# *Azotobacter*: A Potential Biofertilizer and Bioinoculants for Sustainable Agriculture

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**Abstract**

Plant growth-promoting rhizobacteria (PGPR) are best known bacterial species among all other microorganisms that have more influence on physiological and structural properties of soil. PGPR helps to replace chemical fertilizer for the sustainable agriculture production by fixing the atmospheric nitrogen and producing growth-promoting substances. Among PGPR group, *Azotobacter* are ubiquitous, aerobic, free-living, and $N_2$-fixing bacteria commonly living in rhizosphere soil. Being the major group of soilborne bacteria, *Azotobacter* plays different beneficial roles by producing different types of vitamins, amino acids, plant growth hormones, antifungal substances, hydrogen cyanide, and siderophores. The growth-promoting substances such as indole acetic acid, gibberellic acid, arginine, etc., produced by species of *Azotobacter* have direct influence on shoot length, root length, and seed germination of several agricultural crops (soil rhizosphere). Some of the species of *Azotobacter*, viz., *A. vinelandii*, *A. chroococcum*, *A. salinestris*, *A. tropicalis*, and *A. nigricans*, are able to produce antimicrobial compounds which inhibit the growth of plant pathogens, viz., *Fusarium*, *Aspergillus*, *Alternaria*, *Curvularia*, and *Rhizoctonia* species, which can cause

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major plant diseases and economic losses. *Azotobacter* species are efficient in fixation of highest amount of nitrogen (29.21 μg $NmL^{-1}$ $day^{-1}$), production of indole acetic acid (24.50 $\mu gmL^{-1}$) and gibberellic acid (15.2 μg 25 $mL^{-1}$), and phosphate-solubilizing activity (13.4 mm). Species of *Pseudomonas*, *Bacillus*, and *Azotobacter* can grow and survive at extreme environmental conditions, viz., higher salt concentration, high pH environments, and even at higher temperature. *Azotobacter* is found tolerant to a higher NaCl concentration (6–8%), to maximum temperature (45 °C), and also to varied pH ranges (8–9). *A. salinestris* (GVT-1) culture filtrate has increased the paddy seed vigor index or growth and seed germination rate. *Azotobacter* species have maintained maximum levels of viable population at different temperatures in different formulations. *Azotobacter* species can grow and survive for periods in talc- and lignite-based formulations. In view of these properties, *Azotobacter* isolates can be used for sustainable agriculture as biofertilizer and bioinoculants.

**Keywords**
PGPR • Azotobacter • Indole acetic acid • Biofertilizer

## 5.1 Introduction

Soil is considered a storehouse of microbial activity, though the space occupied by living microorganisms is estimated to be less than 5% of the total space. Soil microorganisms play an important role in soil processes that determine plant productivity. Bacteria living in the soil, rhizosphere and rhizoplane, and on plant tissues are called free living as they do not depend on others for their survival. Some bacteria support plant growth indirectly by the production of antagonistic substances or by inducing resistance against common plant pathogens occurring in the vicinity of roots (Tilak et al. 2005). The organic compounds released by bacteria play an important role in the uptake of mineral nutrient. The hormones produced by the rhizosphere bacteria have direct effects on growth and development of plants. The population density status of PGPR depends on the fertility of soil and human activities (Marianna et al. 2005).

Cultivation, production, and consumption of agriculture produce have been increased from the last two decades with the increasing population to sustain food supply within the available land (Chennappa et al. 2013). Asian countries which produce high agriculture productions include China, Korea, India, Pakistan, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, the Philippines, and Japan (FAO 2010). To improve the agriculture production, different types of cultivation practices such as application of chemical fertilizers and chemical pesticides, improved crop varieties and machineries, etc., are being followed. Among them, synthesized fertilizers, chemical pesticides, and other inputs are being excessively applied for the control of plant diseases and insect pests. Farmers use chemical fertilizers to increase production, but the extensive use of these chemical-based inputs or fertilizers leads to contamination of soil and groundwater, depletion of soil fertility, greenhouse effect, damage to the ozone layer, acidification and pollution of water resources, destruction of beneficial microorganisms, acidification of soil, and health hazards (Matin et al. 2011). To overcome these problems, several research works in biodegradation of pesticides have been carried throughout the world in order to minimize the residual toxicity in the food and food products.

However, microorganisms play a major role in the degradation of chemical pesticides, and many soilborne bacteria and fungi have the potentiality to breakdown of pesticides into nontoxic elemental compounds in the soil. For biodegradation of pesticides, numbers of microbes have been employed, and among all, plant growth-promoting rhizobacteria (PGPR) are the widely studied bacterial group. PGPR are not only biodegrade pesticides but they are also involved in nitrogen fixation and produce growth-promoting compounds which can help to replace chemical fertilizer for sustainable agriculture (Castillo et al. 2011; Ahmad et al. 2005). PGPR group includes different species of bacteria; among them, diazotrophic *Azotobacter* are free living in rhizosphere soil ecosystem, which are playing different beneficial roles for the plant growth (Page and Shivprasad 1991; Tejera et al. 2005).

The genus *Azotobacter* has the potentiality to produce different types of amino acids, plant growth hormones, antifungal antibiotics, and siderophore and has a unique ability of atmospheric nitrogen fixation in the soil (Myresiotis et al. 2012; Chennappa et al. 2013, 2014, 2016). *Azotobacter* species happens to be the most dominant species in the rhizosphere soil and can biodegrade chlorine-containing pesticide, viz., 2,4,6-trichlorophenol, simple phenols, and substituted phenols used for the management of plant pathogens causing diseases in agricultural crops (Li et al. 1991). In view of these prominent beneficial applications, the review survey of research articles has been carried to know the complete nature and beneficial properties of *Azotobacter* species.

