CHAPTER 4

Promoting biofertilizer utilization for sustainable crop production: Produce quality and human health implications.

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Abstract

Soil is a repository of diverse microbes many of which play significant roles in nutrients recycling and solubilization, reclamation of perturbed and polluted ecosystem. These microbes serve as soil quality indicator, plant disease suppression, and biocontrol agents. Many soil inhabiting microbes regulate plant growth, serve as source of bioactive compounds; function in the synthesis of hydrolytic enzymes responsible for degrading soil organically fixed nutrients while many others are now being used as biofertilizer. Hence, the widely reported potential positive impact of beneficial microbes in the highly diverse agro-ecosystems facilitated their recommendation and utilization as a component of improved soil-crop management practices. Soil health and quality indicators, including soils ability to guarantee provision of various ecosystem services like promoting sustainable increase in crop yield, guarantee the safety and the quality of produce, are influenced by the diversity and functionality of microbes-soil interaction. However, the possibility and mechanisms of transmission of virulent or infectious microbes via soil-crop-human linkage through consumption of produce from biofertilized fields is unclear. This requires further clarity and proper documentation. In-depth information on the persistence and traceability of beneficial microbes used as biofertilizer through soil application or seed during crop production needs to be carefully understood. We undertake a comprehensive review of the influence of biofertilizer use on soil quality, crop growth, and safety of produce for human and animal health. Lucid understanding of the mechanisms that govern the fate of microbes used during crop production will further provide useful information on safety of agricultural produce obtained, and the quality and safety of human life.

Keywords: Agro-ecosystem, food safety, microbial traceability, nutrient recycling, soil amendment, seed inoculation.

Introduction

Ensuring food security for the ever-increasing population across different nations is topmost in the priority list of many government policies, particularly among the developing and emerging economies. Besides ensuring food security, efforts are also directed at ascertaining that the food produced is nutritious and safe for the teeming populace. Microbial niche via biotechnology has been scientifically explored in agroecosystems to clean polluted environment (Vassilev *et al.*, 2006), rejuvenate degraded soil, recycle nutrient for enhancing soil fertility and improve crop productivity in agro-ecosystems (Adesemoye *et al.,* 2008a). The microbial niche in nutrient recycling through decomposition of organic matter, solubilization and re-distribution of bound nutrients, growth stimulation and as biocontrol agents cannot be overemphasized. This unique functional role informs the utilization of microbes as biofertilizer in agro production system.

Biofertilizers are cultures of artificially multiplied or native soil microbes whose inoculants colonize rhizosphere and/or rhizoplane of crops thereby enhancing supply or availability of essential plant nutrients through symbiosis or solubilization (Laca *et al.,* 2006, Vilchez and Manzanera, 2011), growth stimulus or defense against pathogenic infections through inhibition to the target crops. However, the fate of microbes used as biofertilizers in crop production has not been properly documented. It is known that microbes while performing their obligatory roles engage different principles like plasmids, (pro)phages and conjugative transposons (Juhas *et al.*, 2009), acquisition of new phenotypic traits (Mellmann *et al.*, 2011) and many other mode of actions to deliver their roles. During these processes, gene transfer and alteration may occur through several mutations that may result in emergence of infectious organisms different from the applied non-pathogenic candidate (Eppinger *et al.*, 2011). This might mean that the applied organisms re-design the environment to suit their purpose or change the form of minerals available for uptake by plant among many other possibilities (Chin-A-Woeng *et al.*, 1998). It has been clearly shown that soil-microbe-plant interaction is closely related to crop quantity, quality and safety with implication on health of heterotrophs at different levels (Lugtenberg *et al.,* 2002) because the resulting interactions may present potential hazards to human, animal or plant (Cheuk *et al.*, 2000). The persistence and traceability of the applied organisms in an ecosystem is not well known. This review tries to trace the history of biofertilizer, their benefits, synthesis and safety in agricultural production with a view to ascertaining their roles on soil health, fate in produce and implication of the consumed produce on humans and livestock health.

Development of biofertilizer

In nature, certain soil inhabiting groups of microorganisms perform beneficial roles in improving soil chemical properties through fixation of some plant nutrients, solubilizing unavailable nutrients and promoting plant growths or inhibit pathogenic infection in crops. These organisms exist either as free living in nature or as latent cells of efficient strains of microbes belonging to many taxa of bacteria, fungi, protozoa and cynanobacteria Kingdom (Vessey 2003, Lucy *et al.,* 2004, Smith and Read, 2010). Majority of these organisms interact with plant roots in the rhizosphere to facilitate nutrient uptake (Patil *et al.,* 2014). Groups of microorganisms that perform the above roles are explored for production of microbial-based fertilizers known as biofertilizers (Kloepper *et al.*, 1980a).

The use of these biofertilizers became popular in the twentieth century after the failure of agricultural revolution to take care of environmental consequences of extensive deforestation for farmland, massive utilization of agrochemicals particularly fertilizers and pesticides resulting in ecosystem disturbance, disequilibrium, shift or loss of biodiversity (Saravanan *et al.,* 2009, Abbasi *et al.*, 2011). The environmental challenges that accompanied the drive towards averting crises that could stem up from Malthusian theory of population through commercial agriculture cannot the overemphasized. The impressive outcome of the agricultural revolution notwithstanding was short lived. There was downward trend in the overall harvest with huge environmental perturbation like overall reduction in soil fertility, water and soil pollution, loss of micro-organisms and beneficial insects among many others (Kronening *et al.*, 2001, Katsunori, 2003). This is perhaps in agreement with Newton's Law of action-reactions pairs. It was on this premise that scientists through hindsight did reflect on the age long self-regulating ecosystem and concluded on the need to revert back to pre-agro-industrial revolution system. Some scientists throughout the world worked out substitute to deleterious effects of agrochemicals and found biofertilizer as a right alternative. This made organic agriculture a relevant alternative to salvaging our nearing collapsed ecosystem. Several forms or methodologies such as conservation agriculture, responsible agriculture, sustainable agriculture, organic farming, good agricultural practices, and many others were adapted with a principal focus on sustainable use of renewable resources for the benefits of all living things.

