Chapter 9
Microbial Inoculants for Soil Quality and Plant Health

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Abstract Agriculture is the major economic activity of most developing countries engaging more than 50 % of the population. Low world crop productivity due to low soil moisture, low nutrient capital, erosion risk, low pH, high phosphorus fixation, low levels of soil organic matter, aluminum toxicity pest and diseases, weeds and loss of soil biodiversity has induced the green revolution agriculture which involves high yielding varieties and agrochemicals. The continuous use of fertilizers, pesticides and herbicides has led to low agricultural productivity, low soil fertility, unfavourable economic returns, food poisoning, soil damage loss of biodiversity and serious environmental hazards. Microbial inoculants possess the capacity to enhance nutrient availability, uptake, and support the health of soil and plants to promote sustainable yield and has therefore gained attention of many agriculturist and researchers.

We review the ability of soil through the use of microbial inoculants to supply nitrogen, phosphorus and potassium to crop plants and enhance structural stability. Microbial inoculants such as rhizobium, plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi can be used as biofertilzer to improve soil nitrogen, phosphorus and potassium availability and uptake. Both bacteria and fungi inoculants show potential for use in soil aggregate formation and stabilization and hence,

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E. Lichtfouse (ed.), *Sustainable Agriculture Reviews*, Sustainable Agriculture
Reviews 22, DOI 10.1007/978-3-319-48006-0_9

soil structure enhancement. The ability of microbial inoculants to ameliorate plant stress as a result of drought, soil contamination and salinity are also highlighted. The most commonly used microorganisms as biofertilizers, biocontrol and bioremediators include *Bacillus* spp, *Pseudomonas* spp, *Streptomyces* spp *Trichoderma* spp and *Mycorrhizas*. Microbial inoculants function through various mechanisms such as production of plant hormones, expansion and elongation of the root system, eliciting induced systemic resistance or systemic acquired resistance, production of lytic enzyme and antibiotic 4-hydroxyphenylactic acid, and production of 1-amino cyclopropane-1-carboxylate-deaminase (ACC-deaminase) in plants rhizosphere. These strategies are safe and sustainable in the long run. The use of appropriate carrier material determines the success of microbial inoculation techniques. Microbial inoculants could either be applied directly to the soil or as seed dressing. The fate of microbial inoculants under field application depends largely on both biotic and abiotic factors. The application of some microbial inoculants could cause a change (which could be a decrease or an increase) in the equilibrium of soil microbial communities while some produce no effect at all.

Keywords Agricultural sustainability • Biocontrol • Biofertilizer • Bioremediation • Biotechnology • Food security • Microbial inoculants • Plant growth • Plant growth promoting microorganisms (PGPM) • Soil fertility and health

9.1 Introduction

The increasing demand for food production with shrinking land resources is a major challenge to agricultural sustainability. Sustainable food production requires efficient use of determinate resources (Owen et al. 2015). Attempt to mitigate the problem include the use of high yielding varieties, chemical fertilizers and pesticides to supplement plant nutrition and control plant pathogens for increased agricultural productivity. However the increasing impacts of these agricultural practices on the environment have gradually affected the quality of soil hence, there is a need to optimize soil productivity in such a way that soil capacity to function as a healthy medium is preserved (Trivedi et al. 2012). The use of eco-friendly resources or input has been a major focus of attention in the past three decades. Although reports on the benefits of using microbial inoculants for plant growth promotion and health in agricultural soil have been inconsistent, there is a promising trend for microbial inoculants to meet the sustainable agricultural production needs. Suggestions to replace or supplement the heavy application of chemical fertilizers with inoculants have been reported (Carvajal-Muñoz and Carmona-Garcia 2012). Microbial inoculants application has been in existence for more than 100 years but gained a lot of prominence in the last three decades with several commercial inoculants products in the market (Babalola and Glick 2012).

Fig. 9.1 Maize plant (**a**). Showing Fusarium graminearum infection (**b**). Inoculated with Pseudomonas sp for biocontrol against Fusarium graminearum

Microbial inoculants participate in many ecosystem biological and chemical processes such as biological control of pathogens (Fig. 9.1) and nutrient cycling, thereby improving nutrient availability. Microbial inoculants application increase biodiversity, creating suitable condition for development of beneficial microorganism. They also improve physical properties of soil such as; improve structure and aggregation of soil particles; reduce soil compaction, increase spore spaces and water infiltration. The antioxidant properties of microbial inoculants promote decomposition of organic matter and increase humus content in soil matrix, and are therefore being considered as an alternative way of reducing the use of chemicals in agriculture (Carvajal-Muñoz and Carmona-Garcia 2012). Microbial inoculants techniques ensure biodegradation of complex substances and develop bioremediation processes in soil contaminated with toxics, xenobiotic and recalcitrant substances.

The strategies involved in plant growth promotion by microbial inoculants could be a direct or indirect mechanism. Directly, inoculation of crop plant with microbial inoculants could result in the expansion and elongation of the root system, leading to improved uptake of water and nutrients (Halpern et al. 2015). Production of growth hormones by microbial inoculants impact root morphogenesis such that plant root hairs and lateral roots are over produced resulting in greater uptake of plant nutrients and hence improvement of plant growth (Kumar et al. 2007). Fixations of atmospheric nitrogen, solubilization of minerals such as phosphorus (P) (Babalola 2010), are also some of the direct mechanisms of influence of microbial inoculants. In indirect growth promotion, Microbial inoculants affect the status of plants by eliciting induced systemic resistance (ISR) or systemic acquired resistance (SAR), by improving disease resistance. These acts prevent soil-borne pathogens from inhibiting plant growth (Yang et al. 2009). Ability to trigger a salicylic acid (SA) -independent pathway controlling systemic resistance is a common trait of ISR-inducing biocontrol bacteria. Structural deformities in pathogenic fungi under in vitro culture conditions by the production of diffusible and volatile antifungal compounds have been reported. The bacterial strain successfully restricted the growth of all the test fungi in dual cultures and induced morphological abnormalities such as mycelial and conidial deviations. Also of note is the production of siderophores that solubilize and sequester iron (Hmaeid et al. 2014).