## 5.2 *Azotobacter* Diversity

Beijerinck (1901) was the first person who isolated and cultured species of *Azotobacter*. Later, several other species of *Azotobacter* have been isolated and described as *Azotobacter vinelandii*, *A. beijerinckii*, *A. insignis*, *A. macrocytogenes*, *A. paspali*, *A. chroococcum*, *A. salinestris*, *A. armeniacus*, *A. brasilense*, *A. agilis*, *A. tropicalis*, and *A. nigricans* (Mulder and Brontonegoro 1974; Page and Shivprasad 1991; Kizilkaya 2009). The diversity and beneficial applications of *Azotobacter* species were well documented by different ecosystems from the last two decades because of its plant growth-promoting activity for sustainable agriculture (Aquilanti et al. 2004; Jimenez et al. 2011). Among different species, *A. chroococcum* and *A. vinelandii* are common habitants found in the rhizosphere soils. The *Azotobacter* are ubiquitous in nature, and its occurrence in soil is influenced by many factors, viz., soil pH, organic matter, calcium, phosphorus, potassium content, and other microorganisms present in soil (Rangaswami et al. 1964).

The occurrence and dominance of *Azotobacter* have been discovered from various rhizospheric soils of agricultural crops such as ragi, sorghum, green gram and soybean, sugarcane, rice, and cereals. *Azotobacter* population was found more in black soil than in red soil, and the number may be decreased with depth, but the decrease was more drastic in black soils (Bagyaraj and Patil 1975; Ramaswami et al. 1977).

## 5.3 PGPR Properties

The term PGPR was first described by Kloepper and Schroth (1980). PGPR are a group of bacteria that actively colonizes plant roots and promotes plant growth and increases yield (Bin Zakaria 2009). There are several types of rhizobacteria, and the type is depending on the nutrients provided into the soil systems and mechanism used. PGPR are able to increase plant nutrient uptake by introducing nitrogen-fixing bacteria associated with roots (*Azospirillum*) for nitrogen uptake, iron uptake from siderophore-producing bacteria (*Pseudomonas*), sulfur uptake from sulfur-oxidizing bacteria (*Thiobacillus*), phosphorus uptake from phosphate mineral-solubilizing bacteria (*Bacillus*, *Pseudomonas*), and potassium uptake from potassium-solubilizing bacteria (*Bacillus*).

The PGPR promote plant growth and have the potentiality to produce vitamins (riboflavin), amino acids (thiamine), polyhydroxybutyrate (PHB), and phytohormones (nicotin, cytokinin, IAA, and gibberellins), symbiotic and asymbiotic $N_2$ fixation, production of siderophores, HCN, synthesis of antibiotics and enzymes, and mineralization of phosphates and other nutrients (Gholami et al. 2009; Myresiotis et al. 2012). Enhanced supplies of other plant nutrients such as phytochrome production lead to increases in shoot and root length as well as seed germination of several agricultural crops (Ahmad et al. 2005; Heike 2007). The Production of biologically active substances or plant growth regulators (PGRs) is one of the major mechanisms through which PGPR influence the plant growth and development (Javed et al. 2009). The ability to synthesize phytohormone is widely distributed among plant-associated bacteria, and 80% of the bacteria isolated from plant rhizosphere are able to produce plant growth-promoting substances.

### 5.3.1 Vitamins

Vitamins are essential for physiological functions of living beings which are produced by several groups of bacteria. *Azotobacter* species produces vitamins under favorable conditions, and *A. vinelandii* and *A. chroococcum* strains produced niacin, pantothenic acid, riboflavin, and biotin which belong to B-group vitamins. They are used to maintain metabolic processes of living beings, but the production of vitamins is controlled by several physical factors such as growth conditions, pH, incubation temperatures, and availability of nitrogen and carbon sources (Revillas et al. 2000). Riboflavin is a vitamin B2 required for a wide variety of cellular processes, and it plays a key role in metabolism of fats, ketone bodies, carbohydrates, and proteins, respectively (Almon 1958; Revillas et al. 2000).

### 5.3.2 Amino Acids

Amino acids are also one of the important elements required for the growth and development of cells. Few of the bacterial genera known to produce amino acids, among them *A. vinelandii* and *A. chroococcum*, produced aspartic acid, serine, glutamic acid, glycine, histidine, threonine, arginine, alanine, proline, cysteine, tyrosine, valine, methionine, lysine, isoleucine, leucine, tryptophan, and phenylalanine (Revillas et al. 2000; Lopez et al. 1981).

### 5.3.3 HCN

Many bacterial genera have capability of producing HCN. Species of *Azotobacter*, *Alcaligenes*, *Aeromonas*, *Bacillus*, *Pseudomonas*, and *Rhizobium* produce HCN as a volatile, secondary metabolite that suppresses the growth and development of plant pathogens and that influences the growth of plants (Ahmad et al. 2008). HCN is a powerful inhibitor of many metal enzymes, especially copper-containing cytochrome C oxidases. It is formed from glycine through the action of HCN synthetase enzyme, which is associated with the plasma membrane of certain rhizobacteria.