Biofertilizer is a major component of good agricultural practices aiming at sustainable utilization and recycling of renewable resources without or with minimal detrimental effects on the environment (Pérez-Montaño *et al.,* 2014). The benefits of utilization of biofertilizers in advancing good agricultural practices are significantly related to the health of the soil and quality of the produce from the crops grown on such soil treated with biofertilizers (Malusà *et al.*, 2016). Despite a huge and broad range of benefits associated with the utilization of biofertilizers, there still exists much vagueness that needed to be understood to optimize their use in agroecosystems.

The urgent need to ensure high productivity and maintain environmentally sustainable agro-ecosystem, calls for reduction in synthetic agrochemical usage in order to maintain biodiversity and dynamic ecosystem. Utilization of biofertilizers in agroecosystems need to be heightened by mass producing cultures of microbial inoculants to improve crop production and maintain biodiversity as well as eco-friendly environment. The safety of produce harvested from biofertilized field need to be ensured through quality assurance mechanism and conscious effort to ascertain that the culture of bioinoculants are safe for human and livestock consumption.

Native or indigenous biofertilizers are naturally occurring soil inhabiting microorganisms that respond spontaneously to atmospheric nitrogen fixation, solubilizing phosphorus, and plant growth stimulation through the synthesis of growth promoting substances (Abbasi *et al.,* 2011). The effect of native biofertilizers in improving crop growth is dependent on the vagaries of soil environmental conditions which dictates their efficacy or otherwise. The indigenous biofertilizers belongs to the free-living blue-green algae, bacteria and fungi groups. Crops benefits immensely from nutrient supply and growth promoting potentials of aboriginal biofertilizers since soils are the bank for diversity of microbes. Specificity of non-commercial fertilizers in enforcing soil fertility and nutrient availability might be inconsequential since response of microorganisms to improving rhizosphere is stimulated by exudates from crop roots.

Some of the examples of autochthonous microorganisms belong to the nitrogen fixers like *Rhizobium* spp., *Azotobacter* spp. (Khalid *et al.,* 2005a); phosphate solubilizers such as *Pseudomonas* spp., *Bacillus* spp. *Aspergillus* spp. *Trichoderma* spp. (Tajini *et al.,* 2012, Krey *et al.,* 2013); potash mobilizers like *Bacillus* spp. and Zinc mobilizer including *Rhizobium* and *Pseudomonas* spp. (Filippi *et al.,* 2011, Yu *et al.,* 2011) and many others. Utilization of non-commercial biofertilizers has been extensively limited to manipulation of rhizobia in leguminous crops whereby growth and development of legumes were significantly improved, through nodulation and nitrogen fixation. This is possibly because a broad range of free living soil-borne rhizobia species established symbiosis (Bhattacharjee and Dey, 2014) with legumes (Cooper, 2007). Consequently, rhizobia were considered the best known beneficial plant associated bacteria and the most important biofertilizer. Commercial biofertilizers were later developed from culture of specific microorganisms to effect or stimulate growth and development in specific or target crops.

Biofertilizer classification, types and their roles in agro production.

In separate reports by (Bhattacharjee and Dey, 2014) and (Kumar and Gopal, 2015), biofertilizers may be classified into two unique classes based on the source namely: (i) natural or autochthonous, and (ii) synthetic. The natural biofertilizers are diverse kind of free living soil inhabiting microorganisms belonging to several taxa and kingdoms which colonize plant tissues or the rhizosphere thereby promoting plant growth and nutrient mobilization. They are sometimes referred to as native or indigenous microorganisms. The synthetic biofertilizers on the other hands are artificially multiplied cultures of microbial inoculants of certain soil microorganisms that can improve soil fertility and crop productivity.

In the same vein, biofertilizers could be grouped based on their unique functions in improving soil fertility, enhancing nutrient and water uptake and secretion of bioactive compounds (Figure 1). Based on functional roles, the three major types to which biofertilizer could be grouped are: Nitrogen fixers, phosphorus solubilizers and soil organic matter enrichers (Muraleedharan *et al.*, 2010). Different plant growth promoting microorganisms engage diverse modes of action while performing their unique functions. Types of plant growth microorganisms and the action mode is shown in Table 1.

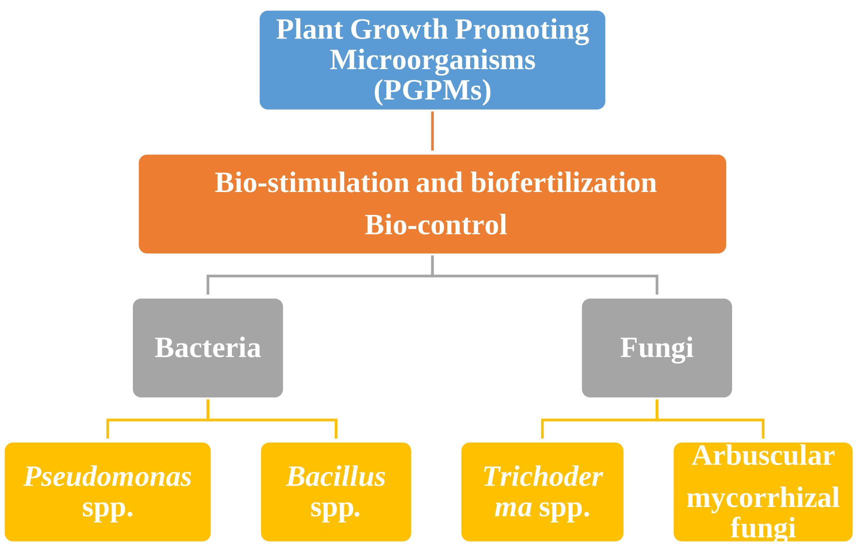

Figure 1. Classes of plant growth promoting microorganisms in agricultural production.

