2 Microbial Inoculants for Optimized Plant Nutrient Use in Integrated Pest and Input Management Systems

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Abstract

The use of fertilizers and pesticides has greatly increased agricultural productivity over the past few decades. However, there is still an ongoing search for additional or alternate tools that can proffer agricultural sustainability and meet the needs of profitability and greater food production for the growing world population. This review examines the enhancement of plant nutrient use efficiency derived from interactions of the diverse microorganisms that live in and around plants such as plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi. These microorganisms form the major bases of the biorational sector of the agriculture industry which has exploded in the last few years with the production of many new microbial inoculant products and the improvement of existing products. Microbial inoculants cannot replace chemical fertilizers now or in the immediate future; thus this review discusses the concept of integrated pest and input management (IPIM), compatibility of inoculants with existing chemicals, and efficacy issues associated with biologicals. Also discussed are inoculant products, the conditions that may affect their success, the untapped potentials for agriculture, and the possible impacts on greenhouse gas emissions and global warming.

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2.1 Introduction

The progress that was made in increased crop production since the middle of the twenty-first century can be attributed in part to developments in plant breeding and genetic engineering and to changes in irrigation and tillage practices. The intensive use of fertilizers and pesticides is another factor that greatly enhanced crop productivity. There is concern, however, that increasing and continuous use of agrochemicals is not sustainable as it leads to the pollution of the environment, especially surface water and groundwater. In addition, applications of agrochemicals may leave residues in foods, thus generating public concern about the impact of agrochemicals on food quality and safety.

The search for additional or alternate tools that will enhance agricultural sustainability while allowing for profitability and more food production for the growing world population is pivotal to agricultural production worldwide. Understanding the diversity of microbes that live in and around plants in different natural environments and modulating their activities offer prospective tools. Microbes interact with plants and support plant health in many different ways such as enhancing plant growth and yield, controlling diseases, and contributing to survival and recovery from adverse environmental conditions, including drought (Cook 2002; Adesemoye and Kloepper 2009; Reid and Greene 2012).

One group of beneficial microbes termed plant growth-promoting rhizobacteria (PGPR) (Kloepper et al. 1989) has been defined in the literature for their ability to live freely in the rhizosphere, stimulate plant growth, enhance root development and architecture, help plants in nutrient acquisition, and provide control of plant pathogens (Kloepper et al. 1991; Canbolat et al. 2006; Adesemoye et al. 2009; Figueiredo et al. 2010). The major genera of PGPR that have been studied or used as inoculants include *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Herbaspirillum*, *Burkholderia*, and *Bacillus* (Glick 1995; Probanza et al. 1996; Artursson et al. 2006; Adesemoye and Kloepper 2009). The direct and indirect mechanisms involved in PGPR activities have been discussed by many authors (Vessey 2003; Glick et al. 2007). Other important groups of plant beneficial microbes include mycorrhizal fungi and *Trichoderma*, a root-colonizing fungus. The role of PGPR and *Trichoderma* as antagonists of soilborne pathogens is well documented. Their use as alternative tools when development of resistance by pathogens to chemical fungicides is possible or where chemical fungicides are not available is one reason why the interest in biological products continues to increase.

The use of PGPR and beneficial fungi to enhance nutrient use is a newer concept than plant growth promotion and biocontrol and comparatively has not been extensively studied. While many of the benefits are known, the potentials for agriculture are only starting to be tapped (Barea et al. 2002; Reid and Greene 2012). Interest in commercial production of microbial inoculants for agricultural use has increased within the last few years. This is important for agricultural sustainability where there is a need to produce more food for the increasing population and limit the amount of fertilizers and pesticides being used.

This review will focus on how PGPR, mainly *Bacillus* spp., and beneficial fungi, particularly mycorrhizae and *Trichoderma* species, help plants in nutrient acquisition. The beneficial roles of rhizosphere microbial populations in soil nutrient availability and how microorganisms applied as inoculants can help plants to improve nutrient use efficiency will be examined. We will explain how microbial inoculants fit within the concept of integrated pest and input management and also look at some of the inoculants which are currently on the market that are based on bacteria and/or fungi. Interactions between PGPR and beneficial fungi used as inoculants will be examined. Also, the conditions that may affect the success of microbial inoculants as well as the untapped potentials for agriculture will be examined.

2.2 Soil Microbial Diversity, Nutrient Dynamics, and Integrated Input and Pest Management

Degradation processes, decline in soil nutrition and productivity, nutrient runoff, leaching, erosion, organic matter depletion, and the negative impacts on groundwater and surface waters are major public concerns. The diversity of microbes in the soil could be used in a sustainable way in agriculture to solve or reduce these problems.