### 5.3.4 Siderophore

Siderophore are iron (Fe)-chelating low molecular weight compounds which are produced and utilized by bacteria and fungi. These compounds are produced in response to iron deficiency which normally occurs in neutral to alkaline pH soils, due to low iron solubility at elevated pH (Johri et al. 2003). Species of *Azotobacter* excretes siderophores under limited iron conditions. *A. vinelandii* produces five different siderophore such as 2,3-dihydroxybenzoic acid, aminochelin, azotochelin, protochelin, and the azotobactin which act as antibiotic in nature (Fig 5.1). Siderophores are used as drug delivery agents, which are important main biotechnological applications, antimicrobial agents, and soil remediation (Page and Von Tigerstrom 1988; Mollmann et al. 2009; Kraepiel et al. 2009; Barrera and Soto 2010). Siderophore-producing PGPR can prevent the proliferation of pathogenic microorganisms by sequestering $Fe^{3+}$ in the vicinity of the root.

### 5.3.5 Polyhydroxybutyrate (PHB)

*Azotobacter* species also produces PHB, alginate, and catechol compounds under determined nutritional and favorable environmental conditions (Barrera and

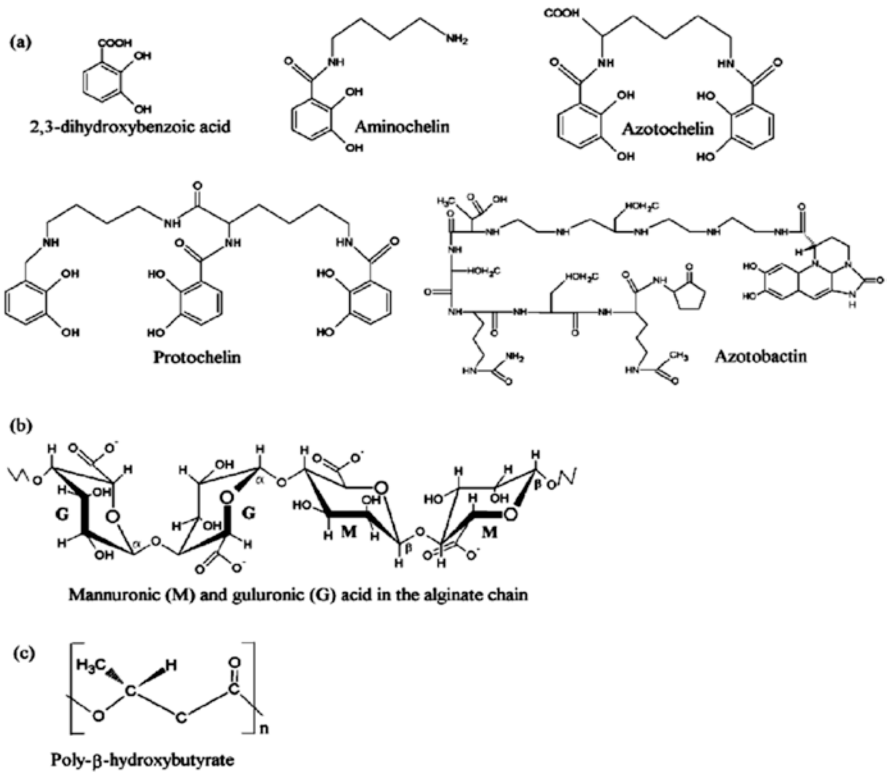


**Fig. 5.1** Different types of antibiotics produced by species of *Azotobacter* (Juan et al. 2014)

Soto 2010). PHB are also used in large-scale production of alginic acid which is a biodegradable and biocompatible thermoplastic used in food industry, for thickening soups and jellies.

### 5.3.6 Enzymes

The production of polyphenol oxidases (PPOs) and phenol oxidases (POs) in members of the family *Azotobacteraceae* is highly presumed and is produced by the group of multi-copper protein bacterial family, respectively (Herter et al. 2011). Few of the reports documented that the production, distribution, occurrence, structural organization, and localization of prokaryotic phenol oxidases seemed to be restricted to some species. *Azotobacter* sp. SBUG 1484 isolated from soil was confirmed for production of phenol oxidases. The presence of phenol oxidases is being exploited in industrial applications such as pulp delignification, textile dye bleaching, and biopolymer synthesis which is highly important. Significant interest in the application of phenol oxidases has also been generated in scientific fields concerning the detoxification and degradation of environmental pollutants and also concerning with the production of fine chemicals (Herter et al. 2011).

### 5.3.7 Antifungal Activities

*Azotobacter* species act as biocontrol agents by the production of antibiotics such as 2,3-dihydroxybenzoic acid, aminochelin, azotochelin, protochelin, and azotobactin for combating plant pathogens (Agarwal and Singh 2002; Mali and Bodhankar 2009; Kraepiel et al. 2009). The production of antibiotics is considered one of the most studied biocontrol mechanisms for combating phytopathogens. The species of *Azotobacter armeniacus* has inhibited root-colonizing *Fusarium verticillioides* which has suppressed fumonisin B1 production. Antifungal activity of *A. vinelandii* showed maximum zone of inhibition (40 mm) against *F. oxysporum* which is commonly known to cause several diseases in agricultural crops, viz., chilli and pigeon pea (Cavaglieri et al. 2005; Bhosale et al. 2013). *Azotobacter* can provide protection against soilborne pathogenic fungi such as *Aspergillus*, *Fusarium*, *Curvularia*, *Alternaria*, and *Helminthosporium* (Khan et al. 2008; Mali and Bodhankar 2009). Nagaraja et al. (2016) have reported the antifungal property of *A. nigricans* against *Fusarium* spp. and its role in decolonizing efficiency against fungal pathogen in rhizoplane soil.

