Chapter 9
Bio-fertilizers: Eco-Friendly Approach for Plant and Soil Environment

Umair Riaz, Shahzada Munawar Mehdi, Shazia Iqbal, Hafiza Iqra Khalid, Ayesha Abdul Qadir, Wajiha Anum, Munir Ahmad, and Ghulam Murtaza

9.1 Introduction

The soil is a living entity because of the presence of a multitude microflora including actinomycetes (Bhatti et al. 2017), algae, bacteria, and fungi (Khanday et al. 2016; Bhat et al. 2017; Sofi et al. 2017). According to an estimate, about 1×10^8 microorganisms exist in 1 g of soil. Majority of these microorganisms are beneficial for agriculture. Some of the organisms are harmful; however, they are very low in number. It has been reported that only 5–7% of soil microorganisms are harmful (Chowdhury and Mukherjee 2006). Soil degradation is the major limitation in achieving higher crop yields in the developing world, especially among farmers with poor resources (Khosro and Yousef 2012). The extensive and imbalanced utilization of pesticides and chemical fertilizers to enhance the crop production has resulted in various social, environmental, and economic concerns (Santos et al. 2012). Chemical fertilizers are technically based materials which consist of known amounts of macro- and micronutrients. The injudicious application of these fertilizers no doubt has improved the crop yield especially in developing countries but

U. Riaz (✉)
Soil and Water Testing Laboratory for Research, Bahawalpur, Pakistan

S. M. Mehdi
Rapid Soil Fertility, Survey & Soil Testing Institute-Punjab, Lahore, Pakistan

S. Iqbal · H. I. Khalid · A. A. Qadir · G. Murtaza
Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

W. Anum
Department of Agronomy, Regional Agricultural Research Institute (RARI), Bahawalpur, Pakistan

M. Ahmad
Soil Science Department, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia

K. R. Hakeem et al. (eds.), *Bioremediation and Biotechnology*,
https://doi.org/10.1007/978-3-030-35691-0_9

also has induced adverse effects on the ecosystem including the contamination of atmosphere and soil and groundwater and increased disease attack by weakening the plant's roots (Chun-Li et al. 2014). Hence, new fertilization strategies with lower cost, more efficiency, and eco-friendly properties are required.

Bio-fertilizers can potentially participate for sustainable agriculture and environment. Recently, the efforts have been made to develop nutrient-rich fertilizer with high quality, called bio-fertilizer, to certify bio-safety. Bio-fertilizer has been known as a substitute for commercial inorganic fertilizer in order to upsurge crop yield by increasing soil fertility in sustainable agriculture. These potential biological fertilizers are eco-friendly as they keep the environment safe and also act as cost-effective agricultural inputs (Khosro and Yousef 2012; Adesemoye and Kloepper 2009).

Bio-fertilizers have arisen as a promising strategy for better nutrient supply in agriculture in recent years. Our whole agriculture is dependent on microbial activities in many ways. A great potential appears for making the use of microbes in enhancing crop yield (Bloemberg et al. 2000).

The term "bio-fertilizer" is defined as "materials consisting of live or cells of effective strains of phosphate-solubilizing, nitrogen-fixing, or cellulolytic microorganism used for seed, soil, or composting area application, for increasing microbial number and to hasten the microbial process which supplements the nutrients that can be simply acquired by plants". The application of bio-fertilizers as soil or seed inoculation multiplies and participates in nutrient cycling and then increases crop productivity (Adesemoye and Kloepper 2009).

9.2 Difference Between Bio-fertilizers, PGPR, and Organic Fertilizers

Though a big difference exists among bio-fertilizer and organic fertilizer, bio-fertilizers have been termed as the organic fertilizer earlier. Bio-fertilizers are microbial inoculants comprising of live cells of microbes like algae, bacteria, and fungi, separately or in combination, which may benefit the crop by increasing productivity, while the organic fertilizers are obtained from or consist of plant sources (green manure) or animal sources (animal manure). Plant growth-promoting rhizobacteria (PGPRs) are microorganisms which make the association with a host plant and enhance the growth of their host (Vessey 2003). However, all the PGPRs cannot be termed as bio-fertilizers. For instance, the bacteria that improve plant growth through the control of harmful organism are termed as biopesticides, but they are not bio-fertilizers. However, some PGPR can improve the growth of plants by working as both biopesticides and bio-fertilizer. For example, *Burkholderia cepacia* strains can stimulate the growth of maize via siderophore production under the low iron condition and also possess biocontrol ability to *Fusarium* sp. (Bevivino et al. 1998). Bio-fertilizers duty comprise of a living cell which enhances the plant growth through enhanced nutrient availability.

9.3 History of Bio-fertilizer

The application of bio-fertilizers in agriculture has begun a long time ago. The acquaintance about microbial inoculum application and its benefits passes from generation to generation in the long history of farmers. The concept of bio-fertilizer emerges from the production of compost on a small scale (Khosro and Yousef 2012; Halim 2009). In this process, microbial culture hastens the decomposition process of agricultural by-products and organic residues and gives healthy crops to harvest (Halim 2009). Beneficial bacterial inoculation with plants can be happening for centuries. Though bacteria were not discovered until 1683, when Von Leeuwenhoek noticed microscopic "animals," the utilization of these bacteria for plant growth stimulation in agriculture has been done since ancient times. Theophrastus (372–287 BC) proposed different soil mixing for the remediation of soil defects (Vessey 2003). From this practice, farmers noticed that application of soil collected from legumes boosted the crop yield, while the application of soil taken from non-legume crops did not affect the crop yield. In the last decades of the nineteenth century, the practice of seed mixing with "naturally inoculated" soil became an endorsed technique of legume inoculation in the USA (Nobbe and Hiltner 1986). In the 1930s, *Bacillus megaterium* was used on a large scale for phosphate solubilization in Eastern Europe. In the 1930s and 1940s, inoculation of legumes with associative, nonsymbiotic, rhizospheric bacteria, like *Azotobacter*, was done on a large scale in Russia (Amutha 2011). Bio-fertilizer's commercial history started with the launch of "Nitragin," a laboratory *rhizobia* culture, by Nobbe and Hiltner in 1895 (Kribacho 2010). In the USA, *Rhizobium* inoculant was first prepared and marketed by the private sector in the 1930s (Smith 1992).

After *rhizobia*, *Azotobacter* was discovered followed by blue-green algae (Kribacho 2010). Vesicular-arbuscular mycorrhizae (VAM) and *Azospirillum* are discovered recently (Rana and Ramesh 2013). In the late 1960s in India, the production of rhizobial inoculum was firstly commenced at IARI, New Delhi, in 1956 (Amutha 2011). In Malaysia, production of microbial inoculants on an industrial scale began at the end of the 1940s. Picking up was started in the 1970s by taking legumes-*Bradyrhizobium* inoculation as a guide. The Malaysian Rubber Board (MRB), a government research institute, has conducted research on young rubber trees in the large plantation by the application of *Rhizobium* inoculums. Bio-fertilizers are generally made as inoculants (carrier based), having active microorganisms (Vessey 2003).

9.4 Mechanisms of Action of Bio-fertilizers

Bio-fertilizers have attracted a significant attention of the researchers in last few years due to their role in improving crop yields, reducing the chemical fertilizers cost, and being less detrimental to the environment (Khan et al. 2010). Bio-fertilizers can stimulate the plant growth either through direct or indirect mechanisms.

Direct mechanism affects the activity of plant growth directly; however these direct ways differ between strains and species. These mechanisms include nitrogen fixation, phosphate solubilization, phytohormones production (auxin, cytokinins, ethylene, gibberellic acid, and abscisic acid), and increasing iron availability through siderophore production. Direct improvement of nutrient uptake has been testified due to increasing influxes of specific ions at the root surface when biofertilizers were applied (Bertrand et al. 2000). Several rhizobacterial genera, e.g., *Agrobacterium*, *Azospirillum*, *Paenibacillus polymyxa*, *Pseudomonas*, and *Erwinia*, are known to produce auxins. *Bacillus* and *Rhizobium* were also found to produce auxin at a different temperature and pH (Ju et al. 2018; Ansari et al. 2013). For instance, many bacteria have established iron uptake systems through siderophore production (DalCorso et al. 2013; Saha et al. 2013; Kundan et al. 2015). Iron is not easily accessible for the plant uptake as it exists as very low-soluble ferric ions (Ganz 2013; Saha et al. 2013; Kundan et al. 2015). Therefore, the microbial siderophores scavenge the iron from minerals by Fe^{3+}complex formation, which is soluble and is taken up by active transport mechanisms. This mechanism is active only under low iron solubility (Saha et al. 2013; Kundan et al. 2015).