Soil microbes with potential biofertilizer roles

Nitrogen fixers

These are bacteria that fix atmospheric nitrogen into soil through symbiotic or asymbiotic relationship with certain plant species especially the leguminosae family. Some of these microbes include:

i. *Azotobacter*

These bacteria belong to the family of Azotobacteriaceae, anaerobic, free living and heterotrophic in nature found in soils with pH ≥7. Apart from *Azotobacter chroococcum* which is a common species found in arable field; other species belonging to this group include: *A. vinelandii*, *A. beijerinckii*, *A. insignis* and *A. macrocytogenes*. Population of *Azotobacter* in an ecosystem is often affected by low organic matter and occurrence of antagonistic microbes. Thus, population of *Azotobacter* rarely exceeds 104 to 105 g^{-1} of soil as their growth is usually controlled by natural mechanisms.

Table 1: Types, characteristics and mode of actions of some plant growth promoting bio-agents

Types of PGPMs	Bio-stimulation and Bio-fertilization	Bio-control
***Bacillus* spp.**	• Phytohormone-like actions (Idris *et al.*, 2004) • Phosphate solubilization (Hariprasad and Niranjana 2009) • Biological N_2-fixation (Rennie and Kemp 1983)	• Competitive colonization of plant surfaces (Bais *et al.*, 2004) • Production of antibiotics (Asaka and Shoda 1996) • Induction of plant's own defenses (Kloepper 1978)
***Pseudomonas* spp.**	• Modulation of phytohormonal balances (Gamalero and Glick 2011) • Phosphate solubilization (Trivedi and Sa 2008) • Biological N_2-fixation (Venieraki *et al.*, 2011) • Siderophore-mediated iron mobilization (Bar-Ness *et al.*, 1992)	Competition for space and nutrients (Kloepper *et al.*, 1980b) • Production of antibiotics (Hamdan *et al.*, 1991) • Induction of plant's own defenses (De Vleesschauwer *et al.*, 2008)
***Trichoderma* spp.**	• Modulation of phytohormonal balances (Martínez-Medina *et al.*, 2014) • Phosphate solubilization (Anusuya and Jayarajan 1998) • Solubilization of micronutrients like. Mn (Altomare *et al.*, 1999) • Enhancement of the plant's nitrogen use efficiency (Harman 2011) • Degradation and buffering of toxins (Doni *et al.*, 2014)	• Competition for resources (Tronsmo and Hjeljord 1998) • Production of antibiotics (Schirmböck *et al.*, 1994) • Inhibition of pathogenesis-related enzymes (Elad 1996) • Hyperparasitism, Mycoparasitism (Chet *et al.*, 1997) • Induction of plant's own defenses (Akram and Anjum 2011)
Arbuscular mycorrhizal fungi	• Spatial acquisition of mineral nutrients (Marschner and Dell 1994) • Improve drought resistance in plants (Augé 2001) • Alleviation of salt and heavy metal stresses (Quilambo 2003) • Stabilization of soil structure (Miller and Jastrow 1992)	• General raise of plant vigor (Castillo *et al.*, 2006) • Induction of plant's own defenses (Khan *et al.*, 2010) • Induction of changes in the composition of soil microbial populations (Srivastava 2009) • Interference with pathogenesis-related signaling pathways (Bari and Jones 2009)

ii. *Rhizobium*

This bacterium interacts with root nodules forming plants in a symbiotic relationship to fix atmospheric nitrogen. The *Rhizobium* species, found in the family Rhizobiaceae are symbiotic in nature with specific mode of action. *Rhizobium* spp. form symbiotic association only with leguminous crops and this limits their effectiveness as only certain or specific legumes benefit from this symbiosis. The association of *Rhizobium* with legumes fixes an average 50-100 kg/ha nitrogen (Mishra *et al.*, 2013). This N rate is optimum for production of wide range of fruit and leafy vegetables like tomatoes, eggplant, amaranthus, lettuce, beetroot, spinach, etc. This explains why this species of N fixers are indispensable in arable field.

Phosphate solubilizers and water uptake enhancers

These are species of *Pseudomonas*, *Bacillus*, *Rhizobium* bacterial genera which have the ability to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate , hydroxyapatite (Richardson *et al.*, 2009), as well as rock phosphate (Owen *et al.*, 2015) and make them available to crops. Similarly, certain fungi aid uptake of water and a variety of mineral elements which are beyond the rhizosphere where crops could reach it. Arbuscular Mycorhizal Fungi (AMF) is the major microbial candidates commonly associated with this function in many arid agroecosystems (Bardi and Malusà, 2012).

Soil organic matter enrichers

Vermicompost, a product of composting process using various worms to create heterogeneous mixture of decomposing vegetable or food waste, bedding materials, and vermincast is useful as biofertilizer for promoting crop growth. Vermicomposting technology involves utilizing earthworms to biologically convert organic wastes into vermicasts through vermiwash. These earthworms feed on organic wastes in which the gut of this worm serves as the bioreactor where the vermicasts are generally produced (Manyuchi *et al.*, 2013). The products from vermincomposting are good source of plant nutrient and growth promoters. Equally, effective microorganisms (EM) are most often incorporated into composting process to enhance their effectiveness and mineralization. The principal classes to which biofertilizer could be categorized is depicted in Figure 2.

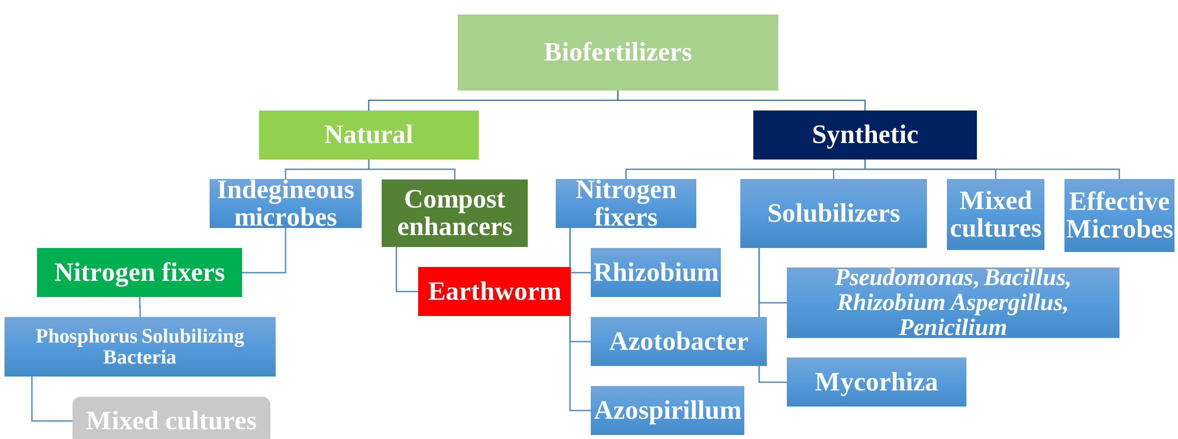

Figure 2: Major classes of biofertilizers

Available commercial and non-commercial biofertilizer products.