### 5.3.8 Plant Growth Hormones

#### 5.3.8.1 IAA

Indole acetic acid (IAA) is the important plant auxin produced by different groups of bacteria commonly living in soil (Barazani and Friedman 1999). Saline soil is a rich source of IAA-producing bacteria, whereas 75% of the bacterial isolates are active in IAA production. Many *Azotobacter* species are found to produce IAA in the range of 2.09–33.28 μg/mL (Spaepen et al. 2007; Chennappa et al. 2013, 2014, 2016). Most commonly, IAA-producing PGPR strains are known to increase root length resulting in greater root surface area which enables plants to access more nutrients from soil. IAA is responsible for the division, expansion, and differentiation of plant cells and tissues and stimulates root elongation (Ahmad et al. 2008). These rhizobacteria synthesize IAA from tryptophan by different pathways via tryptophan-independent and tryptophan-dependent pathways.

In contrast, the indole pyruvic pathway appears to be the main pathway present in plant growth-promoting beneficial bacteria (Patten and Glick 2002). Among PGPR species, *Azospirillum* is one of the best studied IAA producers, and other bacteria genera include *Aeromonas*, *Burkholderia*, and *Azotobacter* (Ahmad et al. 2008). *Bacillus*, *Enterobacter*, *Pseudomonas*, and *Rhizobium* (Ghosh et al. 2010) species have been isolated from different rhizosphere soils.

#### 5.3.8.2 Gibberellic Acid

Another important type of auxin produced by *Azotobacter* is gibberellins. GA production was first discovered by Japanese scientist Eiichi Kurosawa, which was produced by the fungi called *Gibberella fujikuroi* under abnormal growth stage in rice plants. GA includes a wide range of chemicals that are produced naturally within plant rhizosphere and by bacteria and fungi. Gibberellins are important in seed germination and enzyme production that mobilizes growth of new cells. GA promotes flowering, cellular division, and seed growth after germination (Upadhyay et al. 2009).

### 5.3.9 Phosphate Solubilization

Microbes play a significant role in the transformation of phosphorous and referred to as phosphor bacteria. Phosphate-solubilizing bacteria are a group of beneficial bacteria capable of hydrolyzing organic and inorganic phosphorus from insoluble compounds. The P-solubilization ability of the microorganisms is considered to be one of the most important traits associated with plant phosphate nutrition. Phosphate-solubilizing bacteria species such as *A. chroococcum*, *B. subtilis*, *B. cereus*, *B. megaterium*, *Arthrobacter ilicis*, *E. coli*, *P. aeruginosa*, *E. aerogenes*, and *Micrococcus luteus* were identified (Kumar et al. 2000; Garg et al. 2001).

### 5.3.10 Nitrogen Fixation

The Earth's atmosphere contains 78% nitrogen gas ($N_2$), and most organisms cannot directly use this resource due to the stability of the compound. Plants, animals, and microorganisms can die of nitrogen deficiency because nitrogen is one of the important N sources. All organisms use the ammonia ($NH_3$) form of nitrogen to synthesize amino acids, proteins, nucleic acids, and other nitrogen-containing components necessary for life (Lindemann and Glover 2008; Mikkelsen and Hartz 2008). Nitrogen is present in all living organisms, proteins, nucleic acids, and other molecules. It typically makes up around 4% of the dry weight of plant matter.

Inadequate supply of available $N_2$ frequently results in plants that have slow growth, depressed protein levels, poor yield of low-quality produce, and inefficient water use. The sources of nitrogen used in fertilizers are many, including ammonia ($NH_3$), diammonium phosphate ($(NH_4)$ $2HPO_4$), ammonium nitrate ($NH_4NO_3$), ammonium sulfate ($(NH_4)$ $2SO_4$), calcium cyanamide ($CaCN_2$), calcium nitrate (Ca $(NO_3)_2$), sodium nitrate ($N_aNO_3$), and urea ($N_2H_4CO$) (Mikkelsen and Hartz 2008; Rifat et al. 2010; Shakhashiri 2003).

#### 5.3.10.1 Nitrogen-Fixing Bacteria

Following photosynthesis, nitrogen fixation is the second most important process in plant growth and development. Nitrogen fixation occurs by the use of nitrogen gas to form ammonium with the help of nitrogenase enzyme. About 300–400 kg N/ha/