With the increasing use of microbial inoculants for plant growth promotion, this review discusses some of the beneficiary roles of microbial inoculants in plant and soil. It describes changes in soil structure, nutrient solubility as a result of the application of microbial inoculants. We provide an overview of microbial inoculants use for agricultural sustainability, the significance of their application on soil nutrient improvement and soil structure enhancement. Their roles in amelioration of plants stress as a result of drought, soil contaminants, salinity and as biocontrol agents are well explained.

9.2 Microbial Inoculants

Microbial inoculation is one of the major agricultural practices that have been used to acquire desirable characteristics in the soil. Microbial inoculants are the formulations of beneficial living microorganisms that when added to soil, improve availability of nutrient to host plant directly or indirectly, thereby promoting plant growth (Gaind 2011). Most of the microorganisms that are used in the production of microbial inoculants inhabit or are capable of inhabiting the soil and perform various roles and functions in the soil. Microbial inoculants in are applied, singly or in combinations, to seeds, plants and soil to enhance their productivity. Different terminologies such as biostimulant (Halpern et al. 2015), bio-inoculants (Singh et al. 2013), and bio-fertilizers (Ansari et al. 2014) have been used to represent these groups of microorganisms. Microbial inoculants include three major groups: (1) plant growth promoting rhizobacteria (PGPR), (2) arbuscular mycorrhiza fungi and (3) the nitrogen-fixing *rhizobia*, which are usually not considered as PGPR (Yadav and Verma 2014). These groups are known to possess the capacity to enhance nutrient availability, uptake, and support the health of plants to promote plant growth. Microbial inoculants are not nutrients but microorganisms that are able to increase the availability of these nutrients through their biophysical and biochemical activities in soil. Business Communication Co. research report (2011) estimated a compound annual growth rate of about 6.9 % for global microbial inoculants with a market value of $4.5 billion in 2010, $4.9 billion in 2011and projected to reach $6.8 billion by 2016 (Chatzipavlidis et al. 2013).

Microbial inoculation provides an innovative and cost-effective alternative to overcome salinity stress in soils (Tank and Saraf 2010). The system is environmentally friendly, and poses no health risk to either plant, human or animal. Enhance soil nutrient availability to the plants hence their use as biofertilizers (Ahemad and Kibret 2014). Microbial inoculants provide resistance against pathogens. They can

Table 9.1 Examples of some microbial inoculants, the test crops and their beneficial properties

Microbial inoculants	Test Crop	Beneficial properties	References
Chryseobacterium indologenes, Pseudomonas cepacia, P. fluorescens	Wide barley	Salt stress	Hmaeid et al. (2014)
Bacillus subtilis, P. corrugate	Maize	Cool regions (22 °C)	Trivedi et al. (2012)
Paenibacillus yonginensis DCY84	*Arabidopsis thaliana*	Drought, salt stress	Sukweenadhia et al. (2015)
Enterobacter sakazakii	Cowpea	Parasitic weed	Babalola et al. (2007)
Bacillus subtilis	Cotton	Phytopathogen	Pereg and McMillan (2015)
Scutellospora reticulate, Glomus pansihalos (Mycorrhizal fungi)	Cowpea	Soil polluted with Al and Mn	Alori and Fawole (2012)
Pseudomonas putida	Wheat	Cool region	Trivedi and Pandey (2007)
Trichoderma sp., *Gliocladium* sp.	Flowers, ornamentals	Plant pathogens	Julia et al. (2013)
Azotobacter sp + *Pseudomonas* sp	Mustard	Cadmium	Panwar et al. (2011)
Arbuscular mycorrhizal fungi	Citrus	Drought stress	Wu et al. (2013)
Burkholderia cepacia	Yellow lupine	Toluene	Barac et al. (2009)
P. fluorescens strains, CHA0 and Pf1	Banana	Drought stress	Kavino et al. (2010)
Gordonia sp. S2Rp-17	Corn	Diesel (Soil contaminant)	Hong et al. (2011)
Sinorhizobium meliloti	Common reed	Phenanthrene	Golubev et al. (2009)
Bacillus thuringiensis, Rhizophagus intraradices	Trifolium repens	Drought stress	Ortiz et al. (2015)
Burkholderia cepacia	Popular	Toluene	Taghavi et al. (2005)
Arbuscular mycorrhizal	Olive	Salinity stress	Porras-Soriano et al. (2009)
Azospirillum lipoferum	Wheat	Crude oil	Muratova et al. (2005)

therefore be used in biological control against plant pathogens (Sukweenadhia et al. 2015) against weed pest (Biological herbicides) (Babalola et al. 2007) and insect pest (Saharan and Nehra 2011). Microbial inoculants can also be used in phytoremediation of polluted soils (Alori and Fawole 2012; Alori 2015). Waterlogged, compacted, desiccated wind and rain eroded soil are remediated through microbial inoculation. Fungal inoculants protect plants against transplant shock, promote environmental resistance to heat and drought (Sukweenadhia et al. 2015) and vastly improve the quality of the soil (Table 9.1).

9.2.1 Microbial Inoculants and Soil Fertility Improvement

9.2.1.1 Nitrogen (N)

N remains the most limiting element for plant growth. The major sources of N for agricultural soil are mineral fertilizers and biological N fixation carried out by microorganisms. Nitrogen-fixation is the first step for cycling N to the biosphere from the atmosphere, a key input of N for plant productivity (Bernhard 2010). Microbes especially bacteria are important in N cycling. Bacteria are known to exclusively fix atmospheric N either symbiotically or asymbiotically due to their possession of the key enzyme nitrogenase which specifically reduces atmospheric N to ammonia (Wagner 2011).