Integrated pest management (IPM) is an important component of sustainable agriculture, and the definition may vary among scientific disciplines, but the concept is very similar. The Office of Technology (1979) defined IPM in the broad sense as "the optimization of pest/pathogen control in an economically and ecologically sound manner, accomplished by the coordinated use of multiple tactics to assure stable crop production and to maintain pest/pathogen damage below the economic injury level while minimizing hazards to humans, wildlife, and the environment." Integrated nutrient management (INM) is another term that has gained momentum in agricultural sustainability circles. The INM system promotes low chemical input but improved nutrient use efficiency through using natural and man-made sources of plant nutrients for crop production in an efficient and environmentally prudent manner that preserves resources for the future without sacrificing current productivity (Gruhn et al. 2000; Adesemoye et al. 2008b). In recent years, the idea of combining IPM and INM together in a systems approach to become integrated pest and input management (IPIM) continues to emerge and its relevance is expanding. The reasons for this are not farfetched, if we look at the intricate connectivity of soil quality and health to IPM, INM, soil productivity, food quality and safety, and environmental soundness. The functionality of this relationship is better seen from the perspective of a cycle.

Parr et al. (1992) opined that the maintenance or restoration of soil quality is highly dependent on organic matter and the diversity of beneficial macroorganisms and microorganisms that it supports. Their suggestion that reduced input of chemical fertilizers and pesticides and the use of alternative practices that enhance organic matter and soil microbial diversity will improve soil quality and productivity aptly fits the concept of IPIM.

The biorational sector will continue to expand, and the discussion of IPIM will consequently be more relevant. The word biorational will be used through this review, and it is important to define what it connotes as there is no universally accepted definition. Biorationals in agriculture refer to substances or products derived from natural or biological origins that are used in crop production. According to information from the American Society for Testing and Materials (ASTM) International and ATTRA—Sustainable Agriculture, biorationals include biopesticides and nonpesticidal products, such as, but not limited to, those used for crop stress management, enhanced plant physiology benefits, root growth management, enhanced nutrient use, postharvest, or as control agents to pesticides and antimicrobials. Biorationals should not be equated with biologicals or microbial inoculants because microbial inoculant is a component of biorationals as “biologicals” include living organisms. While an extract from a living source, such as neem, is a biorational, it is not a biological. Additional definition for microbial inoculant and some related terms such as biostimulant can be found in Calvo et al. (2014).

There are no scientific data to suggest that biological products will replace chemical fertilizers or pesticides now or in the immediate future, but there is interest in using them to supplement and reduce the amount of chemical products that growers are applying. The concept of IPIM system supposes that biorationals including microbial inoculants will reduce the need for agrochemicals such as fertilizers and pesticides. If this would happen, microbial inoculants and most biorationals must be developed to be compatible with existing agrochemicals.

The biological products in the IPIM systems will have to be locally adaptable and beneficial to the soil-plant systems in terms of the resident beneficial microbial community, overall soil biodiversity, soil structure and health, and enhanced nutrient use efficiency. The improved system should lead to or confer resiliency and sustainability to the agroecosystems in the face of challenges of rapidly changing environmental conditions.

The concept of how microbial inoculants (PGPR and mycorrhizae) can interact with crop roots and be used compatibly with fertilizer in an IPIM leading to a more efficient use of nutrients can be explained by the schematic in Fig. 2.1. Currently in agricultural systems, excess fertilizers (A) more than needed by crops are applied. The amount of nutrient taken up by the crop (B) is far less than applied. Thus, “B” as a percentage of “A” or use efficiency may vary from 10% to 50%. Significant parts of the chemical fertilizer are lost or not available to the crop, and this includes portions lost through runoff (C), immobilization, denitrification (D), leaching (E), greenhouse gas emissions (F), and volatilization (G). How to improve plant available nutrients (I) and make more nutrients available through processes such as mineralization and solubilization (J) and thus maximize the amount of nutrient that is eventually taken up (B) is paramount to IPIM or INM. Part of the goal is to use less fertilizer (i.e., reduce “A”) and reduce the parameters C, D, E, F, and G. All these seem possible in a carefully designed IPIM system where microbial inoculants (H) are better understood and combined with chemicals and appropriate cultural practices, including moisture.

In the diagram, fertilizers (A) refer to different kinds of fertilizers, and microbial inoculants (H) might include the combination of PGPR, mycorrhizae, and/or *Trichoderma* species.

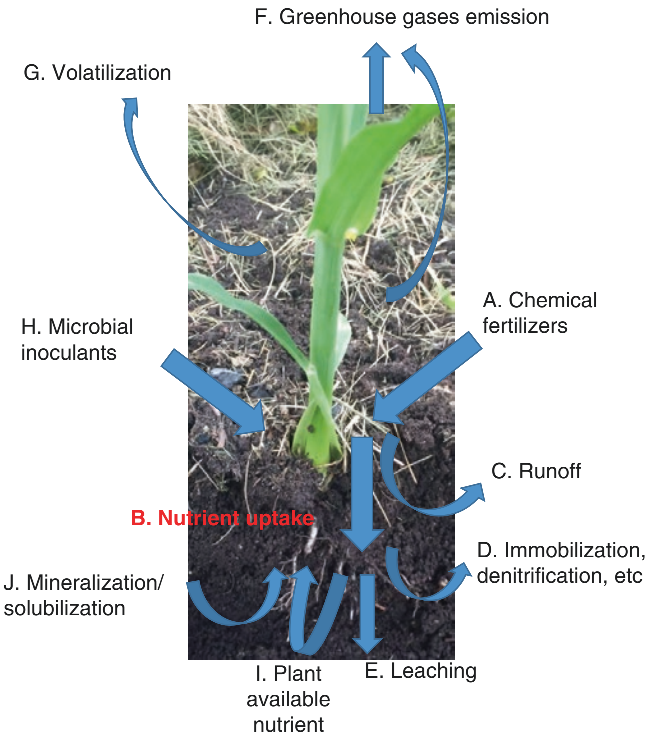

Fig. 2.1 Schematic of the possibilities from the interaction of crop roots and inoculants containing PGPR and mycorrhizae for improved efficient use of nutrients