Indirect mechanisms refer to the inhibition of the functioning of pathogenic organisms of plants. Indirect mechanisms comprise the production of degrading enzymes, ACC deaminase, induced systemic resistance, antibiotics, competition, hydrogen cyanide, quorum quenching, and siderophore production (Balogh et al. 2010; Frampton et al. 2012).

9.4.1 Nitrogen Fixation

Fixation of atmospheric nitrogen into useable nitrogen that is then converted to ammonia is called nitrogen fixation. Biological nitrogen fixation usually occurs at slight temperatures by nitrogen-fixing microorganisms (Bakulin et al. 2007). Rhizobial bacteria lead to root nodule formation by initiating a series of reactions (Gage 2004). In the root nodule, the bacteria do not contain a cell wall (bacteroid). They fix atmospheric nitrogen by the action of an enzyme called nitrogenase enzyme and then produce ammonia (Olanrewaju et al. 2017). Figure 9.1 shows the nitrogen fixation mechanism.

This biological fixation occurs in a nitrogenase complex, which is a complex enzyme. The Nitrogenase complex is explained as a metalloenzyme consisting of two components: (1) dinitrogenase, which consists of a metal cofactor, and (2) dinitrogenase reductase, which is an iron protein. Dinitrogenase reductase supplies high reducing power electrons, while dinitrogenase uses these electrons to reduce N_2 to NH_3. This process utilizes a large amount of energy, necessitating 16 ATP moles for 1 mole nitrogen reduction. For more ATP production, microbial carbon is allocated to oxidative phosphorylation, rather than storing energy in the form of glycogen through the synthesis of glycogen synthesis. An oxygen-sensitive gene, nitrogenase gene (nif), is required for this process. The nif genes also activate

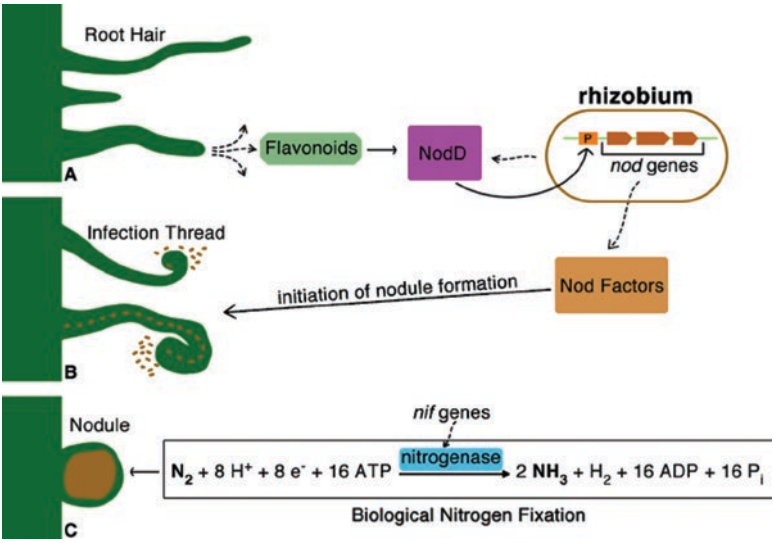

Fig. 9.1 Mechanism of biological nitrogen fixation (*Source*: www.Googleimages.com)

molybdenum, iron, protein, and many other regulatory genes. This gene prevents oxygen from inhibiting the nitrogen fixation and simultaneously supplying enough oxygen for bacteroide respiration inside the nodule. To bind free oxygen, bacterial hemoglobin is introduced (Kundan et al. 2015).

9.4.2 Phosphate Solubilization

Figure 9.2 shows the mechanism of phosphate solubilization.

The main mechanism of phosphate solubilization involves the use of chemicals such as organic acids, siderophores, hydroxyl ion, carbon dioxide, and protons (Rodríguez and Fraga 1999). Organic acids with hydroxyl and carboxyl ions either reduce the pH or make chelates with cations and release the phosphates in a plant-available form (Khosro 2012; Sharma et al. 2017). Organic acid converts tricalcium phosphate to dibasic and monobasic phosphates, and this process boosts phosphorus bioavailability. The type and amounts of organic acid vary with different organisms. Aliphatic acids are more efficient in P solubilization comparative to fumaric acid, citric acids, and phenolics. Tri- and dicarboxylic acids are more efficient compared to aromatic acids and monocarboxylates (Mahdi et al. 2010a). Gaseous "O_2/CO_2" exchange, the release of proton and bicarbonate, lowered pH of the medium (Sharma et al. 2017). Thus, phosphorus availability and rhizosphere pH are inversely related (Olanrewaju et al. 2017).

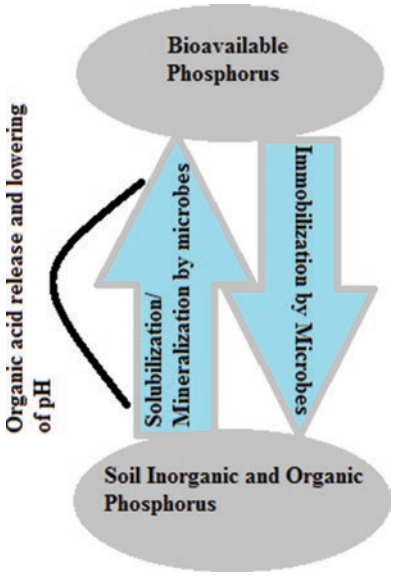

Fig. 9.2 Mechanism of phosphate solubilization by microbes

9.4.3 Zinc Solubilization

The zinc-solubilizing bio-fertilizers acts by secreting organic acids. These organic acids replace the zinc on insoluble chelated compounds and make it accessible for plant uptake (Mahdi et al. 2010b).

9.4.4 Potassium Solubilization

The potassium-solubilizing bio-fertilizers containing potassium-solubilizing microorganisms solubilize silicates through organic acid production and release in the rhizosphere. These organic acids provide H^+ ions and activate hydrolysis. Organic acids such as hydroxyl, carboxylic acids, oxalic acid, citric acid, and keto acids promote the removal of silicates from the cationic complexes into a free or dissolved state. This breakdown of potassium silicate complex also released potassium in the plant-available form (Ju et al. 2018). Figure 9.3 shows the mechanism of potassium and silicate solubilization.

9.4.5 Silicate Solubilization

Some microbial metabolisms produce several organic acids. These organic acids have a double role in weathering of silicate minerals. Organic acids provide H^+ ions and activate hydrolysis. Organic acids such as hydroxyl, carboxylic acids, oxalic

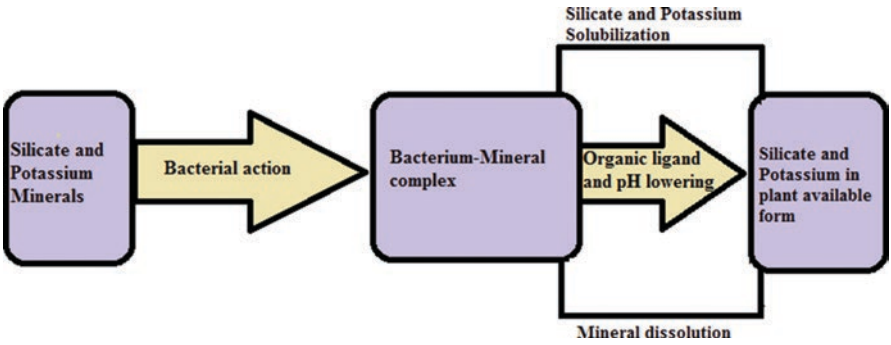

Fig. 9.3 Schematic diagram of silicate and potassium solubilization

acid, citric acid, and keto acids promote the removal of silicates from the cationic complexes into free or dissolved state and then help in silicate retention in the dissolved state in a medium (Rana and Ramesh 2013).

9.4.6 Sulfur Oxidation

Plants uptake the sulfur in the form of sulfates. Sulfur-oxidizing microbes oxidize the sulfur to sulfates (Ju et al. 2018).

