Chapter 2
Orchestration of the Plant Microbiome for Enhanced Agriculture

Shubha Rani Sharma and Rajani Sharma

Abstract The fast rising human population calls for new methods to enhance agricultural production because classical methods such as the excessive use of mineral fertilizers are not sustainable. For instance, recent advances in microbial ecology reveal that many microbial properties can be tuned to favor plant health with positive effects on nutrients availability, root growth enhancement, toxin neutralization, providing resistance to various diseases, enduring stresses such as heat, flood, and drought. Here we review microbial strategies that favor plant health, with focus on the plant-associated microbiome and the use of the soil microbiome for management. Rhizosphere properties influence crop yields through production of exopolysaccharide for soil aggregation, alleviation of crop stress, enhancement of nutrient uptake by soil microbes, and efficient nitrogen mineralization and phosphate solubilization. Plant-microbe interactions increase colonization of plant-beneficial bacteria, which promotes enhanced root growth and phytoremediation.

Keywords Microbiome · Agricultural · Rhizosphere · Phytoremediation · Stress · Bioavailability · Nif genes · Biofertilizers · Mutualism · Phytohormones · Voodoo juice

2.1 Introduction

Science has revolutionized the living standards of the common people. This sounds good that with advanced formulas and technologies death rate has decreased. On the other hand, it is quite alarming as this has resulted in an increase in the consumption

S. R. Sharma (✉)
Department of Bioengineering & Biotechnology, Birla Institute of Technology, Ranchi, Jharkhand, India
e-mail: srsharma@bitmesra.ac.in

R. Sharma
Department of Biotechnology, Amity University, Ranchi, Jharkhand, India

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rate in each field. Increased population has directly influenced the farming trying to enhance it beyond limits. Scientists have tried to formulate the techniques which can increase the crop yield in limited space and time. The major step taken was the use of synthetic fertilizers having all the constituents needed for the high crop yield (Tilman et al. 2002). As the natural manures were not sufficient to meet the demands of the huge agricultural land masses and were slow in their action so we had to take refuge of the synthetic fertilizers.

Synthetic fertilizers were introduced to fulfill the needs of macro and micronutrients for high crop yield as well as chemical pesticides have over-powered the pests and pathogens but the prominent and exhaustive use of both the chemicals actually disturbed the ecological balance (Bhanti and Taneja 2007). On one hand, these chemicals increased the crop yield to great heights in order to quench the demands of the food for the population while on the other hand; it degrades the soil quality (Chowdhury et al. 2008). Some serious steps need to be taken which can increase the crop yield without disturbing ecological balance and the soil physiology (Sharma and Singhvi 2017). Need is the mother of all inventions, this proverb proved right in this case. Severe needs to increase crop production and curb the problem of soil pollution led to the discovery of microbial exploitation to increase the crop yield. Plants parts are known to be associated with multifarious microbiome just like the gut of human beings.

The microbial communities which reside in various plant parts have their own significance. These may be involved in the uptake of nutrients, defense against pathogens and combating abiotic stress (Abhilash et al. 2012). It has been an established fact that, just like animals, plants are not self-sufficient but are highly colonized by an abundance of microbes. Microbes are known to play essential roles in agricultural ecosystems (Gopal and Gupta 2016). The agriculturists have well known that the healthy soils are the foundation for healthy plant life as well as high crop yield. Healthy soil is characterized by sufficient moisture, organic matter and other physical soil attributes (Doran and Safley 1997). In this chapter, we will discuss the role of microbes in enhancing crop yield targeting all the above-mentioned issues.

The excessive use of chemical fertilizer disturbs the soil physiology and frequently leads to accumulation of heavy metals, nitrate and increases the salinity (Pogrzeba et al. 2018; Lominadze and Nakashidze 2016). Increase in salinity also influences the pH level of the soil (Dong et al. 2012). The effect is not limited to soil only; rather it also pollutes the water bodies. The rushing of rainwater to the water bodies may lead to eutrophication (Savci 2012). Uses of biofertilizers have come up with most of the above-mentioned drawbacks. A comparative study between chemical and biofertilizer has been shown in Fig. 2.1.

Actually, the use of earthworms is in practice but microbiomes are preferred for sustainable agriculture. This is due to the emission of carbon dioxide and nitrous oxide by earthworm (Giannopoulos et al. 2010). These two are the main greenhouse gases. On the other hand, the maintenance of earthworm is not cost effective. This directly affects the price index of the crops. For this reason, microbes are targeted to enhance soil fertility and have a positive association with yield improvement.

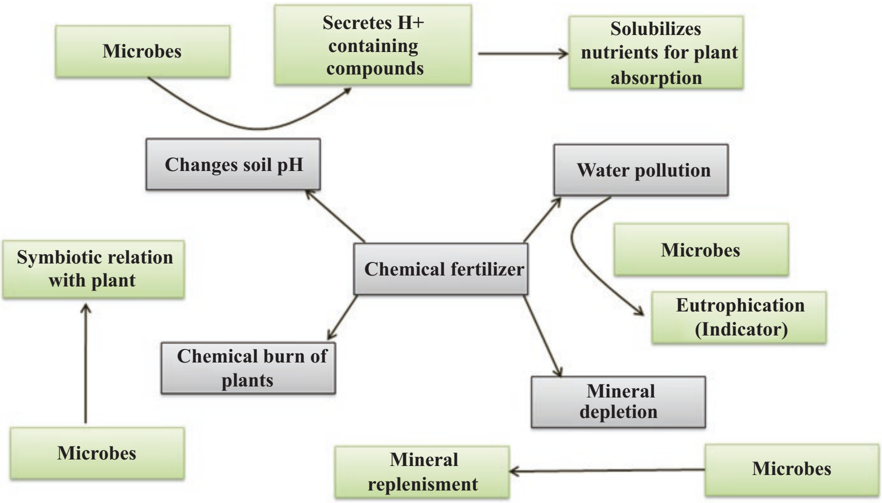

Fig. 2.1 Comparative study of chemical fertilizer and biofertilizer. Chemical fertilizer has adverse effects on soil texture. Biofertilizer meets the entire requirement without disturbing the nature of the soil. Microbes lead to mineral replenishment and increase the bioavailability of unsolubilized minerals

Microbes show symbiotic relation with the plants and may be present as epiphytes or as endophytes. Plant roots create a good rhizosphere by secreting ion and mucilage rich exudates which play an important role in plant microbe interaction Plants are specific for the bacterial colony. This specificity is regulated by the chemical composition of the exudates secreted by roots. Plants root exudates lead to physiological changes in plants that allow the growth of beneficial microbes in its rhizosphere. Microbes support plants by secreting the growth promoting hormones like auxins and cytokinins. Many of the microbes improve the yield by enhancing nutrient uptake, controlling infection; make the plant resistant to biotic and abiotic stresses.