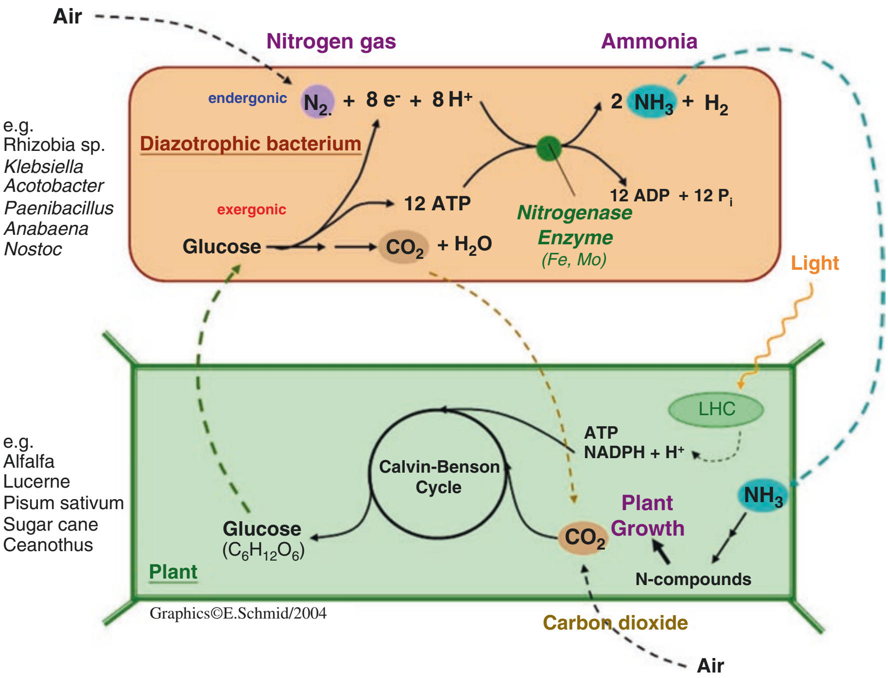


**Fig. 5.2** Mechanism of nitrogen fixation by plant growth-promoting rhizobacterial group (**http://classroom.sdmesa.edu/eschmid/Lecture21-Microbio.htm**)

yr of nitrogen fixation has been fixed by nitrogen fixation process in the soil, and the atmosphere comprises of ~78% nitrogen as an inert gas, $N_2$, which is unavailable to plants. Approximately 80,000 tones of this unavailable nitrogen are present in the soil ecosystem and in the atmosphere. In order to convert to available form of $N_2$, it needs to be fixed through either the synthetic industrial process (Haber-Bosch process) or through biological nitrogen fixation (BNF). Biological nitrogen fixation (BNF) accounts for 65% of the nitrogen currently utilized in agriculture, and out of that, 80% comes from symbiotic associations, the rest from nonsymbiotic and associative systems (Fig 5.2). PGPR root-colonizing microorganisms are known to fix atmospheric molecular nitrogen through symbiotic, asymbiotic, and associative nitrogen-fixing process.

### Symbiotic Nitrogen Fixers

It is estimated that about 80% of symbiotic biological nitrogen fixation available in soil ecosystem and symbiotic nitrogen-fixing bacteria are very specific plant roots of particular legume species for nodulation, invasion, and nitrogen fixation (Chandrasekar et al. 2005). Among different nitrogen-fixing bacteria, *Rhizobia* and *Frankia* have been widely studied, and more than 280 species of woody plants form root nodules which are harbored by *Frankia* (Tilak et al. 2005).

#### Nonsymbiotic and Associated Nitrogen Fixers

Nonsymbiotic nitrogen fixation is known to be of great agronomic significance, and its main limitation is the availability of carbon and energy source for nitrogen fixation process. This limitation can be compensated by several root-colonizing bacteria living closer or inside the plants. Some of the important nonsymbiotic nitrogen-fixing bacteria include the species of *Achromobacter*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Corynebacterium*, *Derxia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, *Rhodospirillum*, *Rhodopseudomonas*, and *Xanthobacter* (Tilak et al. 2005). Among all the species, *Azotobacter* is the most studied diazotrophic nonsymbiotic nitrogen-fixing bacterial species and aerobic soil bacteria with a wide variety of metabolic capabilities (Khan et al. 2008; Mirzakhani et al. 2009).

##### Nitrogen Fixation by *Azotobacter*

Nitrogen fixation is the biological reaction where atmospheric $N_2$ gas is converted into $NH_3$. Ammonia is a form of nitrogen that can be easily utilized for biosynthetic pathways; nitrogen fixation is a critical process in the completion of the nitrogen cycle (Murcia et al. 1997; Barrera and Soto 2010). The species of *Azotobacter* are known to fix on an average 10 mg of N/g of carbohydrate under in vitro. *A. chroococcum* happens to be the dominant inhabitant in arable soils capable of fixing $N_2$ (2–15 mg$N_2$ fixed/g of carbon source) in culture medium. Most efficient strains of *Azotobacter* would need to oxidize about 1000 kg of organic matter for fixing 30 kg of N/ha. Besides, soil is inhabitated by a large variety of other microbes, all of which compete for the active carbon. Plant needs nitrogen for its growth and *Azotobacter* fixes atmospheric nitrogen nonsymbiotically. Therefore, plants get benefited especially cereals, vegetables, fruits, etc., which are known to get additional nitrogen requirements from *Azotobacter* (Tilak et al. 2005; Tejera et al. 2005; Khan et al. 2008; Mirzakhani et al. 2009).

### 5.3.11 Abiotic Stress Tolerance

In soil ecosystem, populations of *Azotobacter* sp. are affected by soil physicochemical parameters (organic matter, pH, temperature, soil depth, soil moisture) and microbiological properties (microbial interactions) (Kizilkaya 2009). Owing to the fact that *Azotobacter* is an aerobe, this organism requires oxygen for the biological activity. As many investigators have noted, aeration encourages the propagation of *Azotobacter*. The initiation of growth of nitrogen-fixing *Azotobacter* species was prevented by efficient aeration but preceded normally with gentle aeration (Gul 2003).