9.5 Production of Bio-fertilizer

Several factors are needed to be considered in the production of bio-fertilizers including growth profile of microbes, formulation of inoculum, types, and optimum conditions of the organism. The inoculum formulation, application method, and product storage are all critical for the accomplishment of a biological product. Generally, six stages are involved in the production of a bio-fertilizer, i.e., (1) selection of active microbes, (2) isolation of target microbes, (3) carrier material selection, (4) selection of propagation method, (5) phenotype testing, and (6) large scale tests. In the first step, the selection of either nitrogen fixer or organic acid bacteria is made, and the isolation of target microbes is done. Next, the isolated organism is streaked on petri dishes. Selection of right carrier material is of critical importance. For powder bio-fertilizer production, peat or tapioca flour is the best carrier material. Microbial culture from petri dishes is transferred into small flasks. In case of large-scale bio-fertilizer production, it is transferred into the fermenter. At the last stage, large-scale testing in a different environment is performed, and its limitations and effectiveness are analyzed (Khosro and Yousef 2012).

9.6 Biochemistry of Bio-fertilizer Production

Anaerobic bio-digestion is the microbial breakdown of biodegradable materials in anaerobic conditions (Ezigbo 2005; Kim et al. 2010). Figure 9.4 shows the process of bio-fertilizer production.

Anaerobic bio-digestion systems can be classified on different categories.

According to the temperature of operation:

1. Mesophilic systems (i.e., 20–40 °C).
2. Thermophilic systems (i.e., 45–70 °C) (Lettinga 1995).

According to the total suspended solid concentration:

1. Dry systems (between 20 and 40% of total solids).
2. Wet systems (dry matter content of approximately 10%) (Braber 1995).

According to the number of stages considered:

1. Single stage.
2. Multistage processes (Vandevivere et al. 2003).

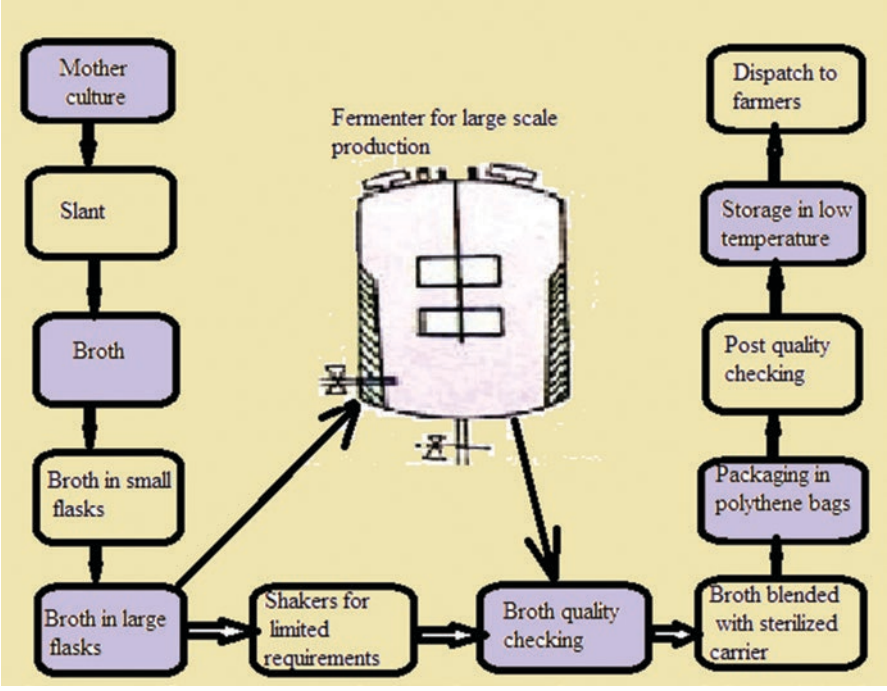

Fig. 9.4 Schematic diagram of bio-fertilizer production

Three biochemical steps are involved in bio-fertilizer preparation that consist of breaking down of complex substances into simpler ones in anaerobic digestion process. Four main stages and three major bacterial groups can be considered in order to simplify the AD process.

9.6.1 Hydrolysis

Hydrolysis is the first step in anaerobic digestion process, in which complex compounds are passed through the cell membrane and then hydrolyzed to monomer compounds (long-chain fatty acids, amino acids, and sugars) through the controlled extracellular enzymes actions, emitted by fermentative bacteria (Ponsá et al. 2008). It is a rate-limiting step for this process. Many groups of anaerobic bacteria take part in this step such as clostridia and bactericides. Some facultative bacteria also take part in this process, e.g., streptococci, etc. (Christy et al. 2014). This is an important step because microorganisms release enzymes to break down large molecules into smaller ones as they cannot use large molecules directly as their food. Extracellular enzymes "cut" the larger compounds into smaller molecules that the microorganism then engulfs and use as nutrition and energy source. Different types of extracellular enzymes are secreted by microorganisms to complete biodegradation and break down a variety of organic materials. Some microorganisms are specific, and they secrete specific enzymes for a specific function. For example, saccharolytic microorganisms secrete enzymes that biodegrade only different sugars; proteolytic microorganism biodegrades only proteins. For biodegradation of proteins, sugars, and fats, different enzymes are secreted (Schnurer and Jarvis 2009). Table 9.1 shows some extracellular enzymes. The rate of hydrolysis reaction varies with the nature of the substrate. Protein decomposition rate is usually faster than cellulose and hemicellulose transformation (Schnurer and Jarvis 2009).

Table 9.1 Some important enzymes, their substrates, and breakdown products (Schnurer and Jarvis 2009)

Enzymes	Substrate	Breakdown products
Cellulase	Cellulose	Cellobiose and glucose
Proteinase	Proteins	Amino acids
Amylase	Starch	Glucose
Lipase	Fats	Glycerol and fatty acids
Hemicellulase	Hemicellulose	Sugars, such as mannose, glucose, xylose, and arabinose
Pectinase	Pectin	Sugars, such as galactose, polygalacturonic, and arabinose acid

9.6.2 A Fermentative Step (Acidogenesis)

In this step, the organic compounds formed in the hydrolytic phase are converted into short-chain volatile fatty acids (VFAs) such as acetic acids, butyric acids, alcohols, carbon dioxide, and hydrogen. Hydrogen is formed as an intermediate product, and it affects the composition of the final product. If hydrogen partial pressure is too low, it would increase the concentration of reduced compounds. Usually, fatty acids, simple sugars, and amino acids are changed into alcohols and organic acids during this phase (Chandra et al. 2012; Gerardi 2003).

9.6.3 Acetogenesis

In this step, acetic acid, hydrogen, and carbon dioxide are produced by the degradation of fatty acids, aromatic compounds, and alcohols (Al Seadi et al. 2008). These acetic acid, hydrogen, and carbon dioxide are used as substrates by the microorganisms active in this phase and carried out anaerobic oxidation (Aslanzadeh 2014). The collaboration of anaerobic oxidation microorganisms is with the methane-forming microorganisms and with the next group. This type of collaboration is dependent on hydrogen partial pressure present in the system (Schnurer and Jarvis 2009; Chandra et al. 2012). When products are transformed into methane, some are converted into volatile fatty acids, alcohols, and methanogenic substrates. Volatile fatty acids with more than 1 unit carbon chain are oxidized to hydrogen and acetate (Al Seadi et al.2008). During the production of hydrogen, protons act as the final electron acceptors, and symbiotic relationship interspecies hydrogen transference happens. Partial pressure plays an important role in this process. Oxidation reactions occur only at low hydrogen partial pressure, explaining the importance of collaboration with the methanogens since they will incessantly utilize hydrogen, to produce methane (Chandra et al. 2012).

9.6.4 Methanogenesis

It is the final, critical (Al Seadi et al. 2008), and rate-limiting biochemical step of the whole anaerobic digestion process. In this step, carbon dioxide and methane are produced by the use of intermediate products through the action of methanogenic bacteria under stern anaerobic conditions (Aslanzadeh 2014).

9.7 Bio-fertilizer Classification

Bio-fertilizers are categorized on the basis of microorganisms' type. Table 9.2 displays the organization of bio-fertilizers.