Commercial fertilizers are artificially cultured inoculants of specific microorganisms that promote crop growth through: biofertilization, rhizoremediation/stress management, phytostimulation and biocontrol. Culture of certain microorganisms species are specifically mass produced in commercial quantity and various forms such as liquid, powder or granules and are made available to growers. These cultures are prepared based on crops requirements and their functional role in promoting crop growth and development. Biofertilizers were commercially introduced in nineteen century by Nobbe and Hiltner when laboratory culture of Rhizobia known as Nitragin was produced (Deaker *et al.*, 2004) but its utilization as agricultural input was poorly adopted by farmers. The principal reason for low adoption was linked to poor access to information or awareness on its potential to improve crop yield and reduce environmental pollution. Recently, awareness has increased on biofertilizer benefits with increase in its patronage in the last decade (Bashan *et al.*, 2014). Reports showed that a number of microbial strains had been registered for use in agro allied operations. Surprisingly, many potential and highly useful strains are yet to be cultured for commercial purposes (Bashan *et al.*, 2014). There are currently over 149 registered microbial strains for agricultural products with steady increase in the demand for cultures of biofertilizer inoculants valued at more than $1 billion USD and predicted to soar to $7 billion USD by 2019 (Research, 2014).

Table 2. Types of Biofertilizers and target crops

Class	Types of Biofertilizers	Characteristics	Micro-organisms	Target crop	Reference
Nutrient supply	Nitrogen fixing biofertilizers	Obtain Nitrogen from the atmosphere and convert this into organic forms usable by plants	*Rhizobium*, *Azospirillum*, *Azotobacter*	Pulses, Oilseeds, Fodder crops, Cereals, Vegetables	(Cassan *et al.*, 2009), (Lucas *et al.*, 2009)
Growth promoters	Plant growth promoting biofertilizers	Increasing the growth and yield of plant	*Pseudomonas* sp.	Vegetables	(Singh and Kapoor 1999) (Upadhyay *et al.*, 2012)
Mineral solubilizers	Phosphorous solubilizing biofertilizers (PSB)	Solubilize insoluble inorganic phosphate compounds	*Bacillus*, *Pseudomonas* and *Aspergillus*	Legumes, Cereals, Root crops	(Bai *et al.*, 2002b) (Bai *et al.*, 2002a)
Mineral mobilizers	Phosphate mobilizing biofertilizers	symbiotic association between host plants and certain group of fungi at the root system	*Mycorrhiza*	Cereals, Legumes, Vegetables	(Bidondo *et al.*, 2011)

Benefits of biofertilizer utilization for sustainable crop production.

There is increasing concerns on the undesirable consequences of synthetic agrochemicals on soil productivity and ecosystem sustainability despite their supposed increasing agricultural production. Synthetic agrochemicals are extremely expensive, inadequate in terms of available quantity to farmers, with harmful environmental impact. The inimical carryover or residual effects of synthetic agrochemicals on the environment evidently in form of bio-accumulation and bio-magnification necessitated the need for alternatives to improving productivity, quality and safety. The use of beneficial microbes as substitutes becomes more imperative (Pérez-Montaño *et al.,* 2014). Biosynthetic potentials, ubiquitous nature and the size of these microorganisms made them useful tools for solving diverse problems associated with agrochemical usage in agroecosystems (Abbasi *et al.*, 2011, Krey *et al.*, 2013). Exploring these potentials has been shown to promote fertile soil with cleaner and safer environment. Some of the benefits of biofertilizers include but not limited to the following:

i. ***Sources of organic nitrogen and bioactive compounds:*** Biofertilizers are good source of stable nitrogen concentration in the soil. In field inoculated with nitrogen-fixing microorganisms as biofertilizers; they replaced chemical fertilizers by 25% and with huge reduction in environmental pollution from agrochemical usage (Franche *et al.*, 2009, Bardi and Malusà, 2012). Metabolic capabilities of some free-living nitrogen fixing bacteria like *Azotobacter* contribute significantly to nitrogen cycle in nature (Fischer *et al.*, 2007).

Furthermore, biofertilizers are potentially situated to synthesize and secrete considerable quantity of biologically active substances that help in modification of nutrient uptake by the plants. Some of these bioactive compounds like nicotinic acid, pantothenic acid, biotin, vitamins, thiamine and riboflavin necessary to enhance diverse metabolic activities are synthates of some biofertilizers. Also, plant growth hormones such as heteroxins, gibberellins are usually synthesized by these biofertilizers which makes them superior to chemical fertilizers. Some microorganisms when applied to soil help synthesize plant growth regulators such as indole acetic acid (IAA) and indole butyric acid (IBA) (Khalid *et al.,* 2005b) and thus accelerate growth and development of plants. These plant hormones are implicated in increasing the rate of mineral uptake through plant roots, resulting improved plant growth and yield.

ii. ***Cheap, affordable and highly effective fertilizer sources:*** The problem of affordability associated with chemical fertilizers especially among the resource poor growers could be overcome when biofertilizer is used. The mode of operation of biofertilizers differed significantly from agrochemicals because they indirectly effect growth and development in plant through subtle means thereby inducing application effectiveness and reduction in cost of application. Biofertilizers are made of culture of microbes, hence minute concentration can effect great improvement in a relatively short time per unit area of land and crop performance. This makes them veritable tools for improving poverty of resource poor farmers as less cost is spent on fertilizer as an agricultural input.