2.2 Plant-Associated Microbiome

The diversity of soil microbes are the most important components of the different factors needed for enhancement of crop production. Among all the soil microbiome, bacteria, fungi, and archaea are the spotlights of the microbiology research. About one million taxa among the 10^9 microbes per g of soil have been documented. The soil is inhabited with millions of types of culturable as well as unculturable microbes (Blackwood et al. 2006). The population of microbes from the rhizosphere flee from the immune responses of the plant and make their way into the plant tissues and reside there as endophytes (Mercado-Blanco and Prieto 2012). These endophytes prove beneficial for the plant in a variety of ways like they assist the plant in defense against pathogens and produce secondary metabolites (Hallmann 2001). Metagenomics of the rhizosphere soil reveals a bizarre of microbes, which reside in the soil around the plant. Also, the molecular analysis of the endophytes exposes the variety of the microbes which reside inside the plants.

The endophyte colonizing the plant tissues includes mycorrhizal fungi, rhizobia, some pathogens and these assist in the absorption of nutrients from soil or atmosphere. These microbes prove to be a boon for plants as they perform a lot of functions that assist the plants eg. they act as decomposers of organic substances, also promote plant growth with the help of rhizobacteria which are designated as plant growth promoting rhizobacteria and are also witnessed to act as antagonists to plant pathogens (Beneduzi et al. 2012). They also help in nutrient cycling by fixing atmospheric nitrogen, mobilization of the phosphate present in the rhizosphere so that these nutrients can be utilized by the plants to the maximum (Sharma et al. 2013).

The nitrogen-fixing bacteria are the versatile group of bacteria which are also designated as 'rhizobia'. They are able to fix nitrogen in legume plants by the residing inside the root nodules of the plants in a symbiotic relationship. The microorganisms belonging to the actinomycetes of the genus *Frankia* of the family Frankiaceae form N_2-fixing nodules on the root (Santi et al. 2013). None of the eukaryotic enzymes can split the triple bond of N_2. Thus, the prokaryotic species are exploited for the reduction of N_2 to NH_3 for nitrogen fixation and this reaction is catalyzed by the enzyme nitrogenase present in the microbes residing in root nodules of plants.

The symbiotic arbuscular mycorrhizal fungi are known to establish mycorrhizal associations with the roots of most angiosperms (Igiehon and Babalola 2017). The formation of arbuscular mycorrhizal formation is a kind of adaptation, in which the plants on association with the arbuscular mycorrhiza are endowed with increased power for nutrient absorption. The additional functions that arbuscular mycorrhiza endows plant include resistance to stresses like pathogen assault, drought, salinity, heavy metals, organic pollutants. It also aids in the development of good soil constitution by aggregate formation (Jeffries et al. 2003). Thus, we can say that arbuscular mycorrhiza has a key role to play in agricultural advancement (Jeffries and Barea 2012). Basically, we harness the microbial benefits by two different approaches, either we apply a different combination of the microbial inoculants or we manipulate the natural microbial flora and fauna to enhance agriculture.

Pseudomonas species is designated as protective bacteria which repress root-fungus disease (Gaffney et al. 1994). An antibiotic produced by *Streptomyces diastatochromogenes* restrains the infection of potato scab disease-causing bacteria (Sarwar et al. 2018). The novel bacteria *Bacillus thuringiensis* protects the plants from fungus gnat. The spores of *B. thuringiensis,* when consumed by fungus gnat larvae, germinate in the gut of the insect and produce the crystal protein known as cry protein. These bacterial inoculants are now marketed in the pelleted form (Kergunteuil et al. 2016).

2.3 Utilization of the Soil Microbiome by Proper Management Techniques

The theoretical and experimental models are very minutely studied and different strategies are designed in order to get the maximum output. One of the wonderful strategies chalked out was to maneuver the exudation produced by root so that we could select the beneficial combination of the microbes for the plant growth and enhancement (Berendsen et al. 2012). A method of plant-microbe co-adaptation is actually followed where the existing pathogens activities are controlled to get the benefit out of it. When a plant is luxuriously thriving in an environment, it develops various relations with various microbes to produce a very rich soil environment so that it happily grows there, but once the same plant is transferred to another environment, it needs to struggle in the new habitat. If we manipulate the new habitat with the help of microbiome present in the previous habitat soil, the plant does not need to fight adverse situation and will thrive better.

The plant or the crop will bracket together with an un-adapted microbe environment, which does not restrict pathogen and thus will become vulnerable to different kinds of diseases. Thus, manipulating the plant environment to attract favorable microbes in its rhizosphere for introducing specific functions like nitrogen fixation, P-mobilization, biocontrol of diseases would prove beneficial for further agricultural developments (Lareen et al. 2016). The main endeavor is to renovate a nominal rhizobiome which will be capable of providing maximum advantage to crops at minimum photosynthetic expenditure (Pérez Jaramillo et al. 2016).

2.4 Bacterial Mechanisms of Plant Growth Promotion

The role of prebiotics is very nicely studied in the context of the microbes in the soil which act as food for them. When we provide prebiotics, it acts as precursors for rooting compounds and allows the bacteria to assimilate the biochemicals and change them into natural rooting compounds which are instrumental in stimulating the growth of the plant. Different types of microbes have different strategies and pathways for processing their foods. Some of the bacteria have pathways exclusively for releasing Plant Growth Promoting biochemicals like auxin-based compounds that are instrumental in inducing and promoting root growth (Costacurta and Vanderleyden 1995). When bacteria combination is provided with appropriate food and nutrition as well as soil extracts, a specialized microbiome can be created which promotes plant growth as well as profuse root development. The following microbial functions can be tapped for the enhancement of agriculture:

1. Bioavailability of the recalcitrant nutrients to the plants by the help of beneficial bacteria to convert them into easily accessible forms (Igiehon and Babalola 2018).

