microbial populations were determined by most

probable number (MPN) and direct plate counting. In strawberry field trials, hydrolysis of FDA was significantly different among treatments at one of four sampling times. Procedures to estimate population size (MPN) did not detect any change in microbial population; however, the use of PGPR inoculants promoted growth and increased strawberry yield.

In greenhouse experiments on tomato, FDA was effective in measuring changes in microbial activity in the rhizosphere following inoculants application, while arylamidase and dehydrogenase procedures were not sensitive in detecting those changes.

Despite detecting changes in microbial activity, no changes in microbial populations, estimated by MPN, were observed. Thus, little or no correlation was detected between microbial enzymatic activity and bacterial population with the procedures used.

Populations of total of culturable and heat-tolerant bacteria were also measured by plate counting at 1, 5, 10 and 15 days after PGPR inoculation. FZB42 and Bioyield treatments generally resulted in significantly greater total populations than the control. FZB42 exhibited a very distinguishable colony morphology, which made it more recognizable on plates inoculated with rhizosphere dilutions.

Induction of soil suppressiveness by PGPR and the relation to microbial activity and population size were also studied. The plant parasitic nematode *Meloidogyne incognita* and tomato were used as a model. Three-week-old tomato seedlings were first inoculated with PGPR products and then challenged with nematode eggs. Harvest was done 45 days after nematode inoculation, and rhizosphere soil samples were taken for microbial activity and population size determinations.

Results showed significant reductions in numbers of nematode eggs per gram of root, numbers of juveniles per ml and numbers of galls in FZB42 and Bioyield treatments. Additionally, increases in population size were detected for those treatments by the direct plate counting, although there was not a correlation between microbial activity and population size.

Overall, population size measured by direct plate counts could be a useful procedure to study root colonization and persistence of introduced microorganisms in the rhizosphere. Knowing that introduced microorganisms are surviving, and which their patterns of growth are will help to determine when and how these PGPR products should be applied. However, because of the lack of consistency, FDA procedure should not be used to decide the frequency of application of PGPR products.