Symbiotic N fixation in soil is a process occurring in legume and non-legume plants. The bacteria *Rhizobium*, *Sinorhizobium*, *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Azorhizobium*, collectively referred to as *rhizobia* are responsible for the legume N fixation while *Frankia* and *Actinobacteria* are responsible for non-legume N fixation in soil (Wagner 2011). It is evident that inoculation of legumes with rhizobia has the ability to increase the soil N status. N fixed annually by legume-rhizobium association was reported to be about 40–48 million tonnes compared to 98 million t year^{-1} of N fertilizer (Jenkinson 2001). This ability to fix high amounts of N into the soil is a great potential of rhizobial inoculant to reduce the cost of industrial N fertilizers, thereby reducing the cost of inputs for farmers. Nitrogen fixation of an effectively nodulated legume is a vital and indispensable aspect of sustainable agriculture. The use of rhizobium inoculant to achieve efficient N fixation in soil requires the compatibility of the legume and rhizobium inoculant and their adaptability to the environment (Wagner 2011). The few inconsistencies about the ability of the rhizobium inoculant to increase the soil N are probably due to compatibility and adaptability to the environment issue. Rhizobium must be able to establish, compete and persist with other microflora to form effective nodules in the introduced environment (Gaind 2011). *Rhizobial* inoculation has played a vital role in legume production in the US and Australia. The legume-cereal cropping system popularized by the International Institute of Tropical Agriculture in sub-Saharan African is also an indication of the growing popularity of the use of *rhizobial* inoculant. Most crop rotation systems use legume in crop sequence because of the understanding that legumes are able to fix N and increase the N status of the soil for the next crop. This has been utilized significantly in soil fertility management for crops to reduce the application of chemical fertilizers. Therefore the proper and efficient use of *rhizobial* inoculants will ultimately benefit sustainable agricultural production.

A group of bacteria that are free living in the soil commonly referred to as plant growth promoting rhizobacteria/plant growth promoting bacteria/plant growth promoting microorganism have the ability to fix N into soil (Calvo et al. 2014), when occupying the rhizosphere of crops both legumes and non-legume. These groups of bacteria include the genera of *Pseudomonads, Azoarcus, Beijerinckia, Cyanobacteria*

(Nostoc and *Anabaena), Klebsiella, Pantoea, Azotobacter, Azospirillum, Bacillus, Burkholderia, Herbaspirillum, and Gluconacebacter diazotrophicus* (Egamberdiyeva 2007). Many of these organisms are used as microbial inoculants for crop growth improvement, singly or in combination with other organisms. Unlike rhizobial inoculants, the use of many of these bacteria as a single inoculant to increase soil N fertility for crop use has not been very effective. Although they may not be as effective as *rhizobium*, there is potential for improvement in their ability to help in sustainable agricultural production. Although the ability of many plant growth promoting rhizobacterial to increase N content of the soil are very inconsistent, some cases of appreciable soil N increase have been observed especially when the inoculants contain more than one of the organisms. The use of *Azospirillum* as helper bacteria used in combination with *rhizobium* increased the effect of *rhizobium* in soil fixation. *Azotobacter* and *rhizobium* contributed 78.8 kg N ha^{-1} $year^{-1}$ total N to soil in soybean-wheat rotation (Rawat et al. 2013).

Plant nutrition for N has also been improved by the application of some fungal inoculants. Arbuscular mycorrhizal fungi that form associations with more than 80 % of plants including most crops have also been identified as a probable N mobilizer for plants (Hodge and Storer 2015; Veresoglou et al. 2012). However, the contribution of arbuscular mycorrhizal fungi to plant N uptake varies widely and the reasons for the variability are still unclear and may likely be resolved by the application of genomics and metabolomics technology (Hodge and Storer 2015). The role of arbuscular mycorrhizal fungi in plant P nutrition and soil structure improvement is well established, and the prospect of involving it in the N nutrition of crops will be a giant stride in tackling soil degradation problems through the use of microbial inoculants. Microbial inoculants such as rhizobium, plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi have the potential to be used as biofertilizer to improve soil N availability and supply for sustainable agriculture

9.2.1.2 Phosphorus

Phosphorus is an essential macronutrient required by plants for their growth and development. It makes up about 0.1 % of the earths' crust (Sanderson 2014). However, most of the P in the earth's crust is in insoluble form and not readily available to plants. Low soil available P limits about 40 % of crop production in arable land worldwide (Bargaz et al. 2012). To compound the problem of P availability, added P fertilizers undergo fixation due to the complex exchanges within the soil limiting the availability of P to plants (Zhu et al. 2011).

The role of microbial inoculants in increasing the availability of soil P for plant growth can be viewed from two perspectives: firstly the solubilization of P from the mineral rock thereby increasing the available P in soil solution and secondly the mobilization of the available P to the plant roots for uptake. Phosphate solubilizing microorganisms and phosphate mobilizing microorganisms (Owen et al. 2015) include the genera of some bacteria and fungi that have been identified to solubilize and render insoluble soil P available to plants with their production of organic acids and enzyme phosphatases and phytase (Calvo et al. 2014). These organisms include *Pseudomonas, Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Rhizobium, Erwinia, Streptomyces, Achromobacter Flavobacterium* and mycorrhiza (Ma et al. 2009). These microorganisms produce organic acids that chelate the cations bound to phosphate and convert them to soluble form (Calvo et al. 2014). They also produce the enzymes phytases and phosphatases that dephosphorylate phytates, the predominant organic P in the soil (60 % of organic P) to release P in a form available to plants (Singh and Satyanarayana 2011).The application of these organisms, either as bacterial or fungal inoculants, has advantage over the P fertilizers that readily form complexes in the soil when applied because the microbes can continuously supply available P to plants over a long range of time. Soil management practices that incorporate microbial inoculant application can really benefit from the sustained P supply to crops.

Fungal inoculants that have P solubilizing/mobilizing potential are well known. The most studied among the P mobilizing fungi is the arbuscular mycorrhizal fungi. Arbuscular mycorrhizal fungi are widespread in the plant kingdom and contribute significantly to plant P nutrition and growth in natural ecosystems (Smith et al. 2011). The mechanism of increased P uptake by Arbuscular mycorrhizal fungi has been attributed to the fungal extra radical hyphae growing beyond the phosphate depletion zone that develops around the root (Smith and Read 2008). Positive effects of arbuscular mycorrhizal fungi inoculation on the growth and P nutrition of crops have been reported (Cozzolino et al. 2013; Dare et al. 2010). Many mycorrhizal inoculants have been produced on a commercial scale, mostly in the US and Europe. *Rhizophagus* (formerly *Glomus*) *intraradices* and *Funneliformis* (formerly *Glomus*) *mosseae* (Kruger et al. 2012) are some of the common mycorrhizal inoculants which have been shown to increase P uptake in diverse crop plants (Dare et al. 2010; Cozzolino et al. 2013). Some other fungi such as *Aspergillus* and *Penicillium* species are able to solubilise inorganic phosphate and mineralise organic phosphate by secreting organic acids and producing phosphatase enzymes (Wang et al. 2015). The significant role of microbial inoculants in increasing the sustainable availability of P to plant is that of P solubilization and P mobilization.