2. Beneficial bacteria for plants produce chemicals and hormones that stimulate growth (Olanrewaju et al. 2017).
3. Beneficial bacteria help prevent infections from pathogens by coating the root surfaces and triggering systemic disease resistance (Kant et al. 2015).
4. Beneficial bacteria for plants help filter out heavy metals and other contaminants from the soil (Khan et al. 2018).
5. The beneficial bacteria act as fertilizer once they die, by releasing helpful nutrients that are absorbed by the plant's roots (Jacoby et al. 2017).

2.5 Rhizosphere Contribution to Crop Yields

The soil is a very versatile habitat for the microbes. The microbes present in the soil can be tapped for the increased production of crops (Andrén et al. 1999). Microbes have a bizarre of applications in the soil and are found to be associated with plants in a variety of ways (Jacoby et al. 2017). They may be present as epiphytes as well as endophytes. The Plant roots prove to build a good rhizosphere. Rhizosphere is the region of soil which is enriched with the exudates produced by roots which actually attracts and communicates with the microbes. Thus, the rhizosphere forms the home of diverse microbes. The microbes present in the rhizosphere prove to improve the crop yield by nutrient uptake enhancement, combating infection and producing resistance in the plant against different types of stresses (Hartmann et al. 2008). Thus, the microbiome present in the plant rhizosphere plays a key role to shoot up crop productivity. It has been witnessed that the microbes play an important role in the biogeochemical cycles and provide the plants, a healthy and improved life.

2.5.1 Exopolysaccharides to Promote Soil Aggregation

Exopolysaccharide is a high molecular weight carbohydrate which is instrumental in promoting soil aggregation. Aggregated soil also provides a high concentration of nutrients and favors proper growth of the plant. Bacteria and fungi both have the property to secrete polysaccharides which adheres the soil particles together. Additionally, extra-radical mycelium of fungi can also enforce the soil particles to come together (Fig. 2.2). Exopolysaccharide secreting microorganisms aggregate soil even in stress condition. It has been seen that exopolysaccharide secretion increases during the stress condition. Increased concentration brings about thermal and osmotic regulation, essential for the survival of bacteria. In 2000, an experiment was conducted on sunflower (*Helianthus annuus* L.) using a strain YAS34 as an inoculum under water stress condition. The bacterial strain not only brought aggregation of soil rather also increased the yield. The inoculum increases the uptake of nitrogen from the soil in the stress condition (Alami et al. 2000).

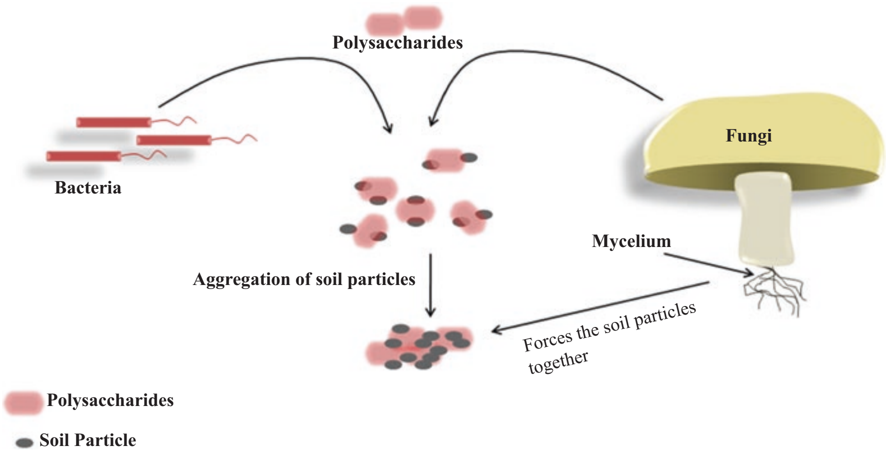

Fig. 2.2 Bacteria and fungi secrete polysaccharides. Polysaccharides adheres the soil particles together. Fungi force the soil particles together by their extra-radical mycelium

A similar study in Chickpea under high salinity condition proved the significance of exopolysaccharide in improving the yield. Under high salinity condition, chickpea was inoculated with salt-tolerant strains *Halomonas variabilis* HT1 and *Planococcus rifietoensis* RT4. Both the strain increased the secretion of exopolysaccharide which helps them to adapt under the stress condition. The increased secretion of exopolysaccharide enhances the aggregation rate of soil (Qurashi and Sabri 2012).

2.5.2 Improving Soil Microbes for Stress Alleviation in Crops

The agricultural scenario always faces a plethora of biotic as well as abiotic stress conditions like stress due to excess salinity, drought, nutrient deficiency, infection, pathogenic diseases and many more. Rhizosphere microbiome benefit plants in many ways. First and foremost, they help the plants to counter the biotic and abiotic stresses (Table 2.1) and boost the productivity of plant as well as their health (Bowen and Rovira 1999). One should have a sound knowledge of the plant-microbe interactions so as to exploit the microbial benefit to the maximum. Researchers have nicely exploited the interactions between *Arabidopsis thaliana* and its rhizobiome (Lundberg et al. 2012). The root exudates initiate and enhance the interactions among the microbiome. The Earth Microbiome Project investigations and results provide us with 2290 plant-associated and 4279 soil microbe data from various collection sites (Gilbert et al. 2014) which overcome the stress.