iii. ***Increase crop yield, reduce production cost and improve soil condition:*** Biofertilizer is a low input technology in agricultural system and at low production cost but with higher returns on investment compared to chemical fertilizers. Unlike agrochemicals, when culture of biofertilizers is applied as seed dressing or soil inoculants, they multiply and contribute to nutrient cycling, thus benefiting the crop productivity without any adverse environmental impact (Franche *et al.,* 2009). Nutrients are supplied to the plant as demanded thus, preventing starvation by providing nutrients reserve to plants. Reports have shown that yield obtained from biofertilizer inoculated field is in the range of 20-30% compared to chemical fertilizers (Lugtenberg *et al.,* 2002, Bhattacharjee and Dey, 2014). Thus, they are more efficient in supplying plant nutrients for improving crop performance than any other means.

iv. ***Enhance water and nutrient holding capacity:*** Biofertilizers increase nutrient and water holding capacity of the soil and also increase the drainage and absorption of moisture in soils, especially in those with structural or nutrients deficiencies. They increase the tolerance of plants to drought and moisture stress (Tajini *et al.,* 2012). In this way, they increase crop yield even in plantations with insufficient natural water supply or irrigation (Krey *et al.,* 2013).

v. **Improve soil fertility and prevent environmental pollution:** Biofertilizers enables microbial population build up and persistence in soil which helps to conserve soil fertility, prevents degradation and contribute to sustainable agriculture. They are environmentally friendly by preventing deleterious effect of excessive use of chemical fertilization on natural resources. Biofertilizers maintain soil environment through diversities of micro- and macro-nutrients via nitrogen fixation, phosphate solubilization and potassium mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Owen *et al.,* 2015).

vi. ***Biocontrol agents as antagonist and antibiotics:*** Biofertilizer utilization in agroecosystems help protect crop against some plant pathogens. They serve as biological control agents in form of antagonist of some phytopathogens. They are useful as antagonists and biological control agent by synthesizing siderophores and secreting antibiotics which act against phytopathogenic organisms like bacteria, fungi and insects both at rhizoplane and rhizosphere. Several fungal endophytes, like *Trichoderma* species have attracted scientific attention as fungi that are able to live part of their life cycle independent of plant, to colonize roots and transfer nutrients to the host plant, through mechanisms that remain unknown.

Perception of safety of biofertilizer utilization in agro production.

Many farmers perceive inoculants and biocontrol microbial products as more costly and less effective than traditional agrochemicals (Parnell *et al.,* 2016). This is possibly because of slow reaction of bio-effector strains to disease epidemic which often act by suppressing pest populations through slower processes. This may allow crop damage to attain economic threshold level rather than quick action on contact by synthetic pesticides. Apart from being slow acting, the need to build growers capacity on effective use of bio-stimulants and bio-pesticides, is another huge obstacle to adoption of bio-effectors. For examples, to use biocontrol strains effectively, there is the need to train farmers on pathogen identification and understanding the lifecycle of the pest or pathogen to be controlled with importance on timeliness of activities; appropriate conditions for preparation and inoculation of biocontrol cultures. This attests to the need for intensifying efforts at incorporating appropriate bioinoculants and biofertilizer products into growers cropping systems. This could be achieved by creating intensive awareness among farmers by all stakeholders. Many growers are skeptical about biofertilizer utilization because of the risk and special skills associated with microbial handling.

Timing of field application of microbial products often contributes significantly to poor perception on utilization of biofertilizers among crop growers (Chutia *et al.,* 2007). The mode of action of microbial products makes them selective and acts only on a specific host. Besides their specificity, microbial inoculants have a shorter life span and sometimes active life than agrochemicals (Van Lenteren, 2012). The combination of selectivity of the microbial strain to host or target substrate and high degradability limits precision of their applicability in the field (Nicot *et al.,* 2011).

Parnell *et al.* (2016) showed that exposure of Bt toxin proteins to rays of sunlight accelerates their speed of degradation. Again, huge cost is incurred as a result of the need for multiple application of Bt based microbial products in order to effect any meaningful result. The efficacy of biofertilizer and or biocontrol agent undermines a trade off between immediate short-lived impact and persistence in the environment (Barea, 2015). To create positive perception among farmers' bio-agents must be reliable in quality, cheap and affordable, available effortlessly, comparably effective, simple to handle, consistent in operation and compatible with the existing farmers' agricultural practices (Selvamukilan *et al.,* 2006, Herrera-Estrella and Chet, 2003).

Soil-plant-microbes interactions and implication for sustainable crop production.

The soil biome is reservoir of diversities of microorganisms with each of the taxon comprising several billions of species. Interestingly, microbial diversity is dependent on rhizosphere (Bulgarelli *et al.,* 2012), crop grown (Lundberg *et al.,* 2012) and habitat (Bulgarelli *et al.,* 2012). Among the several microbes present in the rhizosphere, bacteria are the most widely studied than other rhizosphere inhabitants. Gans *et al.,* (2005) reported that 1 g of soil contained more than 1 million distinct bacterial genomes, while Roesch *et al.,* (2007) obtained 139,819 bacterial and 9,340 crenarchaeotal rRNA gene sequences from four distinct soils. Based on diversity estimators, a maximum of 52,000 operational taxonomic units (OTUs) were identified and Bacteroidetes, Betaproteobacteria, and Alphaproteobacteria were the most abundant bacterial groups in most soils (Roesch *et al.,* 2007). It thus suggests that there are still a lot to be discovered and learnt about diversity of microorganisms across different habitats and ecosystems considering their structural and functional roles in maintaining ecological processes (Kennedy, 1999) and ecosystem stability (Yamanaka *et al.,* 2003). Besides, there are new development surrounding agro production in terms of crop protection, yield improvement, crop quality and climates change. There is the need to intensify research into discovering yet to be identified beneficial microorganisms that could be explored for mitigating these emerging challenges.