#### 5.3.11.1 Salt Tolerance

Many reports related salt, temperature, and pH tolerance of PGPR group of bacteria are available in public database. Among PGPR group, species of *Azotobacter* are known to tolerate maximum salt concentration, and it has been recorded growth rate up to 10% of NaCl concentration. Similarly, *A. salinestris* was tolerant to 8% NaCl concentration, but the total CFU/mL values were reduced at 8% concentration. The NaCl concentration affected the PGPR activity of *Azotobacter* such as nitrogen fixation in soil. *A. salinestris* was isolated from saline soil samples, and because of this activity, the species has been named as salinestris which is sodium-dependent diazotrophic *Azotobacter* species (Page and Shivprasad 1991).

#### 5.3.11.2 Temperature Tolerance

In relation to temperature, a number of microbes can survive at different temperatures, and *Azotobacter* is a typical mesophilic organism. Most research data predicts that 25–30° is the optimum temperature for the growth and for all the physiological properties of *Azotobacter*. The minimum temperature of growth of *Azotobacter* evidently lies a little above 0 °C. *Azotobacter* cells cannot tolerate high temperatures, but in the form of cysts, they can survive at 45–48 °C and can germinate under favorable conditions (Gul 2003). *A. salinestris* survived up to 45 °C and documented a maximum growth rate at 35 °C, and growth was reduced with increasing temperature.

#### 5.3.11.3 pH Tolerance

The presence of *A. chroococcum* in soil or water is strongly governed by the pH value of these substrates. The presence of *Azotobacter* population in soil ecosystem is controlled by pH concentration, and lower pH (<6.0) decreases the population or is completely absent. The optimum pH between 7 and 7.5 is favorable for the physiological functions of *Azotobacter*, and at this pH population number may fall between 102 and 104 per gram of soil (Becking 2006). *A. chroococcum* survived at a pH of 9.0 and did not observe any inhibition of growth at higher pH range. *A. salinestris* was sensitive to pH of above 9.0 and no growth was observed above this range.

## 5.4 Bioformulations and Shelf Life

The scientific term bioformulations generally refer to the development of formulations consisting of microorganisms that may substitute the use of chemical fertilizers partially or completely (Naveen et al. 2010). For the sustained availability of the biocontrol formulations, mass production and development of formulation have to be standardized which also increase the shelf life of the bacterial formulations. This is very important since microorganisms with PGPR cannot be applied as cell suspensions to the field. Therefore, organic carrier materials such as talcum powder, lignite, pyrophyllite, and zeolite are used which support and enhance the survival ability of the bacteria for considerable length of time (Nakkeeran et al. 2005).

The viable population of *Azotobacter* in different carrier materials was determined at different storage conditions. FYM formulation recorded highest population ($25.66 \times 10^5$) by *A. chroococcum*, and the lowest CFU ($18.00 \times 10^5$) was showed by *A. armeniacus* at 35 °C. More than 40 °C has reduced the survivability of bacteria and found only half of the population. All the isolates were survived at 4–45 °C of temperature but varied in the total population. As in case of lignite formulations, *A. salinestris* recorded highest CFU/mL of 22.33 × $10^5$ at 35 °C, and decreased growth trend was observed above 40 °C at 15 days of intervals. Lignite could be considered as carrier material for *Azotobacter* as biofertilizer formulations. Overall, all the isolates survived up to 12 months of incubation period at 35 °C, and decline in population rate was observed.

In talc formulation, *A. salinestris* isolate showed a steady population throughout the year. Among all, *A. salinestris* recorded a highest CFU (23 to 17.35 × $10^5$) up to 12 months of storage at 35 °C. The mean population in FYM formulations, *A. salinestris* and *A. chroococcum* isolate population, was maintained significantly for up to 6 months. Overall, the results depict that talc is the best carrier material to support the *A. salinestris* for longer shelf life at both room temperature and refrigerated temperature conditions, respectively, at the end of a year. Overall, the talc maintained the population *Azotobacter* uniformly.

Talcum-based formulations were developed as method suggested by Vidhyasekaran and Muthamilan (1995). The results revealed the colony-forming units of both *A. nigricans* and *A. salinestris* on Waksman selective media after 6 months of storage in the range of 3 × 107 to 4 × 107, respectively (Nagaraja et al. 2016) (Fig 5.3). This suggests the long-term survival ability of the *Azotobacter* strains and hence can be used as potent biocontrol agents against phytopathogens along with PGPR properties in improving plant growth. The talc-based bioformulation with other bacterial species such as *Pseudomonas fluorescens* strains, *Pseudomonas* strains, and *Rhizobium* sp. has been reported by Vidhyasekaran et al. (1997) and Naik et al. (2013).

## 5.5 What Are Fertilizers?

Plants, unlike all other living things, need food for their growth and development. They require major essential elements like carbon, hydrogen, and oxygen which are available from the atmosphere, water, and soil. The common essential elements like nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, manganese, copper, boron, molybdenum, and chlorine are available from soil minerals or organic matter or by organic or inorganic fertilizers (Al-Khiat 2006). Most of the soils are not fertile and doesn't contain complete elemental nutrients required for the plant growth. The supply and scarcity of these elemental nutrients can be minimized by the use of fertilizers and other chemical inputs for the growth and development of agricultural crops. Based on the production process and usage, the fertilizers can be roughly categorized into three types: chemical, organic, and biofertilizer (Jen-Hshuan 2006).