9.2.1.3 Potassium (K)

Potassium is one of the most important macronutrient for plant growth and the third in fertilizer formulation after N and P. Potassium is one of the seven most common elements in the earth's crust and makes up 2.6 % of the earth's surface layer (Meena et al. 2014). Inadequate supplies of K to plants can lead to poor root growth, slow growth and lower yields in crops (White and Karley 2010).

K-solubilizing microorganisms present in soil and plant rhizosphere are evidently involved in the K cycles (Liu et al. 2012). Potassium-solubilizing microorganisms improve soil nutrients and structure and plant growth by releasing K from insoluble minerals into the soil (Meena et al. 2014). The microorganisms in the soil or rhizosphere solubilize mineral K by synthesizing organic acids (Parmar and Sindhu 2013). A wide range of rhizospheric microorganisms that have been used as inoculants for increasing the K soil content or K plant nutrition include *Acidithiobacillus ferrooxidans, Arthrobacter sp.Bacillus edaphicus, Bacillus circulans, Bacillus mucilaginosus, Burkholderia sp.*, and *Paenibacillus* sp.(Zarjani et al. 2013; Sangeeth et al. 2012) These organisms convert insoluble or mineral structural K compounds into soluble form and make them available for plants.

AM fungal inoculants have also been reported to increase K uptake. Arbuscular mycorrhizal releases proton H^+ or CO_2 and organic anions such as citrate malate and oxalate which increase the solubility of mineral K (Meena et al. 2014). However, the increased K by mycorrhizal has often been linked to increased P availability (Cardoso and Kuyper 2006). Plant growth promoting rhizobacteria and Arbuscular mycorrhizal fungi are responsible for K solubilization and mobilization for sustainable improvement of K availability to plants.

9.2.2 Potentials of Microbial Inoculants in Soil Structure Enhancement

Soil structure is a crucial aspect of sustainability in agriculture and ecosystem functioning because of its influence on the biological, physical and chemical properties of the soil. It refers to the three dimensional arrangement of organic or mineral complexes (aggregates) and pore spaces, which is usually quantified by size distribution of aggregate or the stability of aggregates. Aggregate formation and stabilization are mediated by several factors which include soil microorganisms (Lucas et al. 2013).

The role of microorganisms in the aggregate formation and stabilization of soil is well documented (Lucas et al. 2013; Helliwell et al. 2014). Activities of bacteria and fungi applied as inoculants in the enhancement of soil structure are affected by the aggregate scale (micro- or macroaggregate), soil types and soil mineralogy (Six et al. 2004). Aggregates are divided into microaggregate (<250 μm) and macroaggregate (>250 μm) and this division influences bacteria and fungi differently. While fungi stabilize macroaggregates, bacteria are more involved in the enhancement of microaggregates (Bossuyt et al. 2001). Bacteria play less role in coarse textured sandy soil where only the hyphal network is able to cross-link the abundant sand particles to form stable aggregates, whereas in clayey soil, both bacteria and fungi and their product play the role in aggregation (Six et al. 2004). Fungi are unique in influencing soil aggregate formation and stabilization because of the hyphae development and production of extra cellular polysaccharides. Hyphal networks enmesh and entangle macroaggregates while extracellular polysaccharides help to bind the micro-aggregates into stable macroaggregates (Bossuyt et al. 2001). Bacterial inoculants could play a key role in the soil structural stabilization through their secretions and exudates for microaggregate formation and stabilization.

The most studied fungi in soil structure stabilization are mycorrhizal fungi. Mycorrhizas are well recognized for their role in the improvement of soil structure (Leifheit et al. 2014). According to Rillig and Mummey (2006), mycorrhiza can influence soil aggregation at three main different scales; plant community, individual host plant root and fungal mycelium. Mycorrhizal ability to affect the plant community composition and cause root morphological changes to individual plants is well established (Oláh et al. 2005). However, our focus in this review is on the fungal mycelium which develops with the application of the fungal inoculant. Arbuscular mycorrhiza contribute to soil structure by 1) developing extraradical hyphae into the soil that align soil particles, providing the skeletal structure that enmeshes microaggregates to form macroaggregates; 2) secreting product like glomalin and glomalin related protein, mucilage, polysaccharides, hydrophobins and other extracellular compounds that cement aggregates and 3) delivering plant-derived carbon to aggregate surfaces (Rillig and Mummey 2006; Cardoso and Kuyper 2006). These processes are important for soil aggregation because of the space occupied by arbuscular mycorrhizal fungi in the soil system. Arbuscular mycorrhizal fungi produce significant biomass and represent dominant fungal biomass in agricultural soil (Rillig and Mummey 2006) and this is probably the reason for the positive effect of arbuscular mycorrhizal fungi inoculation on soil aggregation as reported by Leifheit et al. (2014). Considering the agricultural practices that are damaging to the soil structure, the use of mycorrhizal inoculants will not only help in the nutrition of crops, but also enhance the structural stability of agricultural soil. Both bacteria and fungi inoculants show potential for use in sustainable soil aggregate formation and stabilization and hence, soil structure enhancement.

9.2.3 Role of Microbial Inoculants in Crop Tolerance to Drought Stress

By reason of global climate change, drought is becoming more frequent and extreme in most part of the world. In most ecosystems, both fungi and bacteria are capable of resisting drought condition. However fungi show greater resistance than bacteria. Yuste et al. (2011), reported that fungi persisted longer in forest and desert soils during drought than bacteria. Arbuscular mycorrhizal fungi and saprophytic fungi have also exhibited better resistance to a wider range of heat and drought conditions compared to bacteria, However actinomycetes was an exception (Bell et al. 2009). This is not far from the fact fungi have extensive hyphal networks that enable them to access a larger volume of soil. These help fungi to regulate osmotic stress more effectively than bacteria (Leifheit et al. 2014). In the same vein Arbuscular mycorrhizal fungi show greater tolerance to drought than the saprophytic group (Davinic et al. 2013). This is associated with its ability to enhance greater plant nutrient and water uptake, greater carbon assimilation efficiencies. Fungi are also able to breakdown more complex organic structures such as cellulose and lignin (Schwarze et al. 2004).