Although, soil contains pools of microorganisms but the diversity of microbe in soil biomes is a function of abiotic factors such as soil temperature, nutrition and pH. According to Lauber *et al.* (2008) soil physico-chemical properties particularly texture and nutrients have profound influence on microbial pool in soil biomes. Soil acidity is a single most important soil chemical factor that greatly impairs microbial population especially bacteria and fungi in soil ecosystem. The population of bacteria in a given soil is often directly proportional to the pH of the soil. Rousk *et al.* (2010) showed that bacteria diversity were lowest in acidic soil while neutral soil encourages proliferation of microbial population. This implies that bacteria resist acidic environment but tolerant of relatively narrow pH. Similarly, it has been reported that pH is the major driving force that dictates community structures and functions in most if not all soil biomes (Dumbrell *et al.,* 2010). Acidic soil impedes efficacy and ability of microbes to colonize their hosts thus rendering microbes inactive or dormant. This implies that in order to optimize the benefits of microorganisms as biofertilizers there is the need to establish a safe pH for the bioinoculants for effectiveness. Abiotic stresses such as nutrient deficiency, variable soil temperature, drought and others significantly affect symbiotic association between AMF and plant (Antunes *et al.,* 2012). Therefore efficiency of microorganisms as biofertilizers in agroecosystems is dependent on suitability of edaphic factors which creates conducive environment for colonization, multiplication and metabolism.

Introduction or application of microorganisms as biofertilizer into soil tends to trigger shift in microbial niche whereby competition ensue between autochthonous soil microbes and culture of introduced inoculants strains. This explains the importance of understanding the diversity of indigenous microorganisms in a soil to be biofertilized to facilitate effectiveness of such amendment. Although, efforts are ongoing through numerous methods to evaluate impact of these interrelationships on efficacy of biofertilizers. Nevertheless, understanding in this direction as it relates to the interrelationship in the treated soil biome is at infancy stage.

The relationships between indigenous and introduced microorganisms depend largely on the techniques used to influence the dynamics of soil microbial communities (Trabelsi and Mhamdi, 2013). It is not unlikely that the product of the interrelationship could have negative effects on soil health and crop grown on it. Inoculants of fluorescent pseudomonas, symbiotic and free-living N-fixing bacteria, arbuscular mycorrhiza fungi have been shown to impair various taxonomical or functional groups of indigenous soil microorganisms (Trabelsi *et al.,* 2012). It is expedient to understand that appropriate selection and introduction of microbial strains that complement indigenous microbes rather than those that prompt undesirable responses is imperative in order to ensure desire results at improving rhizosphere ability to supply nutrient and other growth substances to crops (Malusa *et al.,* 2010).

The study of genes coding for important enzymatic activities or key genes in the interaction process between the inoculants and native microbial population may contribute to gain such knowledge, which could unveil possible functions for the application of biofertilizers specifically designed for particular soil and/crops.

Plant-microbial interaction impacts various soil processes which changes soil quality with corresponding influence on crop production and safe environment. Soil fertility is directly linked to soil quality that promotes sustainable agricultural production. The effectiveness in soil organic composition of a particular soil is determined by the diversity of microbes in such soil. Apparently, soil biome significantly influences organic matter decomposition, nutrient flux and recycling in the rhizosphere. Plant-microbe interaction is an important factor that regulates soil organic matter composition which indicates the quality of soil.

Diversity of microorganisms in the soil is a function of primary carbon sources: root exudates, plant litters and soil organic matter at varying degree of accessibility and availability to soil microbes. Thus, plant-microbe interaction regulates the quality of soil in terms of its chemical and physical profile that determines its ultimate productivity (Dijkstra *et al.,* 2013). Exudation of soluble metabolites, nutrient mobilization and water uptake through plant-microbe interaction, rhizosphere-mediated soil organic matter decomposition, and the subsequent release of CO_2 through respiration known as rhizosphere processes are the key drivers of soil quality and fertility (Lugtenberg *et al.*, 2002).

Plants interact with soil to effect and enhance soil attributes, such as increased nutrient supply, carbon sequestration and water holding capacity. The nexus between plant-microbe interactions is unique in the sense that the diversity of rhizoplane determines the diversity of rhizosphere. This is because plant species differs in the type of primary metabolites released into the immediate soil environs which informs or dictates the microbes that thrive in such environment. This interaction between plant and soil, changes soil physical, biological and chemical properties in the root zones. The release of plant exudates and plant-fungal-bacterial interactions may result in major differences in local environments and microbial communities. These effects include changes in the species composition of microbial populations that enhance the availability of key plant nutrients. The more diverse the plant community above ground, the more diverse the community below ground. Some of these plant traits that dictate below-ground processes are rarely considered when soil quality within the confines of fertility management is been studied. Exploring the interrelationship among microbial niche and the edaphic variables could play significant role in ensuring soil health as well as animal and food safety (Figures 3 and 4).

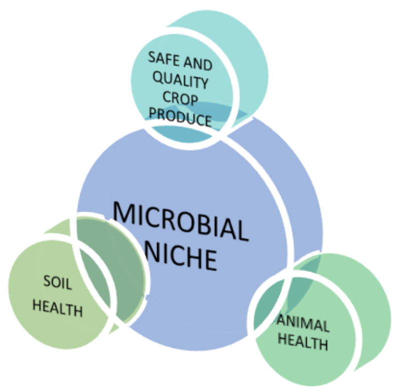

Figure 3: Interrelationship among microbial niche, macroorganisms and edaphic factors

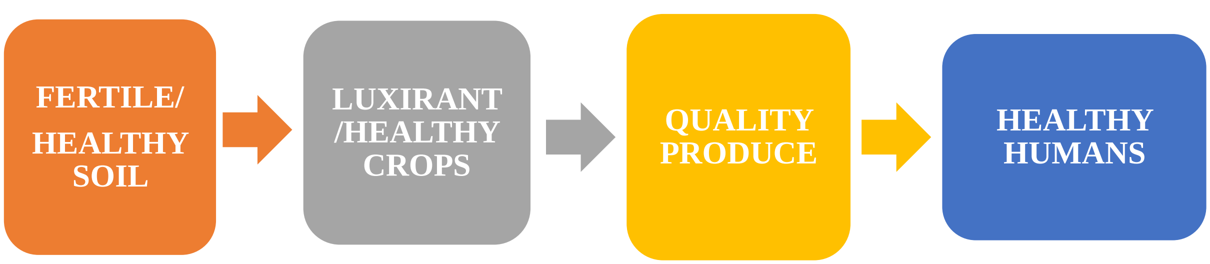

Figure 4: Relationship between healthy soil environment, crop growth and safe produce

Impact of biofertilizer utilization in crop production.