2.3 Activity of Specific Microbial Groups in Enhanced Plant Nutrient Use

Natural beneficial symbiotic relationships formed by different groups of organisms such as mycorrhizae have been known for years (Barea et al. 1993, 2002). Many reports have shown that free-living plant growth-promoting rhizobacteria (PGPR) can form mutualistic relationships with plants (Kloepper et al. 1991; Bashan and Holguin 1998; Compant et al. 2005). When these organisms are introduced into the rhizosphere, they have the potential to alter microbial populations in the rhizosphere and influence nutrient transformation, availability, and uptake by plants (Adesemoye et al. 2008b; Shen et al. 2012). Regardless of whether PGPR and mycorrhizae populations in a location are indigenous or result from purposeful inoculation, neither group will exist or function in isolation. Better understanding of the interaction of PGPR and mycorrhizae as well as other relevant beneficial microorganisms such as *Trichoderma* and their joint influence on crop growth, development and physiology of the plant (Glick and Bashan 1997; Volpin and Phillips 1998; Barea et al. 2002), morphological characteristics of inoculated roots

(Yanni et al. 1997; Biswas et al. 2000), and how improved nutrient uptake occurs (Okon and Kapulnik 1986; Biswas et al. 2000; Adesemoye et al. 2008b) is crucial.

2.3.1 Plant Growth-Promoting Bacillus Species

Diverse effects and mechanisms of plant growth-promoting rhizobacteria (PGPR) have been reported, including phytohormone production (Tien et al. 1979; Hussain et al. 1987; Chabot et al. 1996), production and secretion of siderophores (Joo et al. 2004), N_2 fixation and efficient use of N sources (Yanni et al. 1997) and use of other nutrients (Chabot et al. 1996), and inhibition of plant pathogens (Compant et al. 2005; Haas and Defago 2005; Someya and Akutsu 2006).

Bacillus species are the most widely used PGPR in agricultural products, mainly because of their long-term survival as spores (Yildirim et al. 2006; Adesemoye et al. 2008a). *Bacillus* spp. have wide metabolic capabilities allowing them to play important roles in soil ecosystem functions and processes. Due to their heterotrophic nature, *Bacillus* spp. are also important in soil carbon, nitrogen, and sulfur cycling, as well as the transformation of other soil nutrients (Mandic-Mulec and Prosser 2011). The capacity for survival in constantly changing environments gave *Bacillus* spp. an edge in the potential to alter soil microbial community composition (Compant et al. 2005). Capacity to form stress-resistant endospores, secretion of peptide antibiotics and signal molecules, multilayered cell wall, and extracellular enzymes are characteristics that contribute to their survival and longevity (Kumar et al. 2011).

Bacillus megaterium and *B. muciaraglaginous* co-inoculated with AMF were reported to improve nutritional assimilation of plant total N, P, and K in maize (*Zea mays*) (Wu et al. 2005). *Bacillus polymyxa* was reported with the capacity to fix atmospheric nitrogen (N_2) (Omar et al. 1996), and different *Bacillus* spp. have been identified as phosphorus solubilizers (de Freitas et al. 1997; Rodriguez and Fraga 1999). *Bacillus amyloliquefaciens* FZB45 was shown to contribute to plant growth promotion and produce phytases, the enzymes that solubilize P from phytate, an organic phosphate (Idriss et al. 2002), while *Bacillus licheniformis* and *B. amyloliquefaciens* were found to produce mixtures of lactic, isovaleric, isobutyric, and acetic acids which are organic acids responsible for decreasing the pH of the surrounding soil, thereby releasing phosphate ions (Rodriguez and Fraga 1999). *Bacillus* spp. have also been reported in enhanced K uptake (Sheng and He 2006) and increase uptake of micronutrients (Kohler et al. 2008).

Bacillus PGPR have been shown to help plants in tolerance and survival of abiotic stresses such as drought and salt (Arshad et al. 2008; Vardharajula et al. 2011; Lim and Kim 2013; Egamberdieva and Adesemoye 2016). Drought conditions can elicit various biochemical and physiological reactions in crops, hinder crop growth and productivity, and may lead to death. Lim and Kim (2013) reported growth promotion of pepper plants under drought stress through the inoculation of PGPR *B. licheniformis* K11 and suggested that the strain was able to produce ACC deaminase which reduced the ethylene concentration of the plants by cleaving the precursor ACC (1-aminocyclopropane-1-carboxylate) under drought stress, thereby increasing plant growth. Adaptation and PGPR-induced salt tolerance has been attributed to improved water use efficiency and more efficient overall metabolic processes (Arshad et al. 2008; Yildirim et al. 2006; Egamberdieva and Adesemoye 2016). Though gene expression under these conditions has been studied and drought-responsive gene characterized (Vardharajula et al. 2011), the molecular basis and the detailed physiological changes have not been well understood.

