Chapter 8
Plant Nutrient Management Through Inoculation of Zinc-Solubilizing Bacteria for Sustainable Agriculture

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Abstract The agricultural practices adopted to enhance agricultural productivity have adversely affected our environment and the natural resources. Moreover, food security for the ever-increasing human population also demands improvement in the quality of agri-produce. Due to the very low concentration of micronutrients in cereals, human beings are suffering the deficiency of these micronutrients. Approximately one-third of the total population in developing countries is at high risk of Zn deficiency because they depend on cereals for their daily caloric intake. Indiscriminate use of agro-chemicals and chemical fertilizers to increase crop yield has caused considerably negative impact on environmental sustainability and has resulted in deficiency of micronutrients in soil and plants. The micronutrient deficiency has further resulted in loss of plant enzyme functions, cell damage, oxidative stress and metabolic disturbances and subsequently affected crop productivity. Increased interest in low-input agriculture in recent years has emphasized the use of biological inoculants (bacteria and/or fungi) to increase the mobilization of key nutrients (nitrogen, phosphorus, potassium and zinc) to crop plants. Zinc (Zn) is a crucial micronutrient for plants, microorganisms and humans. Therefore, effective strategies are required to overcome Zn deficiency in edible crops, to enhance the grain Zn content and to minimize the adverse effects of Zn deficiency on humans. Recently, inoculation of zinc-solubilizing bacteria has been recommended to overcome the zinc deficiency in plants and human beings. Zinc-solubilizing bacteria alone or with organic manures has been found to increase the bioavailability of native and applied zinc to the plants. Several bacteria including *Acinetobacter*, *Bacillus* and *Pseudomonas* have been reported to solubilize zinc. Thus, the production and management of biological fertilizers containing zinc-solubilizing bacteria can be an effective alternative to chemical fertilizers. The current knowledge about the characterization of zinc-solubilizing microorganisms (ZnSMs), complexity of the Zn-solubilization mechanisms and the interactions of biofertilizers under the field conditions leading to improved crop productivity is discussed in this chapter.

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8.1 Introduction

Plants require a variety of nutrients for optimum growth and metabolism. The inorganic forms of nutrients are absorbed along with water by the plant roots. Some of the micronutrients play a vital role in balanced crop nutrition and physiological functions and are therefore essential for plant growth and crop production. The common micronutrients important for plant metabolic activities are iron, copper, zinc, boron, nickel, manganese, molybdenum and chloride (Uchida 2000). Deficiency of any one of these micronutrients in the soil could retard plant growth, even if all other macro- or micronutrients are present in sufficient quantity (Yu and Rengel 1999). Most of the soils in world are deficient in micronutrients due to harvesting of micronutrients from the soil by growing of high-yield crops, increased use of NPK fertilizers containing lesser amounts of micronutrients and less use of organic manures and compost.

Among the different micronutrients, zinc is important for healthy growth, reproduction and metabolism of crop plants (Hughes and Poole 1989; Perumal et al. 2017). Zinc serves as an important component in a variety of enzymatic reactions, redox reactions and metabolic processes (Gandhi et al. 2014). Zinc has been reported to perform many critical functions in biological systems, including protection of structural and functional integrity of biological membranes, photosynthesis, biomass production, chlorophyll formation, nodulation, lipid and protein metabolism, carbohydrate synthesis, enhanced stress tolerance and reproductive processes (Thenua et al. 2014; Yu et al. 2017). Zinc is also required for the synthesis of phytohormones like auxins and cytokinins, which help in growth regulation and stem elongation in plants (Hussain et al. 2015). It is used for protection from free radicals and conversion of starches to sugars. It also plays a vital role in regulation of the gene expression needed for the tolerance of environmental stresses in plants (Cakmak 2000).

In areas where zinc deficiency is widespread in crops, there is a high risk for the health of livestock and humans. Zn plays a critical role in humans maintaining the activity of enzymes and is found responsible for controlling over 300 enzymatic reactions (Tapiero and Tew 2003). Solanki et al. (2016) reported that fertility problems have increased in the past few years in humans and animals in areas where zinc deficiency is more pronounced. The deficiency of important micronutrients such as iron and zinc may often lead to impairment in brain development and wound healing, and the person becomes immune-compromised to common infectious diseases such as pneumonia, diarrhoea and malaria (Prasad 2013). Mostly, the zinc and iron deficiencies are caused by a diet deficient in micronutrients or their non-bioavailability (Welch and Graham 2004).