Table 9.2 Classification of bio-fertilizers

Bio-fertilizer groups		Examples
Nitrogen-fixing bio-fertilizers	Free-living	*Azotobacter*, *Anabaena*, *Acetobacter*, *Beijerinckia, Clostridium*, *Klebsiella*, *Nostoc*
	Symbiotic	*Rhizobium (legume)*, *Frankia (non- legume)*, *Anabaena azollae*
	Associative symbiotic	*Azospirillum* sp.
	Fungi	*Penicillium* sp., *Aspergillus awamori*
Phosphate-solubilizing bio-fertilizers	Bacteria	*Bacillus* sp., *Pseudomonas* sp., *Phosphaticum*, *Burkholderia*, *Micrococcus*, *Rhizobium*, *Agrobacterium*, *Achromobacter*, *Aerobacter*, *Flavobacterium*, *Erwinia*
	Fungi	*Aspergillus awamori*, *Penicillium*
Phosphate-mobilizing bio-fertilizers	Arbuscular mycorrhizal fungi	*Glomus*, *Gigaspora*, *Scutellospora* sp., *Acaulospora* sp.
	Ectomycorrhiza	*Laccaria* sp., *Pisolithus* sp., *Boletus* sp., *Amanita* sp.,
	Ericoid mycorrhiza	*Pezizella ericae*
Potassium-solubilizing bio-fertilizers		*Bacillus* sp., *Aspergillus niger*
Silicate-solubilizing bio-fertilizers		*Bacillus* sp., *Bacillus circulans*, *Bacillus mucilaginous*
Zinc-solubilizing bio-fertilizers		*Bacillus* sp., *Pseudomonas* sp., *Acinetobacter, Enterobacter*, *Flavobacterium*, *Serratia*, *Gluconacetobacter*, *Burkholderia*, *Saccharomyces* sp.
Sulfur-oxidizing bio-fertilizers		*Thiobacillus* sp.
Organic matter decomposer bio-fertilizers	Cellulolytic	*Cellulomonas*, *Trichoderma*
	Lignolytic	*Arthrobacter*, *Agaricus*
Plant growth-promoting rhizobacteria ((PGPR)		*Pseudomonas* sp.

9.7.1 Nitrogen Fixing Bio-fertilizers

Nitrogen is an important macronutrient for crop growth purpose. It is present in the atmosphere in a free state. The part of this nitrogen bargains its entry into the soil by fixation that is performed by a special group of microorganisms. This process is called biological nitrogen fixation, and microorganisms that perform this function are called nitrogen fixer or nitrogen-fixing microorganisms. In this process, the nitrogen is converted into a form that is plant usable (Gothwal et al. 2007). Nitrogen fixer microorganisms are used as bio-fertilizer which is able to fix atmospheric nitrogen to meet plants' need of nitrogen. They are grouped into symbionts such as *Azolla*, *Frankia*, and *Rhizobium*; free-living, *Azospirillum* and *Azotobacter*; and the

blue-green algae (Gupta 2004). Some species of nitrogen-fixing microorganisms are shown in Fig. 9.5. Though many genera of nitrogen-fixing microorganisms are reported, only *Azospirillum* and *Azotobacter* have been verified to improve the yield of legumes and cereals under field condition. *Rhizobium* spp., which can fix the atmospheric nitrogen and are mainly associated with legumes, were the first recognized bio-fertilizer and have been commercially used for legumes for more than 100 years (Kannaiyan 2002).

El-Komy (2005) confirmed the advantageous effect of *Bacillus megaterium* and *Azospirillum lipoferum* co-inoculation for improving wheat plant nutrition of nitrogen and phosphorus. The bacterial mixture inoculation gave more balanced nutrition to plants. Improvement in nitrogen and phosphorus uptake by root was the chief mechanism of plants-bacterial interaction.

9.7.2 Phosphate-Solubilizing Bio-fertilizer

Phosphorus is classified as organic P and inorganic P in soil. However, only a little of total P (0.1% or 1 ppm) is available for plants due to low solubility and high soil P-adsorbing capacities. Plants absorb P as anions of phosphate (HPO_4^{-2} or $H_2PO_4^{-}$) from the soil solution, but these phosphate anions are reactive and become inaccessible for plants. When P fertilizers are applied in soil, they often become intricate due to the complex formation with aluminum and iron in low pH soils (Dorahy et al.

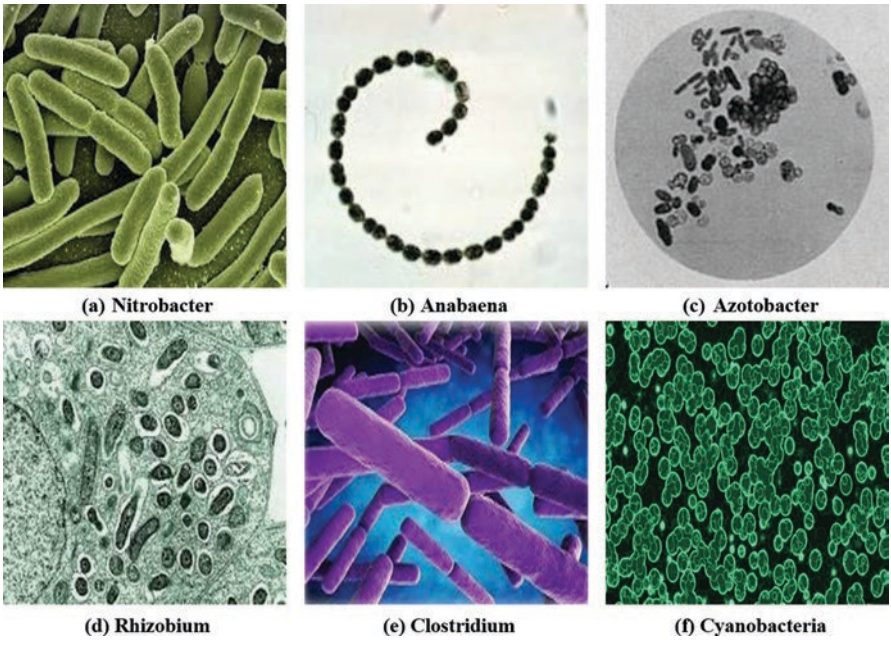

Fig. 9.5 Nitrogen-fixing microorganisms

2005), fixation with calcium and magnesium in high pH soil, and precipitation (Mittal et al. 2008). The overall P utilization efficiency is lower than optimum P utilization efficiency in Pakistani soils (Vance 2001).

Phosphate-solubilizing bio-fertilizers (PSB) contain microorganisms that solubilize the fixed phosphate and make it bioavailable. Many soil fungi and bacteria have the competency to transform insoluble phosphates into soluble forms. This process is accomplished by the excretion of organic acids in the rhizosphere by these organisms. These organic acids decline the soil pH and cause the dissolution of phosphate complexes and make them available to plants (Gupta 2004). Several bacterial species have been found with phosphate-solubilizing ability. These solubilize inorganic phosphate compounds, such as rock phosphate, hydroxyapatite, dicalcium phosphate, and tricalcium phosphate. The more common genera of soil bacteria are *Bacillus* and *Pseudomonas* and fungi. Among other bacterial genera, *Burkholderia*, *Micrococcus*, *Rhizobium*, *Agrobacterium*, *Achromobacter*, *Aerobacter*, *Flavobacterium*, and *Erwinia* are P solubilizer (Subbarao 1988). *Arthrobotrys oligospora*, a nematode fungus, also can solubilize the rock phosphate (Duponnois et al. 2006). The fungus is less effective compared to bacteria in phosphorus solubilization (Alam et al. 2002). Phosphate-solubilizing bacteria exist in large numbers in plant and in the rhizosphere. These bacteria are both aerobic and anaerobic, but aerobic strains are usually found in submerged soils (Raghu and Macrae 2000). Examples include *Bacillus* spp., *Pseudomonas* sp., and *Aspergillus* sp. (Ju et al. 2018) (Fig. 9.6).

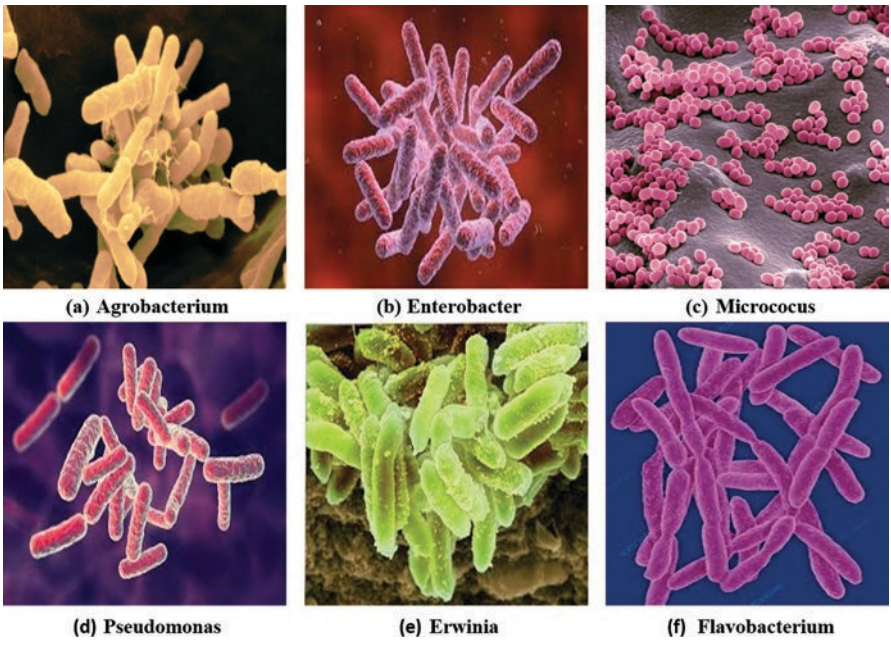

Fig. 9.6 Phosphorus-solubilizing microorganisms

9.7.3 Phosphate-Mobilizing Bio-fertilizers

Phosphate-mobilizing bio-fertilizers work by foraging the soil phosphates and mobilizing the insoluble phosphorus compounds in the soil. Phosphate-solubilizing bio-fertilizer is broad spectrum and also mobilizes the phosphate sometimes (Chang and Yang 2009). Examples are mycorrhiza (Ju et al. 2018).