The *Bacillus* genus is very important as an inoculant and is widely used as active ingredients in many biological products that are available in the United States and many parts of the world. For example, *Bacillus* spp. are components of the commercial inoculants. Accomplish LM and QuickRoots as well as many products are used for biological control of plant diseases. This explains why the genus *Bacillus* is a main interest in this review. Though there is a volume of work on the genus, there is a need for more extensive studies as biology-based products are now more important than ever.

2.3.2 Mycorrhizal Fungi and Interactions with PGPR

Mycorrhizal fungi are key components of the soil microbiota, and in addition to the beneficial relationships with plant roots, they also interact with other microorganisms in the rhizosphere. Mycorrhiza formation changes several aspects of plant physiology and some nutritional and physical properties of the rhizosphere soil, which in turn affects how other soil microorganisms colonize plant roots (Barea et al. 2002).

Mycorrhizal fungi are recognized beneficial organisms that can help improve plant establishment, better nutrient use, biological control of pathogens, and protection against cultural and environmental stresses. The mutually beneficial associations formed naturally with plant roots by ectomycorrhizae and/or endomycorrhizae are known to affect plant physiology including chemical composition of root exudates, better use of nutrient, hormonal balance, and carbon allocation patterns (Schenck 1981; Barea et al. 2002), but these potentials have not been well explored. The dense layer of hyphae (mantle) formed by ectomycorrhizal fungi and the vesicles formed by arbuscular mycorrhizal fungi (the most common among endomycorrhizae) are able to play significant roles in better uptake of nutrients. Other benefits of mycorrhizae listed by Schenck (1981) include enhancement of water transport in plants, decrease transplant injury, and plants' ability to survive extreme temperatures.

In the complex soil ecosystem, it will be impossible for the interaction of mycorrhizae and plant to occur in isolation. The interaction of mycorrhizae and beneficial bacteria especially PGPR in the plant rhizosphere is crucial in understanding the overall effects of microbes in nutrient uptake; however, very little is known about these interactions. It has been shown that bacteria can directly affect the germination and growth rate of mycorrhizal fungi. On the other hand, mycorrhizal fungi affect bacteria community compositions directly or indirectly through plants. The development of the mycorrhizal fungal mycelium can serve as a carbon source to PGPR as well as other rhizosphere microbial communities and introduce physical modifications into the environment surrounding the roots (Barea et al. 2002).

Bacteria and mycorrhizae through these interactions (and some of them may be very specific among strains) have been shown to jointly enhance the growth of plants and better root branching and architecture, thus improving nutrient acquisition (Artursson et al. 2006; Adesemoye et al. 2008b). The functional mechanisms behind the interactions of mycorrhiza and PGPR are not yet clear, but a better understanding is needed to achieve effectiveness for practical application in sustainable crop production.

Applications of single pure cultures of microbial inoculants have recorded little success in the past due in part to low knowledge about the organisms, their colonization and adaptation capabilities, and their interactions with other organisms that are present during the interaction with plant roots. The co-inoculation of PGPR and mycorrhizae and their use as inoculants in optimizing plant nutrient uptake are promising (Adesemoye et al. 2008b). There are indications that the biological sector of the agricultural industry is increasingly interested in combining or exploring multiple organisms or strains in products. This should improve efficacy because overall there is relatively more success in research with joint application of multiple inoculants involving multiple bacteria or bacteria and mycorrhizae.

The benefits of co-inoculation of phosphate-solubilizing PGPR and/or nitrogen-fixing PGPR with mycorrhizae to plants have been demonstrated (Rodriguez and Fraga 1999; Barea et al. 1993, 2002), but the interactions have to be managed to improve on the enhancement of plant nutrient use efficiency. The available knowledge on the tripartite interactions of root, mycorrhiza, and PGPR interactions is still little (Requena et al. 1997; Adesemoye et al. 2008b). In a field study with corn, Adesemoye et al. (2008b) showed improved uptake of nitrogen and phosphorus through co-inoculation of *Bacillus* PGPR and mycorrhizae. In a study involving mycorrhiza, *Rhizobium*, and PGPR, Requena et al. (1997) demonstrated effectiveness of the interactions in improving plant development, nutrient uptake, and root system quality and recommended the use of local isolates due to physiological and genetic adaptation of microbes to the environment. There is a need for more understanding.

It is important that challenges associated with co-inoculation are tackled in research studies or as part of the product developmental process. One of such challenges is compatibility of potential co-inoculants. Stephens and Rask (2000) reported a study where among seven different ways of combining four strains and 16 comparisons made in only one situation was the population of the mixed culture similar to the monoculture. Thus, compatibility of strains must be well tested as there is a possibility that individual strains in a mixed culture may be antagonistic against one another or may find it difficult to reach desired populations.

2.3.3 Other Select Microbial Inoculants

Many organism groups have been well reported in the literature as possible inoculants including bacteria in the genera *Pseudomonas*, *Burkholderia*, *Bradyrhizobium*, *Rhizobium*, *Azospirillum*, and *Lysobacter* and fungi in the genera *Trichoderma* and *Penicillium*. Some of these have been used as active ingredients in biological products currently on the market, and the products are available for use as biopesticide or microbial inoculants in plant growth promotion or nutrition enhancement.