Studies reveal that the soil properties and the type of land-use greatly influence the soil microbiomes (Dignam et al. 2018). These microbes may be either saprophytic or symbiotic like rhizobia which form nodules as well as like the arbuscular

Table 2.1 Soil microbes for various stress alleviation in the host plants

Abiotic stress	Host plants	Microbes involved
Cold stress	Maize plants	*Pseudomonas* sp. DSMZ 13134, *B. amyloliquefaciens* subsp. *plantarum*, *Bacillus* strains
	Tomato	*Arthrobacter*, *Flavobacterium*, *Flavimonas*, *Pedobacter,* and *Pseudomonas*
	Wheat (*Triticum aestivum* L).	*Methylobacterium phyllosphaerae* strain IARI-HHS2–67
	Methylobacterium phyllosphaerae strain IARI-HHS2–67	*Burkholderia phytofirmans*
Heat stress	Wheat (*Triticum* spp.)	*Pseudomonas putida* strain AKMP7
	Sorghum	*Pseudomonas* sp. strain AKM-P6
Salinity	*Zea mays*	*Rhizobium*
	Tomato	*Pseudomonas fluorescens*, *P. aeruginosa*, and *P. stutzeri*
	Rice	*Pseudomonas pseudoalcaligenes*
	Wheat	*Dietzia natronolimnaea*
	Lens esculenta Var. Masoor-93	*Staphylococcus saprophyticus* ST1 and *Oceanobacillus profundus* Pmt2
Water stress resistance	Tomato	*Achromobacter piechaudii* ARV8
	Green gram	*P. fluorescens*

mycorrhizal fungi. Depending upon the host genotype and physiology (e.g., root exudates and metabolites) as well as environmental factors, the microbiota varies (Lundberg et al. 2012). Bacteria like *Azotobacter chroococcum* or *Bacillus megaterium* have been used for the first time in the 1950s to improve growth as well as yield of the crops (Brown 1974). Rhizobia not only fix the soil nitrogen rather it grabs atmospheric nitrogen also (Teintze et al. 1981).

The scientists and researchers are constantly involved in the discovery of ways and means to combat these stresses. To the utter astonishment of the scientists a very interesting fact was discovered that when the plant undergoes certain kind of stress, the interaction of the plant crop and the associated microbiota is altered in the rhizosphere. The beautiful fact is that the alteration of the microbial population due to the stresses is for benefit of the plant species. It has been shown that the environmental stresses on plants induce the production and activation of phytohormones (Rejeb et al. 2014). These phytohormones actually induce better plant growth as well as manipulation and alteration of soil microbes which is beneficial for the plant in all aspects.

A deep insight into the mechanism of the interaction of the phytohormones with plant and the microbiome can provide us with the advantages and disadvantages of the process. This understanding can equip us with the strategy to manipulate the environmental conditions and the microbiome so that it becomes beneficial for the plant. We can design biotechnological strategies with the help of the knowledge so that plant adaptation mechanisms can be optimized and the microbes involved in stress alleviation in crops can be utilized. Plant stress like ‘drought’ is considered as one of the most important matter of concern on agricultural output. The food security is at stake due to unfavorable alterations in the climate. Major research is being targeted on emerging novel strategies to produce crops which are more pliable so that they can endure any kind and any degree of stress. Nowadays root-associated microbiomes with chemical induction are being used which confers plant tolerance to abiotic stresses like drought.

The role of arbuscular mycorrhizal fungi and chemical agents to develop plant tolerance to drought has been highly investigated and still, it is in its adolescent stage (Khan et al. 2018). It was concluded that with an increase in nitrate and potassium ion fluxes in *Pinus pinaster,* the roots were inhabited by the ectomycorrhizal fungus *Rhizopogon roseolus* (Gobert and Plassard 2007). mRNA of several nitrate, phosphate and manganese transporter genes in *Medicago truncatula* roots colonized by *Glamos intraradices* and *Glamos mosseae* were found abundant when the plant was subjected to drought (Gomez et al. 2009). It was demonstrated that arbuscular mycorrhiza symbiosis modulates the root hydraulic properties and thus increases tolerance to drought, cold and salinity stresses (Sharipova et al. 2016). The regulation of PIP2 protein amount and phosphorylation state actually influences the root hydraulic properties (Li et al. 2011). It is possible that each *PIP* gene would be having different function and regulated by arbuscular mycorrhiza symbiosis in stress condition. Investigations have confirmed that the arbuscular mycorrhiza symbiosis can shield host plants against the unfavorable effects of drought stress (Ruiz-Sánchez et al. 2010; Volpe et al. 2018). The microbes of certain plants which help in combating stress are enumerated in Table 2.1. These microbes enhance the crop yield by surpassing abiotic stress.

The beneficial plant-microbe interactions have been researched and explored to define strategies to enhance agricultural yields. The rhizobia have been exploited for their biological nitrogen fixation capability, which launches a symbiotic relation with legumes and crop which contribute to soil fertility (Zahran 1999; Ahemad and Kibret 2014). Internal colonization of the plants by arbuscular mycorrhizal fungi with the help of arbuscular and vesicular part of the hyphae aids in the plant nutrition accomplishment (Berruti et al. 2016). Another more specific symbiotic defensive mutualism between Pooideae grasses and endophytic fungi of the *Epichloe* have been investigated to reveal its importance in the enhancement of crop production (Clay 1988). When some specific microbes are inoculated, we can foretell which plant microbiome interaction will be beneficial and necessary for the enhancement of crop production by just investigating the effects observed.

The composition of the phytomicrobiome is influenced by a variety of factors like the plant genotype, type of pesticide and fertilizers application. In order to enhance the diversity of microbiome constituents, higher plant diversity is used in agriculture like crop rotation technique with the legumes. Other strategies to increase agricultural productivity are the use of different soil combination, fertilizers and pesticides, which will not produce any adverse effects in the population of the microbiome. Critical dosage has a great effect on the microbiome activities as overdosing may produce adverse effects on the microbial population in the soil milieu. In the present scenario when different strategies are being employed for the increased production of the crops, the industries are commercializing the soil microbiome by permutation and combination of the microbes to produce the best combination to enhance the production by natural means (Lupatini et al. 2017). There is a vital requirement to develop processes and application techniques to better recognize the interaction between plants and microorganisms in the soil ecosystem.

2.5.3 Enhancing Nutrient Uptake by Plant Using the Microbiome

Bacteria, as well as fungus, contribute to the bioavailability of nutrients to the plants. The increment of bioavailability is through nitrogen fixation and mobilization of key nutrient (Nitrogen, Phosphorous and Iron) (Rashid et al. 2016). Chemical fertilizer has been formulated to increase the yield. Chemicals affect the soil physiology as well as the environment, so biofertilizers are always preferred. Symbiotic relation of microbes with plants not only avail the nutrients to the plants but also reinstate the fertility of degraded soil. Nitrogen is the major constituent of biomolecules such as protein and nucleic acid. All organism including plants requires nitrogen. Nitrogen present in the environment but it cannot be used directly by the plants. They need to be fixed with the help of microbes. Bacteria directly involve in nitrogen fixation while fungi provide a positive environment to the bacteria. Bacteria shelter inside mycelium and remain protected from oxygen. Bacteria has *nif* gene which helps in the fixation of atmospheric nitrogen to ammonia (Fischer 1994). Table 2.2 shows the list of bacteria involved in nitrogen fixation. Nitrogen not only as a major constituent of biomolecules but itself also regulates the biosynthesis of phytohormones. Phytohormones are regulated through nitrogen signaling. This states that plant growth and development are regulated by nutrients availability.