Mycorrhizae form a symbiotic association with plants. In this association, the fungal partner is penetrated in the root cell and fulfills its carbon necessities from the plant, and in return, the plant is helped by surplus nutrient supply especially phosphorus, copper, calcium, zinc, etc. (Sadhana 2014).

9.7.4 Zinc-Solubilizing Bio-fertilizer

Many nitrogen fixers and phosphorus solubilizer are well accepted as bio-fertilizers nowadays (Subba 2001), but these provide only macronutrients. Soils are also deficient in many micronutrients. The most important of which is zinc because of its low availability. Out of the total, about 75% of applied zinc gets fixed (residual and crystalline iron oxide-bound zinc), and only 1–4%, of totally applied zinc, is used by the plants. Zinc gets fixed by either forming complex by organic ligand or by means of chemisorptions (Alloway 2008). This fixed zinc can be made available by the action of microorganisms such as *Saccharomyces* sp., *B. subtilis*, and *Thiobacillus thiooxidans.* These zinc-solubilizing microorganisms can be used as bio-fertilizers (Raj 2007). In a study, it was recommended that *Bacillus* sp. can be used for increasing zinc availability either alone or in combination with zinc compounds such as zinc carbonate, zinc sulfide, and zinc oxide that are insoluble and cheaper than zinc sulfate (Mahdi et al. 2010b).

9.7.5 Potassium-Solubilizing Bio-fertilizer

These are broad-spectrum bio-fertilizers. Potassium is mostly found in insoluble silicate mineral compounds in the soil. These mineral compounds are unavailable to plants. Only through weathering or solubilization process, these minerals are made accessible for plant uptake (Ju et al. 2018).

9.7.6 Potassium-Mobilizing Bio-fertilizer

These bio-fertilizers mobilize the potassium-unavailable form (bound to silicate minerals). Many phosphate-solubilizing bio-fertilizers such as *Aspergillus* sp. and *Bacillus* sp. carried out phosphate solubilization as well as potassium mobilization (Ju et al. 2018).

9.7.7 Silicate-Solubilizing Bio-fertilizers

Silicate is found in soils as silicate minerals that are unavailable. Many microbes produce several organic acids for converting silicon into an available form (Rana and Ramesh 2013).

9.7.8 Sulfur-Oxidizing Bio-fertilizer

The *Thiobacillus* sp. is a good example of the sulfur-oxidizing microorganism (Ju et al. 2018); commercial bio-fertilizer: Sulfogreen, Sulphomex.

9.7.9 Plant Growth-Promoting Bio-fertilizer (PGPB)

Plant growth-promoting bio-fertilizers are crop specific bio-fertilizers. They produce anti-metabolites and hormones and improve root growth and hasten the process of organic matter decomposition. This decomposition process helps in mineralization and increases the bioavailability of nutrients (Bhattacharyya and Jha 2012). Examples are *Pseudomonas* spp.

9.7.10 Liquid Bio-fertilizers

Liquid bio-fertilizers are usually defined as a "suspensions having agriculturally useful microorganisms." *It is more advantageous than* the carrier inoculants. Liquid bio-fertilizers consisting of microorganisms, such as phosphobacteria *Rhizobium* and *Azospirillum*, are now been used effectively for horticulture crops, vegetables, pulses, sugarcane, rice, millets, and cotton. The reasons behind the increasing use of liquid bio-fertilizers over conventional carrier-based bio-fertilizers are higher competition potentials with native population, quick and easy quality control protocols, longer shelf life (12–24 months), higher populations can be sustained, properties remained unchanged during storage up to 45 °C, more tolerant to temperature, typical fermented smell helps in its easy identification, easy to produce and use for farmers, no contamination, very high enzymatic activity in the meantime contamination is zero, high potential for export, can compete in the global market because of organic crop production, improved soil and seeds survival, their dosages are ten times less than carrier-based powder bio-fertilizers, and cuts the chemical fertilizer use by 15–40% (Rana and Ramesh 2013).

Commercial bio-fertilizer, chitosan concentrate, bass liquid potash, *Azospirillum* bio-fertilizer, liquid consortia bio-fertilizer, potash-mobilizing bio-fertilizer, phosphate-solubilizing bio-fertilizer, etc.

9.7.11 Composting

Compost is used in agriculture as well as in landscaping, as a fertilizer and soil conditioner. Compost is a decomposed remnant of organic matter in the presence of oxygen. This compost making process is called composting. Composting is a biological decomposition of organic waste material in the presence of oxygen at an elevated temperature, carried out by active microorganisms which break down the cellulolytic material. Factors that affect this process include pH, temperature, particle size, oxygen levels, nutrient levels, number, and species of microorganisms (Riaz et al. 2018). Compost is advantageous over chemical fertilizers because of its many useful functions that include means of land reclamation, controls of soil erosion, provides nutrients and support to crops by serving as an absorbent, porous, growing medium and retains soluble mineral and moisture, protects against chemical fertilizers by acting as a buffer, and causes easier till of heavy soils (Somani n.d. www.agriinfo.in).

9.8 Characteristics of Some Microbes Used as Bio-fertilizers

9.8.1 Rhizobium

It has its place in the family *Rhizobiaceae* and forms symbiotic relations (Mahdi et al. 2010a). *Rhizobium* is known to fix atmospheric nitrogen in legumes (Gupta 2004). Rhizobia are special bacteria that live either in the soil or in nodules, formed on the roots especially legumes. The *Rhizobium* colony is whitish, slightly transparent, fast growing, water-soaked, and shiny in nature (Somani n.d. www.agriinfo.in). They can fix nitrogen at the rate of 50–100 kg ha^{-1} with only legumes. It is associated with pulse legumes, red-gram, chickpea, pea, black gram, and lentil; oilseed legumes, groundnut and soybean; and forage legumes, lucerne and berseem. It inhabits on the roots of the legumes and forms root nodules (tumor-like growths), which act as ammonia production factories (Mahdi et al. 2010a).

9.8.2 Azotobacter

Azotobacter belongs to family *Azotobacteriaceae*, is Gram-negative, and is a free-living, aerobic soil-dwelling, heterotrophic nitrogen-fixing bacterium, used as a biofertilizer in most crops (Mahdi et al. 2010a). They range from 2 to 10 μm long and 1 to 2 μm wide in size (Somani n.d. www.agriinfo.in). *Azotobacter* are present in neutral and alkaline soils. Most commonly occurring species of *Azotobacter* is *A. chroococcum* in arable soils (Rana and Ramesh 2013). Other reported species are *A. beijerinckii*, *A. insignis*, *A. macrocytogenes*, and *A. vinelandii* (Subba 2001). *A. chroococcum* is capable of fixing N_2 (2–15 mg N_2 fixed/g of carbon) in culture

media. The proliferation of *Azotobacter* is limited by a lack of organic matter (Rana and Ramesh 2013). The *Azotobacter* number hardly exceeds 105 g^{-1} of soil because of the presence of antagonistic microorganisms and lack of organic matter in the soil (Subba 2001). A plant requires nitrogen for its growth, and *Azotobacter* performs nonsymbiotic nitrogen fixation (Somani n.d. www.agriinfo.in). *Azotobacter* is reported in many crops such as sugarcane, rice, bajra, maize, and vegetables (Arun 2007).