Species of *Bradyrhizobium* and *Rhizobium* are important microbial inoculants but will not be discussed in this review as they are not free living and, therefore, not considered as PGPR. *Azospirillum* species have been well reported to enhance the growth of legumes and nonlegumes and capable of interacting with other PGPR and mycorrhizae (Bashan 1999). The beneficial impacts of *Pseudomonas* species on growth promotion, drought tolerance, and plant nutrient uptake have been demonstrated (Arshad et al. 2008; Kohler et al. 2008; Sharma et al. 2013). However, the inability of *Pseudomonas* spp. to form durable resistant endospores makes it less attractive compared to *Bacillus* spp. (Adesemoye et al. 2008a) in product formulation, especially products in nonliquid forms. Species of *Trichoderma* and *Penicillium* are common in many of the commercially available inoculants on the market. For example, as shown in Table 2.1, Graph-Ex SA, QuickRoots, and SabrEx are examples of inoculants containing *Trichoderma* sp., while JumpStart and TagTeam LCO contained *Penicillium* sp.

Table 2.1 Some examples of inoculants for crop growth and nutrient use enhancement registered in the United States

Microbial inoculant	Active ingredients	Registered crop	Manufacturer
Accomplish LM	*Acidovorax facilis*, *Bacillus subtilis*, *B. licheniformis*, *B. megaterium*, *B. oleronius*, *B. marinus*, and *Rhodococcus rhodochrous*	Corn, soybean	Loveland Products
AGTIV	*Glomus intraradices*, *Rhizobium leguminosarum* bv. viciae	Field crops, potato, peas, lentils, and faba beans	Premier Tech
Bioboost	*Delftia acidovorans*, *Bradyrhizobium japonicum*	Soybean, pea, and lentil	BrettYoung
Cell-Tec	*Bradyrhizobium japonicum*	Soybean, chickpea, pea and lentil, peanut	Monsanto
Dyna-Start	*Bradyrhizobium japonicum*	Soybean, peanut	Loveland Products
Graph-Ex SA	*Bradyrhizobium japonicum*, *Trichoderma* sp.	Soybean, dry beans	Advanced Biological Marketing
HiStick N/T	*Bacillus subtilis*	Soybean, bean (dry/snap)	BASF

(continued)

Table 2.1 (continued)

Microbial inoculant	Active ingredients	Registered crop	Manufacturer
JumpStart	*Penicillium bilaii*	Chickpea, corn, dry bean, sorghum, soybean, sugar beet, sunflower, wheat	Novozymes
Optimize liquid soybean	*Bradyrhizobium japonicum*	Soybean	Novozymes
QuickRoots	*Bacillus amyloliquefaciens*, *Trichoderma virens*	Alfalfa, corn, sorghum, soybean, sugar beet, sunflower, wheat	Monsanto
Regalia Rx	*Reynoutria* spp.	Corn, soybean	Marrone Bio Innovations
Rhizo-Flo	*Bradyrhizobium japonicum*	Soybean	BASF
SabrEx	*Trichoderma* sp.	Corn, wheat, sorghum, rye, and oats	Advanced Biological Marketing
TagTeam LCO	*Bradyrhizobium japonicum, Penicillium bilaii*	Pea and lentil, soybean, dry bean	Monsanto
Vault SP	*Bradyrhizobium japonicum*	Soybean, peanut	BASF

Note: Products shown on this table are specific examples of inoculants in the market, and only products registered in the United States mostly in Nebraska and/or Alabama are shown
Warning: Authors or publishers are not endorsing or approving any product on this table, and, similarly, the nonappearance of any product does not imply disapproval

There is a volume of knowledge on biological control by many beneficial microbes (Zehnder et al. 2001; Buensanteai et al. 2008; Zhang et al. 2010; Zhou et al. 2016), but scientific information is evolving on the role of microbes in helping plants in nutrient uptake (Adesemoye and Kloepper 2009). One common observation from these studies is that one strain of a microbial species may have biocontrol properties and other strains may be effective in enhancing nutrient uptake, but it is less common to find strains that perform effectively in both capacities. These properties may be related but evidently they are separate. Along the path of IPIM, it would be great to have products with strains that are very effective in both properties or combo products with each component having different organisms that have each of these properties. The process to identify high-performance strains for product development and formulation is dependent on the collection and screening procedure. We concur with Fravel (2005) that there is no single correct way for strain collection and screening procedures as the decision is affected by multiple factors, especially cropping system of interest, but the flowchart presented as Fig. 11.1 by Egamberdieva and Adesemoye (2016) is adaptable to a lot of screening programs with different purposes.