The discovery of some soil microorganism associated with natural drought condition in different ecological conditions, has necessitate their use as inoculants in drought season. The mechanisms by wish these inoculants enhance plant drought tolerance include: increased hydric content, decreased antioxidant enzymatic activities, increased nutrient uptake, and decreased stomata conductance. They are also able to maintain indole acetic acid and increase proline production. Arbuscular mycorrhizal inoculants can improve crop drought tolerance in crop via glomalin induced changes in soil structure. Microbial inoculation during drought increased; plant growth, physiological and biochemical plant values that aid adaptive plant response, root growths, water content and plant C, K, Ca and Mg content (Armada et al. 2014). Some microorganisms that have been used to improve crop tolerance to drought include: Arbuscular mycorrhizal fungi such as *Glomus intraradices, Glomus mosseae, Aspergillus niger, Phanerochaete chrysosporium* (Medina et al. 2010; Wu et al. 2013), *Bacillus megaterium* (Armada et al. 2014). *Burkholderia phytofirmans* PsJN*, Enterobacter sp.* FD17 (Naveed et al. 2014), *Pseudomonas putida* (Armada et al. 2014), *Azospirillum* sp (Moutia et al. 2010), *Bacillus thuringiens*, *Rhizophagus intraradices* (Ortiz et al. 2015).

9.2.4 Microbial Inoculants in the Remediation of Contaminated Soil

Bioaugmentation of tolerant crops with microbial inoculants can enhance plant establishment and growth under stress conditions including in the presence of soil contaminants. Phytoremediation of contaminated soil assisted by microbial inoculants enhance plant growth through: Production of plant growth hormones such as indole acetic acid and cytokinins, essential nutrients released by nitrogen fixers' siderophore producers and phosphorus solubilizer and suppression of the production of stress producing ethylene and hence have the potential to aid phytoremediation. Microbial inoculants are capable of remediating both organic and inorganic soil contaminants (Alori 2015). Some plants and associated microbial inoculants in phytoremediation of some soil inoculants are shown in Table 9.2.

Plants, in association with microbial inoculant, can remove or transform contaminants into harmless substance. Microbial populations through the release of chelating gents, acidification, phosphate solubilization and redox changes, affect heavy metal mobility and availability to the plant. The use of microbial inoculants in phytoremediation of polluted soil is cost efficient than alternative engineering-based solutions such as incineration, soil excavation, or land filling of the contaminated materials. Site use and remediation can occur simultaneously. It is an in situ approach, It treats the contamination in place so that large quantities of soil, sediment or water do not have to be pumped out or dug up of the ground for treatment. It is environmentally friendly, i.e, poses no health risk to neither plant, human nor animal. It enhances soil nutrient availability to the plants. Require less equipment

Table 9.2 Some microbial inoculants in phytoremediation of contaminated soil

Remediator (plant)	Microbial inoculants	Contaminant	References
Withania somnifera	*Staphylococcus cohnni subsp urealyticus*	Lindane	Abhilash et al. (2011)
Lolium sp and *Medicago sativa*	*Enterobacter ludwigii*	Hydrocarbon	Yousaf et al. (2011)
Cytisus striatus	*Rhodococcus erythropoli*	Hexachlorocyclohexane	Becerra-Castro et al. (2013)
Brassica juncea	*Azotobacter* sp + *Pseudomonas* sp	Cadmium	Panwar et al. (2011)
Arabidopsis thaliana	*Achromobacter xylosoxidans* F3B	Aromatic compounds	Ho et al. (2012)
Lolium multiflorum	*Pseudomonas* sp. ITRH76, *Rhodococcus* sp. ITRH43	Diesel	Afzal et al. (2011, 2012)
Phragmites australis	*Pseudomonas asplenii* AC	Copper, and creosote	Reed et al. (2005)
Brassica juncea	*Bacillus argabhattai* and *Bacillus megaterium*	Cadmium	Jeong et al. (2013)
Lolium multiflorum	*Pseudomonas putida* PCL1444	Naphthalene	Kuiper et al. (2004)
Arabidopsis thaliana	*Paenibacillus yonginensis*	Aluminium	Sukweenadhi et al. (2015)
Lolium multiflorum	*Pseudomonas nitroreducens* PS-2	Chlorpyrifos	Korade and Fulekar (2009)
Phragmites australis	*Sinorhizobium meliloti* P221	Phenanthrene	Golubev et al. (2009)
Triticum aestivum	*Azospirillum lipoferum*	Crude oil	Muratova et al. (2005)
Vigna mungo	*Pseudomonas aeruginosa* MKRh3	Cadmium	Ganesan (2008)
Pisum sativum	*Pseudomonas putida* VM1441 (pNAH7)	Naphthalene	Germaine et al. (2009)
Phragmites australis	Autochthonous microorganism consortium	Copper	Oliveira et al. (2014)
Juncus maritimus and *Phragmites australis*	Autochthonous microorganism consortium	Cadmium	Teixeira et al. (2014)

and labour than other methods, Phytoremediation using microbial inoculants does not degrade the physical or chemical health of the soil, unlike the soil excavation method that removes the topsoil that is rich in organic-matter- and the heavy machinery used compact the soil that is left behind. Microbial assisted phytoremediation do not require digging up or hauling of soil, hence it saves energy (Alori 2015). The strategies of microbial inoculants in remediation of polluted soil are safe and the effects are sustainable.

9.2.5 Benefits of Microbial Inoculation in Saline Soil

Salination of agricultural soil has become a serious threat to food production and security. According to (Shirmadi et al. 2010) about 5 % of the world soil is currently affected salinity. Vinocur and Altman (2005), predicted that by the year 2050, about 50 % of agricultural soils will be affected by salinity increase. Salinity has a direct effect on both the physical-chemical and biological properties of the soil, rendering such soils unsuitable for crop growth and biological processes. High soil salinity results in disruption in the uptake and transformation of nutrient elements such as Mg^{2+} and Ca^{2+} by plant. More also, it reduces ion activity in soil solution thereby, leading to nutrient deficiency and reduction of overall growth and yield quality of plant.Paul and Nair (2008), stress that plants become vulnerable to soil borne diseases under saline stress In the past, some of the strategies employed to alleviate salt stress include the following: leaching of excess soluble salts from upper to lower soil depth, developing salt resistant cultivars, harvesting salt accumulating aerial plant parts in areas with negligible irrigation (Karthikeyan et al. 2012). These strategies are labour intensive and highly scientific. As a result, cost of cultivation may become increased and sometimes impossible. This has necessitated the need to discover agronomic system that can support plant growth under salinity stress that will not be accompanied by any environmental or health hazard.