Not only nitrogen but P, K and Fe are also solubilized by bacterial as well as fungal activity. Microbes extrude proton and some lower molecular weight organic ions like succinic, citric, gluconic, oxalic acid and α-keto-gluconic acid which lowers soil pH (Marra et al. 2015). With the lowering of pH, the extruded ions got exchanged with P by the process of ligand exchange. Fungi solubilize phosphorous by siderophores. A similar release of an organic acid by bacteria solubilizes potassium by the process of acidolysis, chelation and ligand exchange. Fungi solubilize potassium through mycelia transport of citrate, malate and oxalate. Phosphorus like nitrogen is the structural component of nucleic acid along with phospholipids. Being part of nucleotide triphosphate, the phosphate bond store high energy in it. The soil is rich in the inorganic form of phosphorous like as $Ca_3(PO_4)_2$. Bacterial isolates belonging to genera *Enterobacter, Pantoea and Klebsiella* can solubilize $Ca_3(PO_4)_2$ (Delétoile et al. 2008). These genera of bacteria also show nitrogen-fixing ability.

Table 2.2 Bacterial microbiome for enhanced nitrogen mineralization

S. No.	Bacteria	Host Plant	Reference
1	*Gluconacetobacter*	Sugarcane	Bertalan et al. (2009)
2	*Herbaspirillum*	Rice	Elbeltagy et al. (2001)
3	*Bradyrhizobium, Mesorhizobium, Rhizobium*	*Acacia*	Teixeira and Rodríguez-Echeverría (2016)
4	*Micromonospora, Rhizobium meliloti*	Alfa-alfa	Martínez-Hidalgo et al. (2015)
5	*Rhizobium phaseoli* and *Solanum americanum*	Common bean seed	Mora et al. (2014)
6	*Burkholderia*	Chickpea	Abi-Ghanem et al. (2012) and Wadhwa et al. (2017)
7	*Bradyrhizobium lupini*	Lupin	Schulze et al. (2006)
8	*Allorhizobium, Azorhizobium, Bradyrhizobium Mesorhizobium, rhizobium and Sinorhizobium*	Mesquite	Sprent et al. (2013)
9	*Rhizoctonia solani* and *Fusarium oxysporum*	Lentil	El-Hersh et al. (2011)

Fe is an essential micronutrient which is involved in all the major physiological process in plants. It is also involved in chloroplasts synthesis. On earth, it is predominantly present in an insoluble form ferric (Fe^{3+}). Bacteria convert it into soluble form through the production of low molecular weight protein siderophore. During anaerobic or flooded condition, it is reduced to Fe^{2+} which is the toxic form of iron. This toxicity is controlled by bacterial inoculation like neutrophilic lithotrophs. Such bacteria utilize Fe^{2+} as an electron donor for their metabolism (Nguyen et al. 2015). Microbes which utilizeFe^{2+} from biotite excretes some chemicals like oxalic and tartaric acid. The release of such acid mobilizes potassium from minerals like biotite.

The microbes like bacteria as well as fungus are instrumental in extending their helping hand in making the nutrients bioavailable to the plants. The term bioavailability means the degree and rate at which the nutrients enter the plant system from the soil or atmosphere. Though the nutrients like nitrogen, phosphorous and iron may be present in the milieu, still it is not 100% available to the plant for various reasons. The reasons may pertain to solubility, complex formation, etc. Now if we employ certain strategies in order to solubilise these nutrients so that it is available for the plants to absorb them actually adds to the bioavailability. The recent and magnificent technology that is the best employed is the use of manipulated microbiome.

Though the different chemical fertilizers have been formulated to increase the yield of different types of crops yet we always try to employ certain natural phenomenon so that the extensive chemicals used in the formulation do not add to the soil pollution as well as poison the resources. The biofertilizers have always been a better option over the chemical fertilizers. Vermicompost is one of the biofertilizers which has been applied to the field to increase production. This method successfully uses earthworms to produce natural manures but its high maintenance cost has forced the farmers to prefer the orchestration of the microbiome to enhance the agriculture. This method exploits soil microbes to solubilize soil nutrients and thus facilitates increased nutrient absorption by the plants. The ubiquitous bacteria and fungi not only help the plants to utilize the nutrients but also replenish the fertility of degraded soil. Bacteria and fungi help each other in order to profit the plant crops. The fungi create a positive environment for the bacteria involved in the nitrogen fixation. The fungi protect the bacteria from oxidation by providing them refuge in its mycelium.

Organic ions like succinic, citric, gluconic, oxalic acid and α-keto-gluconic acid as well as protons are released in the soil which makes the soil acidic. This helps in solubilization of phosphorus. The siderophores produced by the bacteria and fungi are used in the phosphorus as well as potassium solubilization. Nitrogen-fixing symbiotic bacteria enhance the absorbance of micro and macronutrients for plant uptake and also lower the salt and water stress. Both the symbiotic as well as free-living bacteria augment the growth of plant crops by Nitrogen fixation. These special microbes are also instrumental in producing antimicrobials and phytohormones like produce auxins, cytokinins and gibberellins so as to endow the plant crops with the property of resistance to diseases. The endophytes like fungi extrude mucilaginous extracellular polysaccharides which are chiefly accountable for the complex aggregates, which actually increases soil porosity and aeration. This mechanism of soil aggregation is shown in Fig. 2.2. Some of the microbes like *Bradyrhizobium japonicum* and *Nocardioides sp*. are involved in the oxidation of the mica enriched with Fe^{2+} (Masuda et al. 2017). Increased production of citric acid, tartaric, oxalic, succinic acid, and keto-gluconic acid can be achieved by inoculation of *B. edaphicus* NBT strains (Hu et al. 2006).