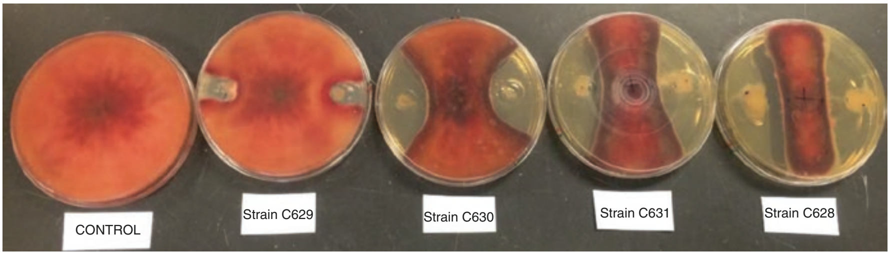

Fig. 2.2 *Burkholderia* sp. antibiosis against *Fusarium graminearum*

It should be added, however, that no screening program should be viewed strictly as a linear process because a precedent level may not correlate with the next level. An example can be seen in Fig. 2.2, where inhibition screening was conducted for four different strains of *Burkholderia* species against one *Fusarium graminearum* by the first author in this review. Based on the inhibition test only, it is correct to conclude with a bigger zone of inhibition, for instance, that strain #4 is more effective than strain #3. However, this trend did not hold true for all the strains after screening with plants in the greenhouse. Therefore, for any screening program to be successful, it should use a collection of data from different screening levels in the flowchart to make a decision of the best strains. One crucial trait that every potential strain to be used in the development of biologicals for nutrient use efficiency or biological control must have though is plant colonization ability. The correlation between root colonization and effectiveness as a PGPR and performance in the field is well established (Zehnder et al. 2001; Vessey 2003; Nelson 2004; Adesemoye and Kloepper 2009). Possible host plant specificity, adaptation to soil types, climatic conditions, and competitive edge against other organisms are some other crucial factors to be considered for any screening process to be effective (Nelson 2004).

Strain C628 has the highest effectiveness and largest zone of inhibition followed by strain C631 and strain C630, while strain C629 has the least effect and barely has any zone of inhibition.

2.4 Potentials of Microbial Inoculants to Reduce Greenhouse Gas Emissions from Fertilizers

Concerns for rising atmospheric concentrations of greenhouse gases (GHG) resulting from human-induced activities continue to be a major issue in the United States and worldwide. Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) have been implicated as the most significant gases of concern because their radiative forcing potential could impact global climate change. While debate exists within the scientific community, as to the extent these emissions have contributed to global climate change, it is a fact that the Earth's surface temperature has increased about 0.8 °C since 1880, with more than two-thirds of this warming occurring since 1975 (Hansen et al. 2010).

Agricultural activities including crop and soil management practices have been identified as a potential source of GHG emissions. Specifically, N fertilization practices have been noted as the greatest contributor via N loss in the form of N_2O flux. Agricultural N_2O emissions are more than twice that of pre-1940 management and about six times greater than native vegetation (Del Grosso et al. 2005). It is estimated that N-fertilization practices account for approximately 75% of the anthropogenic N_2O flux in the United States (US EPA 2012) making it the largest non-fossil fuel contributor, with crop (~51%) and grazing lands (~21%) being the major contributors. Given that most of the world's population depends on crops supplemented with N fertilizer for food, it is crucial to identify alternative N sources and management practices that reduce GHG emissions (Watts et al. 2015).

Recently, there has been evidence showing that PGPR may have a GHG emission reduction effect (Calvo et al. 2013), which has led to a US Patent (US Patent 9,266,786). These GHG emission reductions have been observed under both laboratory (Calvo et al. 2013, 2016a) and greenhouse conditions (Calvo et al. 2016b). This research evaluated the influence of SoilBuilder, a metabolite extract of SoilBuilder, and a mixture of four strains of PGPR *Bacillus* strains [*Bacillus safensis* T4 (previously called *B. pumilus* T4), *Bacillus pumilus* INR7, *Bacillus subtilis* ssp. subtilis IN937a (previously called *B. amyloliquefaciens* IN937a), and *Lysinibacillus xylanilyticus* SE56 (previously called *B. sphaericus* SE56)]. Nitrous oxide reductions of up to 80% were observed when PGPR inoculants were applied to a N-fertilized soil and sand mixture without plants under laboratory conditions (Calvo et al. 2013, 2016a). Similarly, these microbial inoculants exhibited N_2O reductions under greenhouse conditions with corn (*Zea mays* L.) planted in a N-fertilized soil-sand mixture, reducing N_2O flux up to 50% (Calvo et al. 2016b). Moreover, not only did these microbial inoculants decrease N_2O emissions under greenhouse conditions, improvements in plant growth (roots and aboveground biomass) and nutrient uptake were also observed, implying that nutrient use efficiency was also improved (Calvo et al. 2016b).

The exact mechanism involved in N_2O reduction by the microbial inoculants has not been discerned. However, possible mechanisms involved in the reductions are (1) the production or presence of nitrification inhibitors and inhibition of nitrifying and/or denitrifying microorganisms, (3) competition of applied microbial inoculants with native nitrifiers and/or denitrifying community, and (4) immobilization of fertilizer N and root exudates by microbes (when plants are present). It is important to mention that N_2O emissions were also observed when only the microbial metabolite portion of SoilBuilder product was applied. Calvo et al. (2013) suggested that the microbial metabolite portion of SoilBuilder may contain phenolic compounds that inhibited soil nitrifying and/or denitrifying bacterial communities.