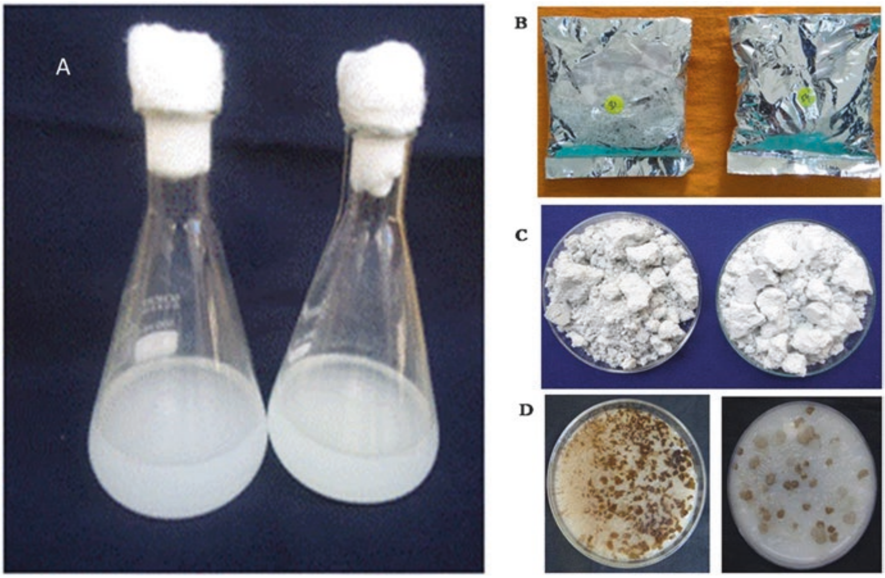


**Fig. 5.3** Mass multiplication and formulation of *Azotobacter salinestris* in Waksman broth (**a**) with lignite and talc formulations (**b** and **c**), viable cells of *A. salinestris* by spread plate count method (**d**)

## 5.6 Types of Fertilizers

### 5.6.1 Chemical Fertilizer (Synthetic Fertilizer)

Fertilizers play an important role in increasing the yield of agriculture produce. The macronutrients present in inorganic fertilizers include nitrogen, phosphorus, and potassium which influence vegetative and reproductive phase of plant growth (Patil 2010). Chemical fertilizer is often synthesized using Haber-Bosch process, which produces ammonia as the end product. Synthetic fertilizers are soluble and easily available to the plants; therefore, the effect is direct and fast. They are quite high in nutrient content; only relatively small amounts are required for crop growth (Jen-Hshuan 2006).

The use of chemical fertilizers alone has not been helpful under intensive agriculture because it aggravates soil degradation. The degradation is brought about by loss of organic matter which consequently results in soil acidity, nutrient imbalance, and low crop yields. The excessive use of chemical fertilizers has generated several environmental problems including the greenhouse effect, ozone layer depletion, and acidification of water. These problems can be tackled by use of biofertilizers (Saadatnia and Riahi 2009; Chennappa et al. 2015, 2016). Due to its high solubility, up to 70% of inorganic fertilizer can be lost through leaching, denitrification, and erosion, reducing their effectiveness (Ayoola and Makinde 2007; Alimi et al. 2007). Overapplication can result in negative effects such as leaching, pollution of water resources, destruction of beneficial microorganisms and friendly insects, crop susceptibility to disease attack, acidification or alkalization of the soil, or reduction in soil fertility, thus causing irreparable damage to the overall system (Jen-Hshuan 2006).

### 5.6.2 Organic Fertilizer

Organic fertilizer refers to materials (manure, worm castings, compost, seaweed) used as fertilizer that occur regularly in nature, usually as a by-product or end product of a naturally occurring process. Organic fertilizers typically provide the three major macronutrients required by plants: nitrogen, phosphorus, and potassium. Organic fertilizers such as manure have been used in agriculture for thousands of years (Thomas et al. 1990). In addition to increasing yield and fertilizing plants directly, organic fertilizers can improve the biodiversity and long-term productivity of soil. Organic nutrients increase the abundance of soil organisms such as fungal mycorrhiza by providing organic matter and micronutrients and can drastically reduce external inputs of pesticides, energy, and fertilizer, at the cost of decreased yield (wikipedia.org/wiki/Fertilizer).

Organic fertilizers are better sources of nutrient in balanced amounts than inorganic fertilizers where soil is deficient in both macro- and micronutrients. Organic-based fertilizer use is beneficial because it supplies micronutrients and organic components that increase soil moisture retention and reduce leaching of nutrients. Organic fertilizers can be used on acid-tolerant and those better suited to neutral or alkaline conditions (Alimi et al. 2007).

### 5.6.3 Biofertilizer

Biofertilizers are commonly called microbial inoculants which contain living microorganisms. When biofertilizers are applied to the seed or plant surfaces, they colonize the rhizosphere or interior of the plant and promote expansion of the root system and better seed germination by increasing the supply of primary nutrients to the host plant (Chandrasekar et al. 2005; Selvakumar et al. 2009). Biofertilizers can add 20–200 kg N ha1 by nitrogen fixation, secrete growth-promoting substances, and increase crop yield by 10–50%. They are cheaper, pollution-free, and based on renewable energy sources and also improve soil health (Saeed et al. 2004). For the last one decade, biofertilizers are used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improve soil fertility status, and enhance crop production by their biological activity in the rhizosphere (Contra 2003; Patil 2010).