Some soil microorganisms have been identified to be capable of alleviating salinity stress in plants and thus improving plants growth and yield. These soil microorganisms include the following genera: *Agrobacteria*, *Azospirillum*, *Bacillus*, *Glomus Gordonia* and *Pseudomonas*. They are environmentally-friendly, economically viable and energy efficient. The application of these groups of microorganisms is therefore a promising approach for alleviating salinity stress in plants.

Microbial inoculants ameliorate salt stress in plant via increased nutrient uptake, induced antioxidative defense system, modulation of the level of plant hormones, and reduction of ethylene level by producing 1-aminocyclopropane-1-carboxylate-deaminase in plants rhizosphere. Inoculation of sunflower with *Pseudomonas fluorescens* biotype F and *Pseudomonas fluorescens* CECT 378^T in sun flower grown in substrate with addition of salt (NaCl) showed that these strains that alleviate salt stress in sun flower produced indole-3-acetic acid and siderophores. The crop plants inoculated had a better developed root and a better K^+: Na^+ ratio in the shoot (Shilev et al. 2012). In the same vein, Jha et al. (2011) discovered that the inoculation of a local paddy rice with *Pseudomonas pseudoacaligenes* and *Bacillus pumilus* in saline soil resulted in a decrease in growth suppression evident by an increased dry weight. The microbial inoculants also induced some osmoprotectants which help to overcome the deleterious effects of salt stress.

9.2.6 *Benefits of Microbial Inoculations as Biofertilizer on Plants Growth*

Some reports in literature collated recently by Babalola and Glick (2012) describe microbial inoculation to improve plant fitness and plant yield components. Microbial inoculation improves most plants growth and vigor. They enhance root growth and exudation (Babalola 2010; Trabelsi and Mhamdi 2013). When applied seeds, plants surface or soil, microbial inoculants increase the availability and supply of essential nutrients to host plants and thereby promoting growth. Microbial-inoculated plants show a reduction in membrane potential, accelerated osmotic adjustment, and enhanced lateral root development due to higher nitric acid and indole-3-acetic acid production (Dimkpa et al. 2009). Fungal inoculants will harmonize with the plant's root system and greatly expand the surface area of the root mass. Production of phytohormones by microbial inoculants can result in modification of root morphogenesis and hence support water uptake to plant roots.

Some common microbial components of biofertilizers include: *Azotobacter, Azospirillum, Bradyrhizobium*, mycorrhizae, phosphorus solubilizing bacteria, and *Rhizobium*. Microbial biofertilizers could be grouped into; Nitrogen fixers e.g. *Rhizobium and Bradyrhizobium*, phosphate solubilizers e.g. *Pseudomonas, bacillus, Aspergillus* etc., cellulose degraders such as *Cytophaga* and phosphate mobilizers such as mycorrhizae. Microbial bio-fertilizers are cost effective and cheaper than the conventional techniques. They provide 25–30 % of chemical fertilizer equivalent of nitrogen. They increase phosphorus and potassium, increase water absorption and keep soil biologically active. In soils cropped with legumes, the application of arbuscular mycorrhizal fungi inoculants tremendously improve growth and yields. More also, inoculation with arbuscular mycorrhizal fungi improved growth of chickpea (*Cicer arietinum* L.) and doubled P uptake at low and intermediate levels of P in a pot experiment on sterilized low-P calcareous soil (Mohammadi et al. 2011). The inoculation of maize with *Trichoderma harzianum* strain T22 as a biofertilizer shortens the plant growth period and time and reduced lignifications hence, enhanced fresh state of maize plant (Akladious and Abbas 2012). An improved grain yield was reported by (N'Cho et al. 2013) when soybean was co-inoculated with rhizobium and fungal inoculants and application of foliar fertilizer.

However, microbial biofertilizers are associated with the following limitations:

(i) The performance and efficacy of microbial inoculants (Biofertilizers) cannot be easily tested in the field i.e. there is a block in biofertilizer development.
(ii) The efficacy of biofertilizers is not reliable. The mechanism of action of the biofertilizers in promoting growth is not yet well understood. In attempting to deal with these issues, research into biofertilizer is increasing.
(iii) The essential nutrient may not be available in sufficient quantities to plants.

Nutritional deficiency could exist due to low transfer of micro and macro nutrients

9.2.7 Benefits of Microbial Inoculants as Biocontrol Agents

Microbial inoculants have offered eco-friendly control mechanism against plant pathogens. Microbial inoculants produce antifungal secondary metabolites such as 2, 4-diacetylphloroglucinol and lytic enzymes. Some also confer plant protection against the activities of dieses causing organism by producing chitinase and protease enzymes. Microbial biocontrol agents also antagonize pathogens by competitive colonization of plant root and by forming biofilms in the hydroponic and soil systems.

Numerous microbial inoculants for control of several diseases especially species of the bacteria *Pseudomonas, Bacillus, Enterobacter, Streptomyces* and the fungus *Trichoderma*-, causing plant diseases as leaf spots, brown patch, *Pythium* blight and root rot, *Fusarium* wilt, dollar spot, summer patch, take-all patch, *Verticillium* wilt and *Typhula* blight have been studied by various researchers. Table 9.3 shows some microorganisms that had exhibited some biocontrol activity against some phytopathogens.