Previous research have shown that N_2O emissions can be impacted differently depending on the N fertilizer source applied. Results from Calvo et al. (2013, 2016a, 2016b) showed that microbial inoculant effects on N_2O emissions are also impacted differently by N fertilizer source. For instance, urea-ammonium nitrate and calcium-ammonium nitrate reduced N_2O emissions, while no effect was observed with urea, and ammonium nitrate increased emissions (Calvo et al. 2013, 2016a, 2016b).

These previous observations from Calvo et al. work suggest that microbial inoculants have promise for reducing agriculture's GHG emission footprint; however, its impact may be dependent on the N fertilizer type used. It is believed that adaption and adoption of microbial inoculants into commercialized products as a N_2O-reducing agent will increase in the coming years once research and development for this new technology's use is refined.

2.5 Better Use of Nitrogen from Fertilizers and the Impact on Nitrous Oxide

Nitrogen is a limiting nutrient which is essential to optimize yield of most cropping systems, and this has resulted in the consumption of nitrogen increasing faster than that of any other plant nutrient source since the 1960s (USDA ERS 2012). This is contributing to rising atmospheric N_2O emissions as nitrous oxide emissions have been correlated with increasing N rates (Halvorson et al. 2014; Snyder et al. 2009), but impacts can vary depending on fertilizer source (Halvorson et al. 2010; Venterea et al. 2010; Sistani et al. 2011). Although N_2O levels reaching the atmosphere are minuscule compared to CO_2, its radioactive forcing is 298 times greater (Myhre et al. 2013) making it a major player in the total GHG emission budget. Abatement strategies to minimize and mitigate N input effects on global warming potential are thus essential.

Soil N_2O production primarily occurs through nitrification and denitrification processes (Firestone and Davidson 1989). Nitrification has been identified as the primary source of N_2O in many aerobic soils and denitrification under anaerobic conditions (Bremner 1997; Dell et al. 2014). Denitrification occurs in anaerobic microsites within partially aerated soils which contribute to the N_2O loss budget (Parkin 1987; Parkin and Kaspar 2006). N-management practices are needed that can better synchronize N supply with crop demand, govern nitrification and denitrification processes, and increase plant N uptake to reduce the potential for environmental loss.

Current fertilizer management practices often exceed plant N needs, where an excess is applied as insurance for crop production (Fig. 2.1). As a result, estimated worldwide N-use efficiency is 20–50% in most agricultural systems with the excess being susceptible to loss through runoff, leaching, volatilization, and N_2O emissions. Consequently, recent fertilizer advancements have facilitated enhanced formulation development for reducing N release rates to soil in efforts to minimize N loss. These enhanced efficiency nitrogen fertilizers (EENFs) are categorized as slow-release, control-release, and/or stabilized N fertilizers (Halvorson et al. 2014) that minimize early season N availability when crop uptake is slow, thereby reducing the loss potential (Akiyama et al. 2010). Some of these EENFs contain chemical formations with nitrification inhibitors to reduce the potential for N_2O emissions.

Recently, there has also been a great interest in the development and implementation of agricultural greenhouse gas (GHG) reduction offset protocols that can be included in cap and trade markets (Millar et al. 2010). Direct strategies or technologies for N_2O reduction are limited, but are expected to include nitrification inhibitors and slow-release fertilizers (Mosier et al. 1998; Singh and Verma 2007) which have had mixed or inconsistent results. However, none of these strategies have included the application of microorganisms, which could play an important role in N_2O reduction by interacting with the native N-cycle microbes. Soil microorganisms are responsible for the mineralization, immobilization, nitrification, and denitrification processes.

Hence, manipulating native soil microbial communities by application of selected inoculants with specific microorganisms can potentially alter N_2O emissions from the soil. Bacteria can transform gaseous nitrogen into ammonia, which can directly be used by plants. It has been well demonstrated that co-inoculation of *Bacillus* PGPR and mycorrhizae or their individual inoculations could enhance nitrogen and phosphorus uptakes in the field (Adesemoye et al. 2008b). The success of inoculants to stimulate uptake of nutrient is affected by many factors, one of which is soil type. Egamberdieva (2007) demonstrated that inoculant did not show significant effects in loamy soil whereas the strains had significant impacts on nutrient uptake in a nutrient-deficient calcisol soil.

2.6 Better Use of Phosphorus from Fertilizers

Soils contain large reserves of P but significant parts of it are not soluble or available for plant uptake (Watts et al. 2010). There are many bacteria in the soil and plant rhizosphere reported to have the capacity to solubilize inorganic phosphates (dicalcium phosphate, tricalcium phosphate, hydroxyapatite, and rock phosphate) or to mineralize organic phosphates, thus making P available to plants; *Bacillus* has been reported as one of the most active groups (Rodriguez and Fraga 1999). Hydrolysis or mineralization of organic phosphates to inorganic forms is carried out through phosphatases. Organic acids produced by bacteria are able to bind P and extracellular phosphatases and release P from organophosphates, making it available to plants. Synergistic interactions of phosphate-solubilizing PGPR and mycorrhizal fungi have been demonstrated (Rodriguez and Fraga 1999; Barea et al. 2002) where mycorrhiza plays a role in increasing the population of phosphate-solubilizing PGPR and the extraradical mycelium acts as a bridge for making phosphorus that was solubilized from nonsoluble inorganic and organic P compounds by the PGPR available.