Impact of biofertilizer on crop yield and quality

Among all the microbes used as biofertilizer, *Bacillus* species a plant growth promoting bacteria (PGPB) has been widely used in agriculture to boost crop yield. The inoculants of these bacteria have a long term survival as spores when applied as soil amendment or as pre-sowing seed treatment (Adesemoye *et al.,* 2008b). *Bacillus* spp. is an efficient microorganism with tremendous metabolic dexterities in soil biome. The organism plays active role in recycling some essential mineral like sulphur, carbon and nitrogen which are transformed into useable form for crop use (Mandic-Mulec and Prosser, 2011). Much more, *Bacllus* spp. is tolerant of diverse biotic and abiotic stresses which made it suitable to adapt under diverse changing environment. Kumar *et al.* (2011) reported that *Bacillus* spp. often secrete peptide antibiotics, extracellular enzymes, multilayered cell walls and sometimes signal molecules to enforce tolerance in rice seedlings to survive adverse conditions. *Bacillus* PGPR were reported to ameliorate drought and salt in pea (Arshad *et al.*, 2008), Maize (Vardharajula *et al.*, 2011); (Egamberdieva and Adesemoye, 2016), pepper (Lim and Kim, 2013) and rice (Kumar *et al.,* 2011). Several strains of *Bacillus* species perform very unique roles in improving crop environment thereby promoting increase crop yield. For instance nutritional assimilation of plant total N, P, and K in *Zea mays* was greatly improved by *Bacillus megaterium and B. muciaraglaginous c*o-inoculated with AMF (Wu *et al.,* 2005). *Bacillus licheniformis* and *B. amyloliquefaciens* were reported to produce mixtures of organic acids such as isovaleric, lactic, isobutyric, and acetic acids which were responsible for solubilizing phosphate ions by decreasing the pH of maize rhizosphere (Rodríguez and Fraga, 1999).

Besides *Bacillus*, the use of rhizobacteria has been widely reported to promote crop growth and yield by improving nutrient uptake (Owen *et al.,* 2015). *Rhizobium* and *Bradyrhizobium* is known to fix atmospheric N_2 through symbiotic relationship with leguminous plants such as soybean, pea, peanut and alfalfa, which is converted into plant usable form: ammonia, as a nitrogen source (Murray, 2011). There are other free-living N_2 fixers such as *Azospirillum, Azoarcus, Azotobacter, Bacillus polymyxa, Burkholderia, Gluconoacetobacter or Herbaspirillum* and many others that have been reported to fix nitrogen into several crops like wheat (Boddey *et al.,* 1986), sorghum (Stein *et al.,* 1997), maize (de Salamone *et al.,* 1996), rice (Malik *et al.,* 1997) or sugarcane (Boddey *et al.,* 2001). Culture of these inoculants facilitate higher biomass accumulation, improves reproductive apparatus and increase grain yield which is attributable to better root development, which allows better rates of water and mineral uptake (Okon *et al.*, 1998).

Phosphorus reserve is abundant in the soil but they are largely available in non-soluble form for crop uptake and thus their deficiency in crop lead to stunted growth and yield loss. Bacteria such as *Azospirillum, Bacillus, Burkholderia, Erwinia, Pseudomonas, Rhizobium* or *Serratiaare* species have the capabilities to solubilize bound phosphate ions (Sudhakar *et al.,* 2000, Mehnaz and Lazarovits, 2006) through acidification (Richardson *et al.,* 2009), chelation or enzymatically (Hameeda *et al.,* 2008) to release plant absorbable phosphate ions. Furthermore, inoculation of PGPR can increase plant uptake of several other nutrients such as Ca, K, Fe, Cu, Mn and Zn.

Mechanisms of action of biofertilizers

Biofertilizers have been found to stimulate synthesis of certain bioactive compounds in crops such as indole-3-acetic acid (IAA), gibberellins (GAs), cytokinins and certain volatiles thereby altering root architecture and promoting plant development. This process known as phytostimulation enhances root cell division and proliferation and thus influencing greatly, uptake of both nutrients and water (Dobbelaere *et al.,* 1999, Khalid *et al.,* 2005b).

Plant growth promotion is achieved indirectly through bio-control activity against plant pathogens. Several mechanisms employed by microorganisms to control bacterial pathogens have been shown to be via antagonism. Members of the bacterial genera *Serratia, Stenotrophomonas*, *Bacillus*, *Pseudomonas*, and *Streptomyces* as well as the fungal genera *Ampelomyces, Coniothyrium*, and *Trichoderma* are known microorganisms with proven microbial influence on plant health (Ikotun, 2011).

Safety of biofertilized produce and human perception

Despite increasing knowledge about the benefits and value of biofertilizers in agro production, little is been published on safety or health implication of produce grown on biofertilized field. In order to guarantee increase in crop yield as well as quality produce; healthy and living soil must be ensured and be preserved from degradation and pollutants.

Application of microorganisms to soil as biofertilizers particularly bacteria and fungi promotes nutrients cycling and water uptake for autothrophs being the primary producers. The partitioned assimilates through photosynthetic process ultimately supplies food to the secondary trophic level in the food chain. There are no empirical facts or information on the adverse or harmful effects with respect to safety assurance and health implication of food grown using autochthonous soil microbes or culture of bioinoculants strains.

There are so many research efforts and reports on beneficial roles of biofertilizers in improving crop soil environment, crop growth, development and yield (Verma *et al.*, 2011, Amprayn *et al.*, 2012, Agamy *et al.,* 2013, Malusà *et al.*, 2016) with scanty information on the their virulence or becoming human pathogens. Adaptation of some of these microbes to plant environment and their persistence in plants could enhance their propensity to becoming harmful to humans and livestock via horizontal genes transfer from native microbial communities present in the arable ecosystem or applied biofertilizers. This should be more of concern as microbes such as those belonging to the family Enterobacteriaceae are adapted to arable habitats without losing their virulence to humans.