9.8.3 *Azospirillum*

Azospirillum belongs to family *Spirilaceae* (Mahdi et al. 2010a) and is a Gram-negative, heterotrophic, motile bacterium and is associated with roots of monocots (Somani n.d. www.agriinfo.in). It lives inside plant roots and does not form root nodules (Rana and Ramesh 2013). *A. brasilense* and *A. lipoferum* are most widely distributed and most beneficial species of this genus. Other species are *A. amazonense*, *A. halopraeferens*, and *A. brasilense* (Mahdi et al. 2010a). The organism multiplies under both aerobic and anaerobic environment. It stimulates the phytohormone production, drought tolerance, and disease resistance. It can fix the substantial amount of nitrogen (20–40 kg N/ha) in non-leguminous plants' rhizosphere such as oilseeds, cereals, cotton, millets, etc. (Rana and Ramesh 2013). The *Azospirillum* forms symbiotic association with C4 plants because they grow and fix nitrogen on salts of organic acids such as aspartic and malic acid (Arun 2007). Thus, it is recommended mainly for maize, sugarcane, sorghum, pearl millet, etc. (Mahdi et al. 2010a).

9.8.4 *Acetobacter*

It is an endotrophic, symbiotic bacteria with the ability of atmospheric nitrogen fixation. It is capable of living inside the sugar plant tissues. It needs high sugar levels that are available in sugarcane tissues. Usage of Acetobacter on a large scale increases crop production (Somani n.d. www.agriinfo.in).

9.8.5 *Beijerinckia*

Beijerinckia is an aerobic, nonsymbiotic free-living, and slow-growing bacteria. The *Beijerinckia* colonies are wrinkled, round, flat, and raised in shape. These microorganisms reside in the rhizosphere of crops and fix the atmospheric nitrogen in acid soil (pH 3.0–4.0). It is commonly used for monocots and applied at 250 g per 10 kg of seeds (Somani n.d. www.agriinfo.in).

9.8.6 *Azolla*

Azolla (*Azolla pinnata*) is an aquatic weed found in shallow ditches, tank, idle pond, and channels. It is found floating on the water surface through small and closely overlapped scale-like leaves and through hanging roots deep in the water. *Azolla* is usually associated with rice cultivation in many countries, for example, the Philippines, Vietnam, Thailand, and China. Azolla bio-fertilizers increased the yield of rice in many experiments. It is known to contribute to 40–60 kg N/ha per rice crop (Rana and Ramesh 2013). Azolla also forms a symbiotic association with blue-green algae (*Anabaena azollae*). They both are applied as co-inoculation. This symbiotic association of *Azolla pinnata* and *Anabaena azollae* is termed as *AZOLLA-ANABAENA COMPLEX*. In this association, blue-green algae fix atmospheric nitrogen for *Azolla*, and *Azolla* provides food and shelter to the algae in return. This ability gives this association a great potential as bio-fertilizer for the agricultural field. It can serve as an alternative fertilizer to chemical nitrogenous fertilizers. It is reported that Azolla application increased rice yields by 0.5–2 t/ha in a field trial (Gupta 2004).

9.8.7 *Cyanobacteria*

Cyanobacteria are symbiotic, free-living, aquatic, and one-celled to many-celled and are red, brown, or purple in color. They cannot live in acidic conditions (Rana and Ramesh 2013). They form a symbiotic association with ferns, fungi, liverworts, and plants, but the most common symbiotic association is formed by *Anabaena azollae* with the ability of nitrogen fixation (Mahdi et al. 2010a). This is only used in paddy fields. BGA bio-fertilizers are applied by a broadcast method in standing water as an algal mass in a paddy field after 1 week of transplantation (Somani n.d. www.agriinfo.in).

9.8.8 *Mycorrhizae*

Mycorrhiza signifies “fungus roots.” Among fungi, arbuscular mycorrhizal (AM) fungi are more abundant and account for 5–50% of soil microbe’s biomass. Out of 150 species of fungi in class *Zygomycetes*, order *Glomales*, an insignificant magnitude is assume*d* to be *mycorrhizal.* Only six genera of fungi produce *arbuscular mycorrhizal fungi* (AMF). Four genera, *Acaulospora*, *Gigaspora*, *Entrophospora*, and *Scutellospora*, form spores, similar to zygospores. Two genera (*Glomus* and *Sclerocytis*) yield only chlamydospores. Arbuscular mycorrhizal fungi (AMF) form a symbiotic relationship with host plants at the root system, first evolved 400 million years ago (Sawers et al. 2008) (Table 9.3).

Table 9.3 Different crops recommended bio-fertilizer and their application method

Crops	Bio-fertilizer	Method of application
Chickpea, pea, groundnut, soybean, beans, lentil	*Rhizobium*	Seed treatment
Rice	*Azospirillum*	-do-
Oilseeds	*Azotobacter*	-do-
Maize and sorghum	*Azospirillum*	-do-
Tobacco	*Azotobacter*	-do-
Rubber, coconuts	*Azotobacter*	-do-
Fruit plants	*Azotobacter*	-do-
Leguminous plants/trees	*Rhizobium*	-do-

Singh et al. (2015)

9.9 Application Methods

Mainly three types of bio-fertilizer application:

(a) Seed treatment or seed inoculation.
(b) Seedling root dip.
(c) Soil application.

9.10 Advantages of Bio-fertilizer over Chemical Fertilizer

Industrially formulated materials, which comprise known quantities of macro- (nitrogen, phosphorus, potassium) and micronutrients or combination of two or more of these nutrients, are called chemical fertilizers. The practice of chemical fertilizers may lead to air and groundwater pollution as a result of eutrophication of water bodies (Youssef and Eissa 2014). According to Chun-Li et al. (2014), soil acidification as well as atmospheric and groundwater contamination increases due to heavy use of chemical fertilizers and pesticides. These heavy doses reduce immunity of plant roots and make them prone to unwanted diseases. In this scenario, use of nutrient-rich high-quality fertilizers such as bio-fertilizer is a safe and healthy approach to pledge bio-safety.

Bio-fertilizer has been recognized as a competitive option to chemical fertilizer to increase soil fertility and crop production in sustainable farming. These eco-friendly and cost-effective inputs help the farmer to increase productivity of soil in a sustainable way Bio-fertilizers are able to fix nitrogen, solubilize and mobilize phosphate, and promote rhizobacteria (Bhat et al. 2010). The effectiveness of bio-fertilizer depends on selective microorganism that may be useful for the soil suitable packaging for a longer shelf life and adaptable to environment and user (Brar et al. 2012). Microorganisms are not applied directly to the field; instead these are settled

Table 9.4 Replacement of chemical fertilizer by bio-fertilizer

Sr. no.	Bio-fertilizer	Substitutes/ha per year
1	*Rhizobium*	108.6–217.3 kg of urea
2	*Azolla*	20–40 kg urea/10 mg
3	*Azospirillum*	60 kg urea in maize
4	BGA	54–65 kg urea
5	*Frankia*	195 kg urea

Bhowmik and Das (2018)

on some material. This material not only makes the application easier but also increases shelf life and facilitates rapid growth (Mahdi et al. 2010a).

Chemical fertilizers alters the metabolic activities that may be due to drop in osmotic potential. The chemical fertilizers releases more salt ions to the growth media, thus the osmotic pressure outside the embryo organs increases, and, consequently, water is osmotically bound, and thus salt concentration enhances, and water accessibility decreases for the embryo germination (Rafiq et al. 2010).

The bio-fertilizers act as a soil conditioner, and the conditioning property increased organic matter contents to the soil which in turn improves soil structure, prevents oil erosion as well as desertification and increases oil and water retention capacity (Swathi 2010). A functional relationship developed within rhizospheric microorganisms, and due to this, holistic system plant flourishes and grows fruitfully (Ju et al. 2018).

The high cost of chemical fertilizer and unavailability at the time of application further aggravate the economic conditions of farmers. Bio-fertilizer practice considers not only economical but also environment-friendly. Similar to chemical fertilizers, bio-fertilizers increase the soil fertility, crop production, and productivity without causing environment problems (Yadav and Sarkar 2019). Human, plants, and the environment are protected to pollution as well as save wages through bio-fertilization. Additionally, it upgrades soil biota and reduces the use of synthesis fertilizers (Jalilian et al. 2012) (Table 9.4).

Preventive Measures in the Use of Bio-Fertilizer:

1. Bio-fertilizers should not be blended with nitrogen fertilizers.
2. Bio-fertilizers should not be applied with fungicides.
3. Bio-fertilizers should not be exposed to sunlight directly.
4. Bio-fertilizers should always be stockpiled at room temperature, not below 0 and above 35 °C.
5. Used solution should not be kept overnight (Hari and Perumal 2010) (Table 9.5).