Table 9.3 Microbial inoculants used as biocontrol agents

Biocontrol agents	Plant disease/Pathogen	Crop	References
Bacillus subtilis HJ5	*Verticillium* wilt	Cotton	Li et al. (2013)
Bacillus subtilis SQR9	*Fusarium* wilt	Cucumber	Cao et al. (2011)
Bacillus subtilis	*Fusarium* wilt	Maize	Cavaglieri et al. (2005)
Trichoderma asperellum T-34	*Rhizoctonia solani*	Cucumber	Trillas et al. (2006)
Trichoderma harzianum SQR-T 037	*Fusarium wilt*	Cucumber	Yang et al. (2011)
Paenibacillus polymyxa, Trichoderma harzianum	*Fusarium* wilt	Water melon	Wu et al. (2009)
Streptomyces sp strain g10	*Fusarium* wilt	Banana	Getha et al. (2005)
Pseudomonas spp	*Verticillium* wilt	Cotton	Erdogan and Benlioglu (2010)
Bacillus pumilus SQR-N43	*Rhizoctonia solani*	Cucumber	Huang et al. (2012)
Streptomyces mutabilis NBRC 12800	*Rhizoctonia solani* damping-off	Tomato	Goudjal et al. (2014)
Bacillus amyloliquefaciens	Panama disease	Banana	Xue et al. (2015)
Streptomyces	*Phytophthora* root rots	Alfalfa	Xiao et al. (2002)
Streptomyces	Damping-off	Sugar beet	Sadeghi et al. (2006)
Actinoplanes campanulatus, Micromonospora chalcea and *Streptomyces spiralis*	*Pythium aphanidermatum*	Cucumber	El-Tarabily et al. (2008)
Streptomyces	*Sclerotium rolfsii*	Sugar beet	Errakhi et al. (2007)
Streptomyces	Damping off	Tomato	Dhanasekaran et al. (2005)
Micromonospora aurantiaca, Streptomyces griseus	Damping off	Wheat	Hamdali et al. (2008)
Bacillus pumilus, *Pseudomonas alcaligenes*, and *Rhizobium* sp.	Wilt disease	Lentil	Akhtar et al. (2010)
Bacillus subtilis	Stem-end rot	Avocado flowers	Demoz and Korsten (2006)

9.3 Characteristics of Good Inoculants

Most microbial inoculants have received attention because of their catabolic versatility (Hmaeid et al. 2014), excellent root-colonizing ability which includes; motility, adhesion and growth rate (Hmaeid et al. 2014). They have capacity to produce a wide range of enzymes and metabolites, the ability to produce auxin or indole acetic acid, solubilize phosphate, produce siderophores (Hmaeid et al. 2014), survive and multiply in microhabitats associated with the root surface, in competition with other microbiota (Nivedhitha et al. 2008). They can persist in soil, are stable in storage and culture and are able to tolerate environmental constrains such as stress caused by fluctuating soil water conditions, use of fertilizers or agrochemicals (both organic and conventional) and soil disturbance such as cultivation (Hungaria et al. 2005). The success of microbial inoculation depends largely on the following: the plant species and cultivar, soil type, soil moisture and temperature conditions, the number of pathogens present in the soil around the plant and how the inoculants were prepared and applied (Babalola et al. 2007).

9.4 Properties of Good Carriers for Microbial Inoculants

The use of appropriate carriers for microbial inoculants preparation cannot be over emphasized. Good microbial inoculants carrier should (i) be easily handled and stored for a long period of time. (ii) have the capacity to deliver the right number of viable microbial cells in appropriate physiological condition at the right time, (iii) protect microbial cells from various biotic and abiotic stresses they will face once applied to the soil, (iv) retain microbial Plant-Growth Promoting abilities after a long period of storage, (v) be of low cost and locally available, (vi) be mixable and package able, (vii) permit gas exchange, particularly oxygen and have high organic matter content and water holding and retention capacity and it should be more than 50 %, (viii) be easy to process (mixing, curing and packaging operations) and free of lump- forming materials, (ix) be easy to sterilize by autoclaving or gamma-irradiation, (x) have good adhesion of seeds (xi) have good pH buffering capacity and (xii) be nontoxic to plants (Ferreira and Castro 2005).

Bacterial inoculants should be kept under a cool temperature, between 1.1 °C and 21.1 °C is best, away from extreme heat, direct sunlight or exposure to the elements or repeated freezing and thawing. Fungal inoculants are best kept dry. Excessive heat or cold is never of benefit. Also to be noted is the fact that agronomic practices have profound effects on soil organisms. They should therefore be designed to work in harmony with microbial inoculants and biological processes in order to support sustainable agricultural systems. Table 9.4 shows some materials that have successfully been used as carrier for microbial inoculants and the associated microbes. The use of appropriate carrier material determines the success of microbial inoculation techniques

Table 9.4 Types of carriers used for inoculants production

Carrier material	Inoculants	References
Sterilized oxalic acid, sludge, industrial waste, alginate-perlite dry granules, soybean oil or peanut oil added with lyophilized cells, composted sawdust, nutrient supplemented pumice, mineral soils, diatom, porosil mp, microcel, vermiculite, agriperlite, expanded clay, kaolin, celite, wheat bran, sugarcane bagasse, coal/charcoal, granular inoculants amended with nutrient and perlite	*Rhizobium, Sinorhizobium, Bradyrhizobium, Azospirillum, Agrobacterium*, Phosphorus solubilizing fungi, and *Aspergillus niger*,	Zaidi et al. (2014)
Alginate beads supplemented with skim milk, charcoal based, broth based	*Bacillus subtilis*, *Pseudomonas* corrugate	Trivedi et al. (2012)
Talc powder +carboxyl- methyl cellulose	*Pseudomonas fluorescence*	Negi et al. (2005)

9.5 Methods of Application of Microbial Inoculants

The soil environmental conditions for crop production can be optimized by introducing friendly environmental microbial formulations to the soil. The use of appropriate carriers for microbial inoculants preparation is critical for the success of microbial inoculation (Babalola 2010). Microbial inoculant formulations are sold as wet able powders, granules or liquids sprays (Babalola and Glick 2012). The following methods can be used (Table 9.5). Microbial inoculants could be made of a singular strain of microbe. This approach is called the monoculture approach, where as an inoculant made of two or more strain of microbe or different types of organism is referred to as a co- culture or multiple culture approach. Microbial inoculants could either be applied directly to the soil or as seed/seedling dressing.

9.6 Factors that Determine the Performance of Microbial Inoculants Under Field Condition

The fate of introduced microbial inoculants includes: the ability to survive inoculation on seed, multiply in the spermosphere in response to seed exudates, attach to the root surface and colonize the developing root system. The ability of microbial inoculants to compete with indigenous microorganism present in the rhizosphere and the soil, for successful colonization of a developing plant depends on a number of biotic and abiotic factors. The survival, colonization and establishment of the inoculated microbes depend largely on these factors.