2.5.4 Microbiome for Efficient Nitrogen Fixation

Nitrogen is one of the major limiting elements for crop growth. Plants cannot utilize the free atmospheric nitrogen, so the nitrogen has to be fixed. **Nitrogen fixation** can be defined as any natural or industrial procedure that causes free atmospheric nitrogen (N_2), to chemically combine with other elements to form more-reactive nitrogen compounds which can be very well utilized by the plants. For time immemorial, the farmers have been using a profuse number of chemical fertilizers to promote crop production. This is the case with the developed nations that have surplus money to account for the cost of fertilizers, but on the other hand in the still progressing countries inorganic fertilizers are often not available and small-holder farmers suffer the resultant poor yields.

Natural and cost-effective alternatives have been probed in the countries where the use of microbiome present in the soil is exploited for nitrogen fixation. A lot of research has been performed in the area of nitrogen fixation by the microbes. The investigations revealed that bacteria and archaea have the capability to fix atmospheric nitrogen to ammonia, which can be readily utilized by the plants. Thus, the nitrogen uptake by the plants can be improved by either introducing nitrogen-fixing bacteria into the crops or adding nitrogenase enzyme to the crop soil environment for nitrogen fixation. The mechanism of nitrogen fixation is shown in Fig. 2.3. The scientists have come up with biotechnological solutions to solve the problem of nitrogen availability.

Plants, specifically legumes, are found to promote facilitate colonization of nitrogen-fixing rhizobacteria in their root nodules to create a suitable oxygen-limited environment for nitrogen fixation (Mus et al. 2016). As flavonoids are released, the rhizobial bacteria trigger the production of the Nod factor that starts the plant processes essential for the symbiotic association (Abdel-Lateif et al. 2012). Whereas legumes fix nitrogen by the technique of nitrogen-fixing symbiosis, the special association of mycorrhiza is omnipresent within the whole plant kingdom and not restricted to only legumes (Mus et al. 2016; Santi et al. 2013).

Companion planting is the method that was used by the Native American farmers for nitrogen fixation when the use of rhizobial inoculants was not known. In this technique, different plants were grown together to augment each other's health (Cunningham 1998). Hereby planting leguminous crops like corn, squash, and beans, the Americans residents utilized the ability of legumes to attract nitrogen-fixing bacteria. Nowadays harnessing the beneficial properties of more Plant Growth Promoting Bacteria such as *B. thuringiensis* are being put to use (Macdonald and Singh 2013).

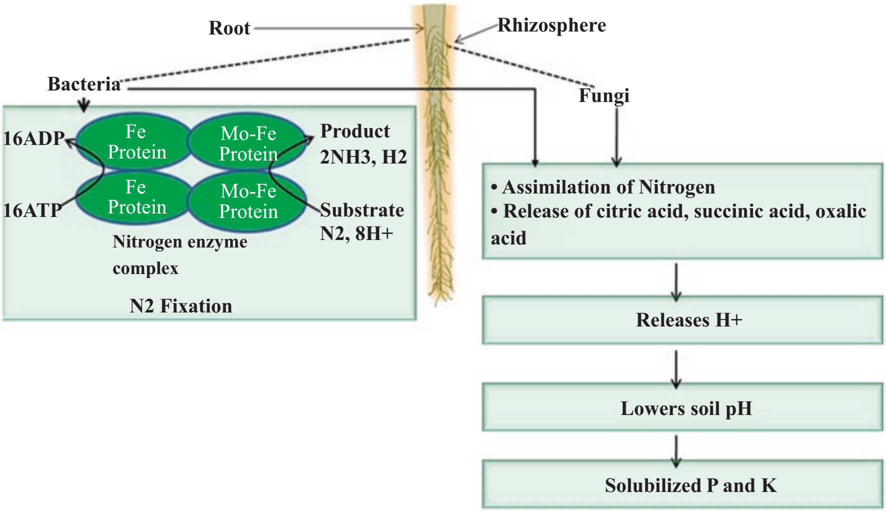

Fig. 2.3 Bioavailability of nutrients by bacteria and fungi. Bacteria have tetramer units of the nitrogen enzyme complex. This complex is involved in fixation of atmospheric nitrogen with the involvement of 16 molecules of ATP. Fungi are not directly involved in nitrogen fixation but it favors bacterial environment for nitrogen fixation. Both fungi and bacteria release certain acidic compounds in soil which solubilizes phosphorous and potassium to be consumed by plants

2.6 Plant–Microbiome Interactions to Improve Phytoremediation

Phytoremediation refers to a promising technology that utilizes synergistic interaction of living plants and microbe to cleanse the contaminated soils. There are a number of different types of phytoremediation techniques. One of them being rhizosphere biodegradation, in which, the plant roots secrete bio-chemicals and make nutrients available to the microbes. The microbes get attracted by the exudates and form aggregates in the rhizosphere. They in turn work for the biological biodegradation of the contaminants present in the soil.

The other is phytostabilization where the biochemicals produced by the plant roots just immobilize the contaminants and thus they are removed from the soil replenishing the fertility and freshness of the soil (Lone et al. 2008). The next to be mentioned is phytoaccumulation which also known as phytoextraction where the plant roots absorb the pollutants besides the nutrients and water. The contaminants accumulate in the plant parts rather than being destroyed. This process is more applicable for wastes containing metals. One common example is plants capable of absorbing water-soluble metals like 'lead'. The aerial shoots of these plants which store the metals like lead are then exploited to recover the metal by the process of smelting (Sheoran et al. 2011). Phyto-volatilization needs a special mention because here the plants absorb the aqueous organic pollutants and then release them into the atmosphere in gaseous form through their leaves. The phyto-degradation process employs plants to metabolize and demolish the pollutants in the tissues of the plant itself.

The phytomicrobiome like bacteria and Arbuscular Mycorrhizal fungi are exploited for their capabilities of phytoremediation of the soils profoundly contaminated with radionuclides, xenobiotics and heavy metals. The associated microbes actually use different types of mechanisms to get rid of the pollutants. These mechanisms comprise of a number of functions like plant growth enhancement, nutrient supply, assembly of Fe-binding siderophores, plant hormones production, ACC-deaminase activity enhancement, etc. Bio-augmentation of plant-associated microbiome has also been exploited for phytoremediation. Bioaugmentation is a technique where a pregrown microbial culture is added to boost microbial populations which aid in speeding up the removal of contaminants saving time and cost.