Zinc deficiencies are commonly found in 30% of the global soils (Sharifi and Paymozd 2016) and have resulted in large losses in yield and quality of several crops and legumes worldwide. The low solubility of zinc in spite of its high abundance in soils is mainly responsible for widespread occurrence of zinc deficiency problem in crop plants (Cakmak 2008). In India, up to 50% of the agricultural land, particularly the whole of the Indo-Gangetic belt, is reeling under zinc deficiency and expected to further increase up to 63% by 2025 (Sunitha Kumari et al. 2016). The deficiency of zinc results in remarkable reduction in plant height and occurrence of whitish brown patches, which turn necrotic subsequently. This led to serious consequences when crop plants were grown on zinc-deficient soils, which resulted in grain yield reduction of up to 80%. Zn deficiency is very common in rice cultivation, and it stands next to nitrogen and phosphorus deficiency. Severe deficiency causes a decrease in the number of tillers and delay in crop maturity (Wissuwa et al. 2006). Mostly, chemical fertilizers are applied to overcome these nutritional constraints, and the impact of zinc application on increasing crop yields has been recorded on most crops, both under irrigated and rainfed conditions. Usually, the addition of 25 kg/ha $ZnSO_4$ heptahydrate, equivalent to 5 kg/ha zinc, is generally recommended for every year or alternate years for soil application. But, they are not cost-effective, and added fertilizers readily get converted into non-accessible insoluble form to plants.

Availability of zinc from insoluble sources is regulated by many factors, among which biochemical reactions of rhizospheric microorganisms play an important role in converting unavailable forms of zinc into available forms (Singh et al. 2005; Bapiri et al. 2012; Zamana et al. 2018). From the exogenous application of soluble zinc sources, only 20% of applied zinc is available for plant uptake (Bapiri et al. 2012). The unavailable or immobilized zinc, i.e. zinc phosphate, zinc oxide and zinc carbonate, is reverted to available forms by the inoculation of bacterial strains which can solubilize it by release of organic acids and decrease in pH (Wang et al. 2013; Sharma et al. 2014).

8.2 Importance of Zinc (Zn) in Metabolism of Plants, Humans and Microorganisms

The essentiality of zinc as a micronutrient in plants and animals is phenomenal (Das and Green 2013), and Zn is observed as the 23rd most copious element on Earth with five stable isotopes (Broadley et al. 2007). Zn^{2+} has distinct characteristics of Lewis acid and is considered to be redox-stable (Barak and Helmke 1993; Sinclair and Kramer 2012; Hafeez et al. 2013). Interestingly, Zn plays a prominent role in many biochemical reactions because it is a structural constituent or a regulatory cofactor for different enzymes and proteins. At the organism level, the significant role of ‘zinc finger’ as a structural motif is well established in regulation of transcription (Klug 1999; Englbrecht et al. 2004; Broadley et al. 2007).

8.2.1 Responses of Zinc in Plant Metabolism and Growth

Zinc performs several important functions in different plants. It is involved in the regulation of carbonic anhydrase for fixation to carbohydrates in plants and also promotes metabolism of carbohydrate, protein and auxin and pollen formation (Marschner 1995). Zinc has been found to govern the functioning of biological membranes and to perform defence mechanism against harmful pathogens. The presence of Zn in superoxide dismutase and catalase as a cofactor has been shown to protect plants from oxidative stress. Moreover, Zn is the component of all the six enzyme classes, i.e. oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases, which perform catalytic role in various biochemical reactions in plants.

Zinc is a component of the Rubisco structure, and therefore, it activates several biochemical reactions in the photosynthetic metabolism (Brown et al. 1993; Alloway 2004a, b). Zn has been found to inhibit the production of high toxic hydroxyl radicals in Haber–Weiss reactions in the thylakoid lamellae, due to its high affinity with cysteine and histidine (Brennan 2005; Disante et al. 2010; Tsonko and Lidon 2012). The uptake and availability of water to plants have also been found to be affected by the availability of Zn (Barcelo and Poschenrieder 1990; Tsonko and Lidon 2012). In addition, Zn is also involved in the formation of complexes with DNA and RNA (Pahlsson 1989; Coleman 1992). Due to its involvement in the tryptophan synthesis (precursor for indole acetic acid production), Zn has been reported to play an active role in signal transduction (Brown et al. 1993; Alloway 2004a, b; Hansch and Mendel 2009). By combining with phospholipids and sulphydryl groups of membrane proteins, Zn is also involved in the regulation of membranes. Based on its prominent role in different functions, the Zn concentration required for proper growth of the plant is estimated to be 15–20 mg Zn kg^{-1} dry weight (Marschner 1995). The Zn deficiency in plants may cause different symptoms and responses including necrosis at root apex and inward curling of leaf lamina, mottled leaf due to inter-veinal chlorosis, bronzing and internode shortening and size reductions in leaf. Significant losses in crop quality and quantity have been reported worldwide due to Zn deficiency in crops and legumes.