Equally, *Pseudomonas aeruginosa* has been shown to promote plant growth and used by many as biofertilizers. However, they are found to be infectious to plants and humans. Perhaps some microorganisms used as biofertilizers to improve crop performance could strongly contribute to human health (O'Callaghan, 2016). For instance, certain *P. aeruginosa* strains used as crop growth promoters have been reported to be infectious to mouse (Rahme *et al.,* 2000). It was indicated that microbiomes of different environments are not isolated but show interplay. Hence, a non-infectious plant growth promoting microorganism could become pathogenic when transferred to human gut through consumption. This could results in heavy outbreaks of infectious diseases by transferring possible pathogens (Van Overbeek *et al.,* 2014)

Persistence and traceability of virulent microbes in biostimulants and biocontrol agents.

Utilization of biostimulants and biocontrol agents become more interesting because of their alleged high degradability when applied. Although, persistence of bio-agent used in agriculture varies depending on the stains involved but most of these products become highly non-traceable after a few weeks of application. Biocontrol agent like *Trichoderma harzianum* as well as *Bacillus amyloliquefaciens* FZB42, a plant growth and health promoting microbes become nondetectable shortly after application (Kröber *et al.*, 2014). Other biostimulant strains persist for a longer time in the rhizosphere though become less infectious and reduced in abundance than when applied. Prominent among these strains are bacteria belonging to nitrogen fixing groups like *Rhizobium phaseoli* and *Bradyrhizobium japonicum* (Narożna *et al.,* 2015).

It is commonly considered that plant growth promoting microorganisms only effect beneficial changes in plants and therefore, are not harmful to humans or livestock. This may not be absolutely true as there are evidences from several experiments that demonstrated the possibility of development of phytotonosis suggesting a human pathogen transmitted through infected crop product (Van Overbeek *et al.,* 2014). This is important as infections of plants by pathogens may seriously impact on plant health, human and livestock health in many ways. Generally, some phytopathogens may be infectious when in contact with human or livestock cells as this normally does not occur. There is scanty empirical evidence on the pathogenic role of plant microbes in humans (Colson *et al.,* 2010). However, a study reported that *Pepper mild mottle virus* may react with the immune system of humans and induce a clinical symptom (Balique *et al.,* 2015).

Some plant growth-promoting bacteria (PGPB) perhaps cause a potential threat to human, animal or plant health. No doubt, some soil inhabiting microbes beneficial to plants are human pathogens hence there is thin line between families of plant beneficial microbes and human/livestock pathogenic microbes. Many soil microbes have been reported to effect beneficial effects in plants while a host of others belonging to the same family are pathogenic. Some fungal species that secretes mycotoxins such as *Aspergillus flavus*, *Fusarium* spp. and *Penicillium* spp. have been isolated and identified to cause disease in plant, animal and humans. Aflatoxins are one of the most common and serious groups (B1, B2, G1 and G2), of mycotoxins which are produced by some *Aspergillus* species. Aflatoxin B1 is one of the most serious mycotoxins, because it is lethal at high doses and carcinogenic to humans at low doses. Mycotoxin produced by this stain of *Aspergillus* can cause hepatic malfunction, nauseating, inflammatory infections and so forth. Several secondary metabolites produced by some fungi produce toxins which have been traced to some leguminous and cereal crops produces such as peanuts, pistachios, cocoa, and maize. In addition, mycotoxins can be consumed indirectly by humans through the consumption of meat from animals fed on food contaminated with mycotoxins. It thus implies that there are some plants and animal pathogenic microbes that are closely related to beneficial microbes. Humans are considerably exposed to pathogenic microbes which when entry is gained into the mammalian guts and cells via consumption may induce some negative changes in mammalian cells resulting in immune responses in humans leading clinical symptoms (Al-Sadi, 2017).

According to Al-Sadi (2017) plant diseases affect humans either directly or indirectly through consumption of plant products contaminated by toxic metabolites secreted by pathogenic fungi. Although the fungi producing these mycotoxins infect plants and not humans, however these mycotoxins directly affect animals that consume such contaminated produce, resulting in diseases and death. Studies have shown that gut microbiota are involved in human diseases, and that microbes can biosynthesize phytohormones with implication on humans and animal health. Therefore, investigating animal-microbe interactions using plant is very germane (Chanclud and Lacombe, 2017). Ingestion of plant product with trace of PGPB that induces absicic acid (ABA) synthesis have been reported to cause inflammatory bowel disease. Although, the mechanism is still not clear as there are still many grey areas that need to be understood.

Conclusion and recommendations.

It is important to take cognizance of humans, animals and the environment safety while applying biofertilizer in form of soil amendment or pre-sowing seed treatments. Efforts should be directed at promoting harmonized, reliable principles for evaluating safety of these strains of bacteria and fungi used as biostimulant or biocontrol in agro-production. This must follow widely applicable methods and internationally acceptable benchmarks.

There is an urgent need to evolve an efficient procedure with high precision for predicting safety of PGPB used in agricultural system. The evolvement of harmonized methods for ascertaining bio-safety of crop bio-stimulants and bio-control in order to forestall health hazard to humans, animal and the environment is very expedient. To this end, development of unified protocols with high efficiency and reliability would suffice for this purpose. More researches are required on the direct impact of biofertilizers, biocontrol agents on humans and livestock. Special attention should be focused on biofertilizers derived from families of microbes that have been implicated in causing plant diseases, secretion of poisonous secondary metabolites such as mycotoxin and their presence in human food.

Public awareness on relationship between plant diseases, crop safety and human health is also important. In order to ensure safer environment and safety of humans and animals, adequate regulatory procedures to prevent cross infection from plant pathogens to humans and animals must be put in place. Ensuring quality control and quality assurance of biofertilizer is critical to its widespread adoption in agricultural system. Enforcement of standardization and quality control measures for production of quality biostimulants and biopesticides products is expedient. Biofertilizers are living organisms with mutation inclination therefore, the need for quality examination and certification prior utilization in sustainable agroecosystems is highly essential.

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