The Arbuscular Mycorrhizal fungi have different strategies to check the toxic metal ions to enter into the intracellular environment (Millar and Bennett 2016). This is done through the process of metal immobilization, extracellular metal sequestration as well as the chelation of metallic ions. They are also equipped with strategies to resist the oxidative stress produced by heavy metals. Heavy metals can be permanently confiscated by Glomalin-related soil proteins which are produced by Arbuscular Mycorrhizal fungi (Yang et al. 2017), leading to the stabilization of heavy metals. This property of heavy metals stabilization or removal is shown by Arbuscular Mycorrhizal fungi due to the virtue of heavy metals transporters present in them. Heavy metal-adapted rhizobacteria and Arbuscular Mycorrhizal fungi interact with each other and due to co-inoculation resulted in the accumulation of huge amounts of heavy metals and also enhanced plant growth, growth hormone production, and enzymatic behavior. One more strategy to remediate heavy metal contaminated soils was to inoculate Arbuscular Mycorrhizal fungi with plant growth-promoting rhizobacteria, in the agro waste residue which resulted in altered bacterial population and improved phytoextraction. Once we focus on improving the condition of the soil, bio-availability and easy access of contaminants, exploitation of soil and plant-associated microbes for better plant growth, we can achieve the best model for phytoremediation.

2.7 Disease-Induced Assemblage of Plant-Beneficial Bacterial Consortia

After the epidemic of a disease, it has been witnessed that a community of disease protective microbiome is developed which proves to be resistant to the disease in the rhizosphere. This can be considered as the development or establishment of the immune system of the plant. Stimulation of the host plant immune system by the protective rhizosphere microbes, present in rhizosphere produces the induced systemic resistance (Yuan et al. 2018). It has been demonstrated that three bacterial species colonized the rhizosphere post infection of downy mildew pathogen *Hyaloperonospora arabidopsidis* on *Arabidopsis thaliana* (Cabral et al. 2011). It was found that all the three bacteria in synergism with each other were able to form biofilm *in vitro* and resulted in resistance against downy mildew and promoted growth of the plant. From the above finding, it was concluded that the plants can regulate the type microbial community in the soil of the root region. This regulated growth of some specific microbiota confers disease resistance and promote plant growth and develops an environment in the plant crops potentiating the plants to grow luxuriously without the fear of some dreaded disease. The negative soil-feedback technique involves the interplay of the pathogens, promoting the plant biodiversity which is now equipped with the property of disease resistance and thus a better variety of plant is obtained.

When crops like wheat and sugar beet are continuously cultivated in an area, it can induce soil suppressiveness to disease, which is caused by special microbiota that can inhibit the growth as well as the activity of the pathogens present in the soil (Mazzola 2002). This observable fact is due to the manufacture of antimicrobials produced by the existing microbes that specifically inhibit the growth of the pathogen. The phytohormones salicylic acid and jasmonic acid are important modulators of the rhizosphere microbiome assembly of *Arabidopsis thaliana* (Kniskern et al. 2007). Jasmonic Acid is normally effective against necrotrophic pathogens while Salicylic Acid is effective against pathogens which prove to be biotrophic. Both the phytohormones are seen to be produced in response to infection by certain pathogens, so they actually influence the diversity of the microbial population.

2.8 Microbes to Enhance Root Growth

A very healthy as well as luxuriant root system confers plant a strong and healthy living. A good root mass is a requirement for a high agricultural yield. Either it is for breeding or making a clone, a root mass on seedlings is large is always desired. The question is that why do need such a profusely growing root. We need them for proper uptake of nutrients, prevention of diseases and disease resistance. There are various biotic and abiotic factors which regulate growth in plants. Phytohormones play a vital role in the regulation of growth. Auxin, cytokinin, ethylene and gibberellins control the plant growth positively while abscisic acid controls cell growth.

Apart from the plant, few of the microbes are also involved in the biosynthesis of phytohormones. More than 80% of bacteria present in rhizosphere show the production of auxin (Spaepen and Vanderleyden 2011). *Agrobacterium spp., Pseudomonas spp., Azospirillum spp.* are well-known bacteria involved in auxin biosynthesis (Glick 2012; Somers et al. 2005). *Agrobacterium* has Indole Acetic Acid gene in its T-DNA. It controls both the steps of IAA synthesis (Zhao 2010). One is the conversion of tryptophan to IAM while another is hydrolysis of IAM to IAA. Tryptophan independent pathway is also there for auxin synthesis (Woodward and Bartel 2005). Though there is no solid evidence of the presence of such genes in plants but in *A. brasilense, a* tryptophan independent pathway has been observed (Prinsen et al. 1993).

Though synthetic hormones have been used for this purpose time immemorial but the after effects and the high cost involved cannot be denied. Synthetic rooting products have also been used as regulators of plant growth to induce rooting in plants. But these products when probed into were found to be potent carcinogens. So, the hunt for a natural product was the main aim of agriculturists. After much of investigation, the researchers have found that the microbiome of the soil surrounding the plants can be very well used for inducing roots in the plants. The interplay of the microbes present in the soil can be studied and the mechanism of root induction can be tapped for inducing roots in the plants so that the plants are equipped with better nutrition absorption capacity and better grip in the soil.

Cultured Biologix, LLC, Colorado, United States, has come up with rooting products those utilize beneficial microbes to augment luxuriant growth of roots. The mechanism that they have adopted may be by shielding the plant from pathogens or extruding natural biochemicals which is instrumental as rooting agents. These produce signals that encourage the plant to generate larger and faster roots. This product is then paired with particular plant extracts obtained from soybeans and Aloe vera which are organic in nature and produce stimulants which signal for profuse rooting.

A specific product of Cultured Biologix, Dr. Root is a redimix of mycorrhizae and bacteria which cooperate with each other to produce endomycorrhizae and promote healthy root growth. In this magnificent product, spores of the bacteria and mycorrhizae are combined with a growth-inducing biochemical present in the cottonseed meal to provide enhanced sticking and coverage of the root system. It is also enriched with seaweed and humic acids which accounts for stimulation of microbial growth, and extracts of Aloe vera which is a rich source of organic compounds (Goudarzi et al. 2015). Voodoo Juice is considered to be ultra-premium, a patented combination of beneficial bacteria demonstrated to enhance root mass and crop yield. The bacterial combination in Voodoo Juice is shown in Table 2.3. The composition is used as biofertilizer.