Biofertilizers include mainly the nitrogen-fixing, phosphate-solubilizing and plant growth-promoting microorganisms. Among the most extensively used biofertilizers are *Azotobacter*, *Azospirillum*, blue-green algae, *Azolla*, *P*-solubilizing microorganisms, *mycorrhizae*, and *Sinorhizobium* (Selvakumar et al. 2009). Among

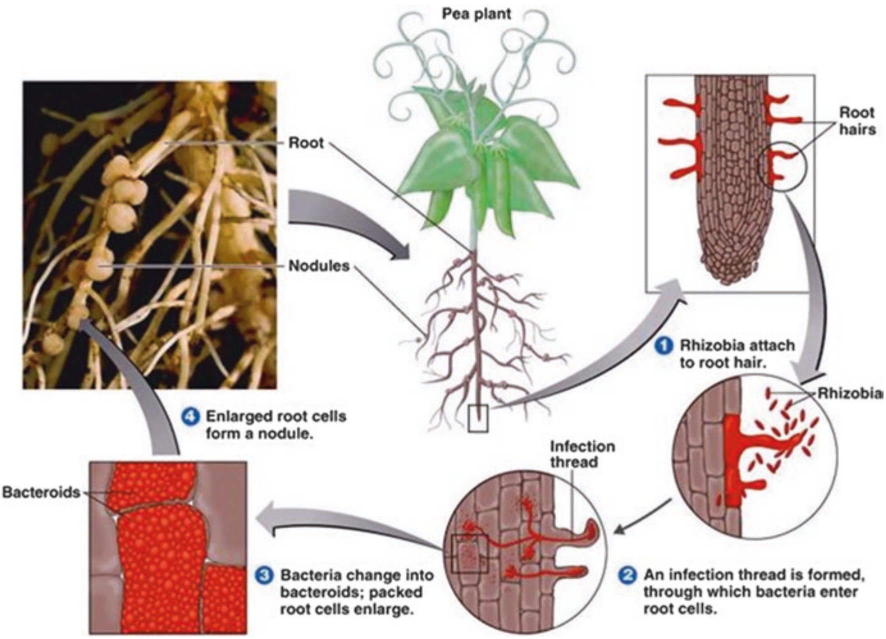


**Fig. 5.4** Schematic representation of biofertilizer applications and their mechanisms in plant root ecosystem (https://image.slidesharecdn.com/soilmicrobiologyzarrin-1-140807003503-phpapp01/95/soil-microbiology-33-638.jpg?cb=1407373113)

biofertilizers, *Azotobacter* strains play a key role in harnessing the atmospheric nitrogen through its fixation in the roots (Fig 5.4).

#### 5.6.3.1 *Azotobacter* as Biofertilizer

*Azotobacter* species are used as a biofertilizer for the cultivation of most agricultural crops such as cereals and pulses by direct application, by seed treatment, and by seedling dip methods because of its high nutritional conditions. *Azotobacter* increases seed's germinating ability, and it can increase germination by 20–30% because of the production of the plant growth-promoting compounds, which reduce chemical nitrogen and phosphorus by 25%, stimulating the plant growth. The direct promotion of plant growth by PGPR may include the production and release of secondary metabolites such as plant growth regulators or facilitating the uptake of certain nutrients from the root environment (Glick 1995; Polyanskaya et al. 2002).

The strains of *A. chroococcum* showed their ability to invade the endorhizosphere of wheat and higher production of cellulase and pectinase. *A. chroococcum* is beneficial for plantation as it enhanced growth and induced IAA production and phosphorus solubilization when compared with that of agrochemicals and other biofertilizers on agricultural crops (Sachin 2009). The higher concentration of agrochemical application, the lower is the plant growth (Matin et al. 2011). Different kinds of formulations have been developed from carrier material such as talc, lignite, and vermicompost which are being readily used all over the world. Among different carrier materials used, vermicompost was the best carrier material for the survival of *A. chroococcum*, and their cells have the most significant effect on improving the growth and yield parameters of summer rice cv. IR-36 (Roy et al. 2010).

Application of PGPR and phosphate-solubilizing bacteria (PSB) combination resulted in a positive effect on plant growth. Combined application of *Azotobacter* and *Azospirillum* bacteria at different levels of nitrogen for sunflower plant showed that these two bacteria increased plant growth characteristics and reduced the application of nitrogen fertilizer by 50%. Similarly, the application of *Azotobacter* can reduce nitrogen fertilizer consumption (Yousefi and Barzegar 2014).

### 5.6.4 Benefits of Biofertilizers over Chemical Fertilizers

Biofertilizers are used as inoculants and alternatives to chemical fertilizer, and these inoculants increase crop yield, soil fertility, permeability, and organic matter decomposition for sustainable agricultural systems (Silva and Uchida 2000). Biofertilizers maintain the natural habitat of the soil and increase crop yield by 20–30%, and it replaces chemical nitrogen and phosphorus by 25% in addition to stimulating the plant growth. Finally, it can provide protection against drought and some soilborne diseases. They are cost-effective relative to chemical fertilizer and reduce the costs toward fertilizer use. It is an environment-friendly fertilizer that not only prevents damaging the natural source but also helps to some extent clean the nature from precipitated chemical fertilizer and can provide better nourishment to plants.

Biofertilizers provide in addition to nitrogen certain growth-promoting substances like hormones, vitamins, amino acids, etc. On the other hand, biofertilizers supply the nitrogen continuously throughout the entire period of crop growth in the field under favorable conditions over chemical fertilizer (Al-Khiat 2006). Continuous uses of chemical fertilizers adversely affect the soil structure, whereas biofertilizers when applied to soil improve the soil structure. The effects of chemical fertilizers are that they are toxic at higher doses. Biofertilizers, however, have no toxic effects. Chemical fertilizers are expensive; they disturb the ecological balance of agroecosystems and cause pollution to the environment.

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