Table 9.5 Benefits and drawbacks of diverse application methods

Method	Benefits	Drawbacks	Reference
Carrier-based inoculation	Readily available Readily prepared Inexpensive	Carrier can contaminate the inoculants by unwanted microbes such as peat. No uniformity on the carrier. Short-term storage ability	Smith (1992); Brockwell (1980); Brockwell (1977); Bezdicek et al. (1978)
Seed coating and covering	Easy application No need of specific machine Practiced by farmers in case of pesticide application to seeds	Application of pesticides to the seeds. Sticking agents harmful to bacteria. Flexibility in seeding is less	Brockwell (1977); Brockwell (1980); Bashan and Carrillo (1996); Bashan and Holguin (1997); Bashan and Levanony (1990)
Pelleting	Easy application Preferred by farmers Adaptable in seeding and application Lime pellets can be used for acid soils	Less moisture hindered the survival of bacteria. Need special machinery to prepare thus expensive	Brockwell (1977); Bezdicek et al. (1978); Bashan and Levanony (1990); Bordeleau and Prevost (1981)
Direct soil application	Injection in the root zone is possible Easy and simple	Expose to sun Dehydration problems Require more volume	Brockwell (1977)
Root dipping	Require nursery Simple and easy	Liquid media and bacterial cells needed in large quantity Easily contaminate from environment	Brockwell (1977); Bordeleau and Prevost (1981); Bashan and Levanony (1990)

References

Adesemoye AO, Kloepper JW (2009) Plant–microbes interactions in enhanced fertilizer-use efficiency. Appl Microbiol Biotechnol 85:1–12

Al Seadi T, Ruiz D, Prassl H, Kottner M, Finsterwaldes T, VolkeS JR (2008) Handbook of biogas. University of Southern Denmark, Esbjerg

Alam S, Khalil S, Ayub N, Rashid M (2002) In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganism (PSM) from maize rhizosphere. Int J Agric Biol 4:454–458

Alloway BJ (2008) Zinc in soils and crop nutrition. International Zinc Association, Brussels

Amutha AI (2011) The growth kinetics of Arachis Hypogaea L. Var. TMV-7 under the inoculation of biofertilizers with reference to physiological and biochemical studies. PHD thesis. Department of Botany and Research Centre Scott Christian College (Autonomous). Manonmaniam Sundaranar University, Nagercoil

Ansari MW, Trivedi DK, Sahoo RK (2013) A critical review on fungi mediated plant responses with special emphasis to piriformospora indica on improved production and protection of crops. Plant Physiol Biochem 70:403–410

Arun KS (2007) Bio-fertilizers for sustainable agriculture. Mechanism of P-solubilization, 6th edn. Agribios, Jodhpur, pp 196–197

Aslanzadeh S (2014) Pretreatment of cellulosic waste and high rate biogas production. Doctoral thesis on resource recovery. University of Borås, Borås, pp 1–50

Bakulin MK, Grudtsyna AS, Pletneva AY (2007) Biological fixation of nitrogen and growth of bacteria of the genus Azotobacter in liquid media in the presence of perfluorocarbons. Appl Microbiol Biotechnol 43:399–402

Balogh B, JonesJB IF, Momol M (2010) Phage therapy for plant disease control. Curr Pharm Biotechnol 11:48–57

Bashan Y, Carrillo A (1996) Bacterial inoculants for sustainable agriculture. In New horizons in agriculture: agroecology and sustainable development; J. Pérez-Moreno and R. Ferrera-Cerrato(eds.), pp. 125–155, Proceedings of the 2nd international symposium on agroecology, sustainable agriculture and education. San Luis Potosi, Mexico, 16-18.11.1994. Published by Colegio de Postgraduados en ciencias agricolas, Montecillo, Mexico

Bashan Y, Levanony H (1990) Current status of Azospirillum inoculation technology: Azospirillum as a challenge for agriculture. Can J Microbiol 36:591–608

Bashan Y, Holguin G (1997) Azospirillum-plant relationships: environmental and physiological advances (1990–1996). Can J Microbiol 43:103–121

Bertrand H, Plassard C, Pinochet X, ToraineB NP (2000) Stimulation of the ionic transport system in Brassica napus by a plant growth-promoting rhizobacterium. Can J Microbiol 46:229–236

Bevivino A, Sarrocco S, Dalmastri C, Tabacchioni S, Cantale C, Chiarini L (1998) Characterization of a free-living maize-rhizosphere population of Burkholderia cepacia: eject of seed treatment on disease suppression and growth promotion of maize. FEMS Microbiol Ecol 27:225–237

Bezdicek DF, Evans DW, Abeda B, Witters RE (1978) Evaluation of peat and granular inoculum for soybean yield and N2 fixation under irrigation. Agron 70:865–868

Bhat M, Yadav S, Ali T, Bangroo S (2010) Combined effects of rhizobium and vesicular arbuscular fungi on green gram (*Vigna radiata* L.) under temperate conditions. Indian J Ecol 37:157–161

Bhat RA, Dervash MA, Mehmood MA, Bhat MS, Rashid A, Bhat JIA, Singh DV, Lone R (2017) Mycorrhizae: a sustainable industry for plant and soil environment. In: Varma A et al (eds) Mycorrhiza-nutrient uptake, biocontrol, Ecorestoration. Springer International, Berlin, pp 473–502

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiotechnol 28:1327–1350

Bhatti AA, Haq S, Bhat RA (2017) Actinomycetes benefaction role in soil and plant health. Microb Pathog 111:458–467

Bhowmik SN, Das A (2018) Biofertilizers: a sustainable approach for pulse production. In: Legumes for soil health and sustainable management. Springer, Singapore, pp 445–485

Bloemberg GV, Wijfijes AHM, GEM L, Stuurman N, Lugtenberg BJJ (2000) Simultaneous imaging of Pseudomonas fluorescens WCS 3655 populations expressing three different autofluorescent proteins in rhizosphere: new perspective for studying microbial communities. Mol Plant Microb Int 13:1170–1176

Bordeleau LM, Prevost D (1981) Quality of commercial legume inoculants in Canada. In Proceeding of the 8th North American Rhizobium Conference, K.W. Clark and J.H.G. Stephens (eds.), pp. 562–565, University of Manitoba, Winnipeg, Canada

Braber K (1995) Anaerobic digestion of municipal solid waste: a modern waste disposal option on the verge of breakthrough. Biomass Bioenergy 9:365–376

Brar SK, Sarma SJ, Chaabouni E (2012) Shelf-life of biofertilizers: an accord between formulations and genetics. J Biofertil Biopestic 3:109

Brockwell J (1977) Application of legume seed inoculants. In A Treatise on Dinitrogen Fixation, ed. R. W. F. Hardy & A. H. Gibson. John Wiley and Sons, New York, Section IV, pp. 277–310

Brockwell J (1980) Experiments with crop and pasture legumes—principles and practice. In Methods for Evaluating Biological Nitrogen Fixation. Ed F J Bergersen, pp 417–488, Wiley, Chichester, U.K.

Chandra R, Takeuchi H, Hasegawa T (2012) Methane production from lignocellulosic agricultural crop wastes: a review in context to second generation of biofuel production. Renew Sustain Energy Rev 16:1462–1476

Chang CH, Yang SS (2009) Thermotolerant phosphate solubilizing microbes for multifunctional bio-fertilizer preparation. Biorese Technol 100:1648–1658

Chowdhury A, Mukherjee P (2006) Biofertilizers. Newslett, ENVIS Centre Environ Biotechnol. 9:2–7

Christy PM, Gopinath LR, Divya D (2014) A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms. Renew Sust Energ Rev 34:167–173

Chun-Li W, Shiuan-Yuh C, Chiu-Chung Y(2014) Present situation and future perspective of bio-fertilizer for environmentally friendly agriculture. Annu Rep 1–5

DalCorso G, Manara A, Furini A (2013) An overview of heavy metal challenge in plants: from roots to shoots. Metallomics 5:1117–1132

Dorahy CG, Rochester IJ, Blair GJ (2005) Response of field-grown cotton (*Gossypium hirsutum* L.) to phosphorus fertilisation on alkaline soils in eastern Australia. Soil Res 42:913–920

Duponnois R, Kisa M, Plenchette C (2006) Phosphate solubilizing potential of the nemato fungus Arthrobotrys oligospora. J Plant Nutr Soil Sci 169:280–282