The four different strains of bacteria in the Voodoo Juice work in synergism so as to develop a fine cannabis root hairs system that enhance the absorption of water and nutrients, inhabit the vascular systems of the plants, boosting nutrient transport into and within the crop, manufacture plant growth factors that enhance growth, solubilize nutrients and phosphates so that they become easily available for the plants. The best part of this juice is that it can be applied at all the year round in the life cycle of the plant. It works best during the first 2 weeks of both grow and bloom phases. The advantages of using Voodoo juice are immense like maximal-development of roots on the seedlings, transplants, and clones, roots have enhanced branching, root density, root mass, increased efficiency of nutrient intake which saves time and money and maximize growth and flowering. The favorable microbes break down old root material to provide nutrition to the plants. The microbes provide aeration so as to make way for the extra oxygen into the roots. They also sequester the atmospheric nitrogen and help make phosphorus more accessible to the plants (Gougoulias et al. 2014).

Tarantula, a product of Company called Advanced Nutrients comprises of a combination of about 11 strains of advantageous bacteria that boost lateral root development, produce natural growth cofactors and defend against harmful bacteria in the root zone. When Tarantula and Voodoo Juice are combined together, they provide a multifaceted variety of advantageous bacteria that maintain more vigorous root development increasing the transport of necessary nutrients into the plants and help fight plant disease caused by harmful pathogens and destructive bacteria strains. Some of the commercial bacterial mix for plant growth enhancement is given in Table 2.4.

Table 2.3 Synthetic fertilizer (Voodoo juice) containing bacterial colonies

Voodoo juice: Bacterial composition	Colony-forming unit/ml
Bacillus subtilis	10,000,000 cfu/ml
Bacillus megaterium	5,000,000 cfu/ml
Bacillus amyloliquefaciens	250,000 cfu/ml
Bacillus pumilus	5,000,000 cfu/ml
Bacillus licheniformis	10,000,000 cfu/ml
Bacillus mycoides	50,000 cfu/ml
Paenibacillus azotofixans	100,000 cfu/ml
Bacillus laterosporus	250,000 cfu/ml
Bacillus macerans	25,000 cfu/ml
Bacillus polymyxa	5,000,000 cfu/ml
Bacillus cereus	250,000 cfu/ml
Paenibacillus polymyxa	500,000 cfu/ml

Table 2.4 Commercially available advanced fertilizers and their function

Advanced fertilizers	Function
Tarantula	1. Shorter stems, more flowers, and have increased branching in plants
Voodoo juice	1. Maximum-development of roots on seedlings, transplants and clones with roots have enhanced branching, root density, root mass. 2. Enhanced efficiency of nutrient intake, saving time and money. 3. As well as maximize growth and flowering.
Nirvana	Stimulate root function, cell replication, and flower.
Piranha (fungi based)	1. Beneficial fungi maximize root absorption of oxygen, nutrients and water, also maximizes root growth for optimum yields. 2. Helps plant roots gain surface mass as well as maximizes production of floral essential oils.
Sensizyme organic	1. Strengthens the roots of the crops and keeps them clean, white and bright boosting the amount of bioavailable nutrition in your root zone, giving them more fuel to reach their full genetic potential as well as provides a broad spectrum of enzymes to more closely replicate the most fertile natural soil conditions.

2.9 Metabolic Potential of Endophytic Bacteria

Endophytic bacteria are directly involved in enhancing the metabolic potential of plants. However, endophytic bacteria also indirectly assist in the metabolism in plants. The bacterial endophytes robustly affect the performance, growth and stress tolerance of plants. *Actinobacterium*, *Pseudonocardia sp*. strain YIM 63111 are few endophytic bacteria which enhance the production of the anti-malarial compound artemisinin in its host plant *Artemisia annua* (Li et al. 2012). The endophytes induce secondary metabolite production in aromatic and medicinal plants. The endophytes sometimes not only enhance the secondary metabolite production but produced them along with the plants in combination.

One of the examples may be quoted where the flavor of strawberries is due to the furanoids which produce a typical fragrance. This unique feature is due to the plant-associated methylobacteria which actually influence the quality and quantity of the flavor (Verginer et al. 2010). The bilateral biosynthesis of the polyamine pavettamine of South African Rubiaceae in association with nodulating plants is one of the wonderful features (Brader et al. 2014). Nodulating plants devoid of bacteria do not produce pavettamine. The mangrove tree Kandelia *candel* having endophytic *Streptomyces* sp. HKI0595, produce multicyclic indolosesquiterpenes (Xu et al. 2014) while the endophytic actinomycete *Streptosporangium oxazolinicum* K07-0450^{T} associated with orchids produce antitrypanosomal alkaloids spoxazomicins A-C. The metabolites produced by the plant which associated with bacteria in roots and the rhizosphere is generally in low quantity. Future research in this area pertains to the enhancement of these metabolites. Direct analysis of metabolites in situ has been achieved for antibiotic lipopeptides from several *Bacillus subtilis* and for pyrrolnitrin, 2, 4-diacetylphloroglucinol and phenazine-1-carboxylic acid from *Pseudomonas fluorescens* strains (Thomashow et al. 1990).

2.10 Conclusion

The microbial population is one of the magnificent gifts from God which actually have a plethora of advantages for mankind. As there are two faces of the same coin these microbes may also be harmful as much, they prove to be beneficial. It is upon mankind as to how we want to utilize them. The microbes are adorned with so special features that on one side they can be used as bio-weapons and on the other hand they can serve as antibiotics to fight the different infections. Now we need to decide where we are going to use them. From the above review, we can well witness the bizarre of advantages the microbes bestow in the field of agriculture. Agriculture remains the backbone of primary sector and may guarantee nutritional security for the mankind. So, we need to expedite the technologies to enhance agriculture to get the maximum yield. There are numerous microorganisms which play a very crucial role in the life of plants in different ways. Each type of microbe is unique in its kind. Now if exploit these microbes in different permutation and combination we can really orchestrate them to produce maximum yield in the agricultural scenario.

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