For microbial inoculants to survive competition, they must be able to sense chemo attractants like lipopolysaccharide such as O-antigen chain. However, lipopolysaccharide in colonization is strain dependent. O-antigenic side chain of *Pseudomonas fluorescens* PCL1205 is involve in tomato root colonization whereas

Table 9.5 Methods of application of microbial inoculants

Methods	Mechanism of application	Advantages and limitation	References
Directly to the soil	After seed germination, they are applied directly to the soil at the plant base near the plant roots	Withstand low moisture conditions better than carrier based inoculants. A less expensive method.	Mokone and Babalola (2013)
Seed application	Seeds are coated with microbe-carrier slurry. Adhesive solution such as sucrose solution is recommended.	Adequate loading of bacterial cells. Seeds treated with microbial inoculants may come in direct contact with any seed applied with chemicals which may adversely affect the survivability of the inoculated organism. Microbial culture may move away from rooting zones after application and could be exposed to agrochemicals after planting (Zaidi et al. 2014)	Mokone and Babalola (2013), Babalola et al. (2007), Babalola (2010) and Akladious and Abbas (2012)
Seedling root dip method	The seedling root dip method is mostly used for transplanted crops like vegetables. The roots of the seedling are dipped in a mixture of microbial culture and water for 5–10 min. The seedlings is then removed and transplanted almost immediately.	A less expensive method compare to carrier base inoculants	Babalola et al. (2007)
Field/Soil application	The direct application of inoculants to soil. Generally the granular inoculants are placed on the furrow under or alongside the seed. This enhanced inoculated microbe is in contact with the plant root.	less time consuming than the seed inoculation method	(Babalola et al. 2007)
Broadcasting method	Microbial inoculants could also be mixed with farmyard manure before broadcast.	Rapid and greater colonization of inoculants per unit area	(Akladious and Abbas 2012)

O-antigenic aspect of lipopolysaccharide of *Pseudomonas fluorescens* WCS374 does not contribute to rhizosphere colonization. Other factors include; high microbial growth rate and the ability of the inoculants to produce vitamin B1 and exude nicotinamide adenine dinucleotide dehydrogenases (NADH) and their ability to secret site specific recombinase (Dennis et al. 2010).

The association between inoculated culture and host plant play a vital role in determining the success of microbial inoculation technique in the field. More also, Different inoculants produce different level and types of organic acid. The organic acids also vary in their ability to form complexes with cations to release inorganic nutrients for plant use. For instance, the ability of organic acid to complex with cation and liberate inorganic phosphorus varies with oxalic and citric acid. Gluconic acid has a limited ability to chelate and release phosphorus complex with calcium. Other factors include: Physico-chemical properties of soil such as soil pH, organic matter content and moisture content, presence of environmental pollutants such as xenobiotics and composition of root exudates. Further, management practices such as irrigating; grooming and fertilizing also influence microbial activity and growth. To overcome these short comings, applications must occur at times when environmental conditions strongly favor activity of the inoculant and inoculant must be formulated in a way that favors its activity and survival. The fate of microbial inoculants under field application depends largely on both biotic and abiotic factors.

9.7 Effects of Microbial Inoculants on the Resident Microbial Community

In the inoculation of seed and soil large quantity of efficient and viable microbial cells are introduced to the soil to cause a rapid colonization of the host rhizosphere. This may greatly disturb the equilibrium of soil microbial communities (Babalola 2014). These changes could either be by reason of direct trophic competitions or because of antagonistic or synergetic interactions between the introduced microbes and the resident microbes. It could also be indirect effect mediated by enhanced root growth and exudation. These changes could be in the taxonomic group or in the functional capabilities of the soil microbial community.

Depending on the technique used to address the effect of microbial inoculants on soil microbial communities, microbial inoculation may cause tremendous changes in the composition and number of taxonomic groups. While some researchers reported a long term effect, some other ones observed a transient or no effect at all. Plant and soil are affected by both the temporal and long term effects of inoculants. These effects result in unpredictable reactions (Trabelsi and Mhamdi 2013). For instance, Probanza et al. (2002) observed alteration in microbial rhizosphere composition when *Pinus pinea* L was inoculated with *Bacillus licheniformis* CECT 5106 and *Bacillus pumilus* CECT105. Conn and Franco (2004) observed that the introduction of a non-adapted (mixed commercial inoculants) microbial inoculums to the soil cropped with wheat disrupted the natural actinobacterial endophyte population thus reducing the diversity and colonization level. In contrast addition of a single actinobacterial endophyte to wheat plant increase colonization level and the indigenous endophyte population was not adversely affected (Yousaf et al. 2011). When maize was inoculated with *Azospirillum brasilense*, according to

Herschkovitz et al. (2005) the inoculants did not disrupt or alter the diversity and structure of root associated bacterial group both when universal bacterial primer and polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) approach in conjunction with group-specific primers techniques were employed. The application some microbial inoculants could cause a change (which could be a decrease or an increase) in the equilibrium of soil microbial communities while some produce no effect at all.

9.8 Conclusion

This review has undoubtedly shown that microbial inoculants could improve biological management of nutrients and plant diseases resulting in improved plant performance in integrated plant management systems. They can contribute to a possible reduction of overuse of agro-chemicals and their environmental impacts. Though there are inconsistencies in the ability of many of the microorganisms that are used in the inoculant formulations to promote plant growth, the prospects of the inoculants like *rhizobium*, arbuscular mycorrhizal fungi and some *rhizobacteria* outweigh the lapses in meeting the goal of sustainable agricultural production. Apart from plant growth promotion, soil degradation is a serious problem in agriculture and the use of microbial inoculants is potentially part of the solution to this problem. More research effort is needed to elucidate the complex soil-plant-microbe interaction in order to reach the goal of completely substituting the environmentally degrading agro-chemicals with environmentally enhancing microbial inoculants. A combination of microorganisms in inoculant formulations has been shown to be helpful in many cases and can be made more efficient with research. Biotechnological research for effective and efficient microorganisms that are compatible with crops and adaptable to the soil environment will also be very helpful. In meeting the goal of sustainable agriculture, the use of microbial inoculants technology could be adopted for safe, increased production and sustainable agriculture.

Acknowledgements North-West University is gratefully acknowledged for ETA and MOD postdoctoral supports. OOB would like to thank the National Research Foundation, South Africa for grant (Ref: UID81192) that have supported research in her laboratory.

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