Vassileva et al. (2010) explained that mineralization of lignocellulosic agro-industrial wastes by microbial processes and simultaneous solubilization of inorganic insoluble phosphates will provide the plant with an organic amendment rich in polysaccharide compounds and make P and nutrients available to plants but could also enhance soil enzyme activities and quality. In this system, more efficiency was reported with association of arbuscular mycorrhizal (AM) fungi with P-solubilizer/agro-waste-amended treatments. In the effort to understand how inoculants can affect the use of phosphorus by alfalfa, Piccini and Azcon (1987) inoculated three different endomycorrhizal fungi—*Glomus mosseae*, *G. fasciculatum*, and *Glomus* sp.—with or without co-inoculation of phosphate-solubilizing bacteria (PSB) in the presence or absence of Bayovar rock phosphate. The researchers reported that in the presence of Bayovar rock phosphate, PSB increased the dry weight of alfalfa in all inoculated treatments but dual inoculation of PSB and mycorrhizal fungi stimulated alfalfa dry weight more than either organism alone. The results also showed that alfalfa plants reached the maximum yield in the presence of rock phosphate plus PSB and mycorrhizal colonization.

There is substantial evidence that P-solubilizing bacteria have great potentials for the inoculant sector. What is needed is further investigation to improve their performance and develop them for compatibility with other bacteria and/or mycorrhizae for effective co-inoculation and practical application to enhance nutrient uptake in agricultural systems. For this to happen, each lead P-solubilizing strain must be well studied in terms of their survival and establishment, stability of their P-solubilization trait following inoculation, and their competitiveness and resilience in the soil system. Adequate phosphorus (P) availability for plants stimulates early plant growth, proper maturity, and consequently good yield. As a limiting nutrient, too little P will hinder plant development, and too much will contribute to agro-environmental pollution through leaching or runoff with surface water and contribute to eutrophication (Adesemoye and Kloepper 2009). Effective use of inoculants in an IPIM would help ensure application of appropriate amounts of P, which will be less than current recommended amounts, and ensure availability of adequate amount to plants.

2.7 Examples of Microbial Products on the Market for Optimized Nutrient Use

The interest of consumers, farmers, and research information in recent years has been driving the evolution and development of the biorational sector in the agriculture industry. The factors, among others, include (1) growers/farmers demand for information on alternative strategies and products that can help ensure better and sustainable use of soil resources, (2) how to reduce the dependence on chemical inputs, (3) development of resistance by pathogens and pests against pest control chemicals, and (4) there is increasing consumers' interest in foods that are free from chemical residues. The agricultural industry has exploded in the last few years with many new inoculant products or fortification of existing products. There has been a lot of investment going into the sector, and this includes the big six pesticide companies—Bayer, BASF, Dow Chemical Company, DuPont, Monsanto, and Syngenta. Some specific examples of inoculant products on the market that are registered nationally or in certain states in the United States are provided in Table 2.1. Readers should note that the appearance of any product on this list does not imply endorsement by the authors or the publishers, and, similarly, the nonappearance of any product does not imply disapproval.

Conclusion

There are indications that the inoculant market will continue to grow and expand. There is a correlation between inoculant and organic production and demand of customers for organic products.

Progress is being made in understanding the role of microbes in nutrient use, but how to translate the volume of scientific information into practical field applications is still elusive. One of the reasons for this is that most studies were based on the impact of individual strains on plants, but field situations are far more complex, with many organisms interacting with the plant at the same time. Future studies need to address the interactions of multiple beneficial microorganisms with plants concurrently to provide better understanding of the complexity of microbial interactions in plant nutrient utilization and make findings more applicable in the field.

One of the challenges with inoculants is the inconsistent results under different conditions. Acceptance of these products by farmers is increasing, but there are concerns from farmers that the efficacy of many of the products currently available on the market is not consistent. Results from university studies have confirmed this concern on many occasions. Efficacy is improving and there is optimism that improvement will continue with more investments in related research. Molecular technology, especially metagenomics, has been evolving and helping to understand the complex interactions that occur in the soil-plant systems, especially the root microbiome.

Another concern is that products may not be compatible with existing chemicals and/or farming practices. What is the worth of a new agricultural product that is not compatible with common agricultural practices or cannot be delivered with equipment that are commonly used by farmers? Compatibility and efficacy are determined in part by the form in which the product is made available and applied. Is the product going to be coated onto the seed before going to the market or mixed with the seed shortly before planting? Is the inoculum to be delivered in-furrow onto the seed during planting or later onto the seedling? These are considerations that should be paramount in the formulation processes for a new product or improvement of an existing product.

Recent major investments in the biological sector are an indication of the role that these products will play in crop production going forward. Following many acquisitions, realignments, new ventures, and launch of new product platforms in this sector, market watchers have projected that the biological sector should experience double-digit growth between now and 2020 from the current estimated $2 billion market, and inoculants and biostimulants will be a crucial part of this growth. The investment in research is increasing and no doubt, this will improve efficacy. The interests in inoculants and overall biology-based technology will continue to expand as it is good for conventional agriculture as well as organic farming.

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