El-Komy HMA (2005) Co-immobilization of A. lipoferum and B. megaterium for plant nutrition. Food Technol Biotech 43:19–27

Ezigbo U (2005) Studies on the production of biogas from droppings and cow dung. Unpublished B.Sc. Thesis. Department of Botany, University of Jos, pp 110–26

Frampton RA, Pitman AR, Fineran PC (2012) Advances in bacteriophage-mediated control of plant pathogens. Int J Microbiol 326–452

Gage DJ (2004) Infection and invasion of roots by symbiotic, nitrogen- fixing rhizobia during nodulation of temperate legumes. Microbiol Mol Biol Rev 68:280–300

Ganz T (2013) Systemic iron homeostasis. Physiol Rev 93:1721–1741

Gerardi MH (2003) The microbiology of anaerobic digesters. Wiley, Hoboken, pp 89–92

Gothwal RK, Nigam VK, Mohan MK, Sasmal D, Ghosh P (2007) Screening of nitrogen fixers from rhizospheric bacterial isolates associated with important desert plants. Appl Ecol Environ Res 6:101–109

Gupta AK (2004) The complete technology book on biofertilizer and organic farming. National Institute of Industrial Research Press, Delhi, pp 242–253

Halim NA (2009) Effects of using enhanced bio-fertilizer containing N-fixer bacteria on patchouli growth. Thesis. Faculty of Chemical & Natural Resources Engineering, University Malaysia, Pahang. 145, 42, pp 913–920

Hari M, Perumal K (2010) Booklet on bio-fertilizer (phosphabacteria). Shri Annm Murugapa Chettiar Research Centre, Chennai, pp 1–6

Jalilian J, Modarres-Sanavy SAM, Saberali SF, Sadat-Asilan K (2012) Effects of the combination of beneficial microbes and nitrogen on sunflower seed yields and seed quality traits under different irrigation regimes. Field Crop Res 127:26–34

Ju I, Wj B, Md S, Ia O, Oj E (2018) A review: biofertilizer—a key player in enhancing soil fertility and crop productivity. J Microbiol Biotechnol Rep 2(2)

Kannaiyan S (2002) Biofertilizers for sustainable crop production. Biotechnology of biofertilizers. Narosa, New Delhi, p 377

Khan RU, Rashid A, Khan MS, Ozturk E (2010) Impact of humic acid and chemical fertilizer application on growth and grain yield of rainfed wheat (*Triticum aestivum* L.). Pak J Agric Res 1:23

Khanday M, Bhat RA, Haq S, Dervash MA, Bhatti AA, Nissa M, Mir MR (2016) Arbuscular mycorrhizal fungi boon for plant nutrition and soil health. In: Hakeem KR, Akhtar J, Sabir M

(eds) Soil science: agricultural and environmental prospectives. Springer International, Berlin, pp 317–332
Khosro M (2012) Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. Resour Environ 2:80–85
Khosro M, Yousef S (2012) Bacterial bio-fertilizers for sustainable crop production: a review. APRN J Agric Biol Sci 7:237–308
Kim MD, Song M, Jo M, Shin SG, Khim JH, Hwang S (2010) Growth condition and bacterial community for maximum hydrolysis of suspended organic materials in anaerobic digestion of food waste-recycling wastewater. Appl Microbiol Biotechnol 85:1611–1618
Kribacho (2010) Fertilizer ratios, Krishak and Bharati Cooperative Ltd. J Sci 5:7–12
Kundan R, Pant G, Jadon N, Agrawal PK (2015) Plant growth promoting rhizobacteria: mechanism and current prospective. J Fertil Pestic 6:1–9
Lettinga G (1995) Anaerobic digestion and wastewater treatment systems. Antonie Van Leeuwenhoek 67:3–28
Mahdi SS, Hassan GI, Samoon SA, Rather HA, Dar SA, Zehra B (2010a) Bio-fertilizers in organic agriculture. J Phytology 2:42–54
Mahdi SS, Dar SA, Ahmad S, Hassan GI (2010b) Zinc availability—a major issue in agriculture. Res J Agric Sci 3:78–79
Mittal V, Singh O, Nayyar H, Kaur J, Tewari R (2008) Stimulatory effect of phosphate-solubilizing fungal strains (Aspergillus awamori and Penicillium citrinum) on the yield of chickpea (Cicer arietinum L. cv. GPF2). Soil Biol Biochemi 40:718–727
Nobbe F, Hiltner L (1986) Inoculation of the soil for cultivating leguminous plants. US Patent. 570813
Olanrewaju OS, Glick BR, Babalola OO (2017) Mechanisms of action of plant growth promoting bacteria. World J Microbiol Biotechnol 33:197
Ponsá S, Ferrer I, Vázquez F, Font X (2008) Optimization of the hydrolytic–acidogenic anaerobic digestion stage (55 C) of sewage sludge: influence of pH and solid content. Water Res 42:3972–3980
Rafiq MA, Ali A, Malik MA, Hussain M (2010) Effect of fertilizer levels and plant densities on yield and protein contents of autumn planted maize. Pak J Agric Sci 47:201–208
Raghu K, Macrae IC (2000) Occurrence of phosphate-dissolving microorganisms in the rhizosphere of rice plants and in submerged soils. J Appl Bacteriol 29:582–586
Riaz U, Murtaza G, Farooq M (2018) Influence of different sewage sludges and composts on growth, yield, and trace elements accumulation in rice and wheat. Land Degrad Dev 29: 1343–1352
Raj SA (2007) Bio-fertilizers for micronutrients. Biofert Newslet (July), pp 8–10
Rana R, Ramesh KP (2013) Biofertilizers and their role in agriculture. Pop Kheti 1:56–61
Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339
Sadhana B (2014) Arbuscular mycorrhizal fungi (AMF) as a biofertilizer—a review. Int J Curr Microbiol App Sci 3:384–400
Saha R, Saha N, Donofrio RS, Bestervelt LL (2013) Microbial siderophores: a mini review. J Basic Microbiol 53:303–317
Santos VB, Araujo SF, Leite LF (2012) Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems. Geoderma 170:227–231
Sawers RJH, Gutjahr C, Paszkowski U (2008) Cereal mycorrhiza: an ancient symbiosis in modern agriculture. Trends Plant Sci 13:93–97
Schnurer A, Jarvis A (2009) Microbiological handbook for biogas plant. Swedish Waste Management, Swedish Gas Centre, Malmö, pp 1–74
Sharma S, Kumar V, Tripathi RB (2017) Isolation of phosphate solubilizing microorganism (PSMs) from soil. J Microbiol Biotechnol Res 1:90–95

Singh M, Kumar N, Kumar S, Lal M (2015) Effect of co-inoculation of B. japonicum, psb and a fungi on microbial biomass carbon, nutrient uptake and yield of soybean (*Glycine max* L. Merril), pp 14–18

Smith R (1992) Legume inoculant formulation and application. Can J Microbiol 38:485–492

Sofi NA, Bhat RA, Rashid A, Mir NA, Mir SA, Lone R (2017) Rhizosphere mycorrhizae communities an input for organic agriculture. In: Varma A et al (eds) Mycorrhiza-nutrient uptake, biocontrol, ecorestoration. Springer International, Berlin, pp 387–413

Somani LL (n.d.) Biofertilizer: commercial production technology & quality control. www.agri-info.in

Subba RNS (2001) An appraisal of biofertilizers in India. In: Kannaiyan S (ed) The biotechnology of biofertilizers. Narosa, New Delhi. (in press)

Subbarao NS (1988) Phosphate solubilizing microorganism. In: biofertilizer in agriculture and forestry. Regional Biofert. Dev. Centre, Hissar, pp 133–142

Swathi V (2010) The use and benefits of bio-fertilizer and biochar on agricultural soils" unpublished B.Sc. Doctoral dissertation, Thesis, Department of Chemical and Biological Engineering, Chalmers University of Technology, Goteborg, Sweden

Vance CP (2001) Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiol 127:390–397

Vandevivere P, De Baere L, Verstraete W (2003) Types of anaerobic digesters for solid wastes. In: Biomethanization of the organic fraction of municipal solid wastes. IWA, London

Vessey JK (2003) Plant growth promoting Rhizobacteria as bio-fertilizers. J Plant Soil 225:571–586

Yadav KK, Sarkar S (2019) Biofertilizers, impact on soil fertility and crop productivity under sustainable agriculture. Environ Ecol 37:89–93

Youssef MMA, Eissa MFM (2014) Biofertilizers and their role in management of plant parasitic nematodes. A review. J Biotechnol Pharm Res 5:1–6