8.2.2 Effect of Zinc in Humans

Zinc is a structural component of several body enzymes in the human body. Deficiency of Zn may result from unsatisfactory consumption and inappropriate absorption of Zn in the body. More than 30% of world's population is found to suffer from severe Zn deficiency (Welch 2002), and Zn deficiency is the fifth most important risk factor responsible for illness and death of humans in the developing world (Cakmak 2009). Zinc has been reported to improve the immune system of humans (Walker and Black 2007; Gibson et al. 2008). Due to the deficiency of Zn, human body suffers from hair and memory loss, skin complications and weakness in body muscles. Insufficient Zn intake during pregnancy may cause stunted brain development of the foetus (Graham 2008; Benton 2008). Moreover, infertility has also been perceived in Zn-deficient men. Zinc deficiency may also cause congenital diseases like acrodermatitis enteropathica (Zimmermann and Hilty 2011; Kumar et al. 2016; Sharma et al. 2016). Zn deficiency in human beings is widespread in India, Pakistan, China, Iran and Turkey, and interestingly, these are the regions with Zn-deficient soils (Hotz and Brown 2004; Joy et al. 2015).

The detection and diagnosis of zinc deficiency in the human body is usually carried out by measuring zinc concentration in serum and other tissues (Hambidge and Krebs 2007). A common recommendation for an average male is for intake of 11 mg Zn per day, whereas an average female needs 9 mg of Zn daily. A female needs 13–14 mg of Zn on a daily basis during pregnancy and lactation because the requirement for zinc intake increases during this period (Hotz and Brown 2004). Zn has been found abundant in the rice husk and grains. Zn-rich foods include beef, pork, chicken and breakfast cereals; nuts like roasted peanuts, almonds, walnuts and oats; and dairy products such as yogurt, cheese and milk (Cakmak 2002; Masood and Bano 2016; Velazquez et al. 2016).

8.2.3 Role of Zinc in Microorganisms

The role of zinc in the nutrition and physiology of both eukaryotic and prokaryotic microorganisms is widely studied (Hughes and Poole 1989). Zinc deficiency in fungi and bacteria is accompanied by impairment of the formation of pigments such as melanin, chrisogenin, prodigiosin, subtilisin and others (Chernavina 1970). A few fungal genera possess immense potential of solubilizing zinc and tolerating a high zinc level. *Aspergillus niger* was found to grow under 1000 mg Zn, and this fungus is used to quantify zinc in soils containing low zinc (2 mg kg^{-1} available zinc) (Bullen and Kemila 1997). Lichens and conifers are conspicuous for their high zinc content, and the highest concentration of zinc has been found in poisonous mushrooms (Vinogradov 1965). Some bacteria, viz. *Thiobacillus thiooxidans*, *T. ferrooxidans* and facultative thermophilic iron oxidizers, have been reported to solubilize zinc from sulphide ore (sphalerite) (Hutchins et al. 1986).

8.2.4 Zinc Tolerance and Toxicity in Plants and Microbes

Zn is toxic to cellular organisms at high concentrations, but it is an indispensable component of thousands of proteins in plants, humans and microorganisms. Hence, adequate supply of Zn is critical for growth and development of organisms. Therefore, further efforts are required to understand the concept of application, acquisition and assimilation of zinc in plants. The exposure of leaf with elevated level of Zn, i.e. above 0.2 mg g^{-1} dry matter, has been found to cause multiple abnormal functioning in plant. This toxicity level resulted in deterioration of leaf tissue, and the productivity of plant is lowered by making their growth stagnant. Soybean and rice plants were found to show sensitivity toward toxic Zn concentration (Chaney 1993). Similarly, leafy vegetable crops, viz. spinach and beet, tend to accumulate a high concentration of Zn, and therefore, effect of Zn toxicity was observed in these crops (Boawn and Rasmussen 1971).

Zinc is also toxic to prokaryotic and eukaryotic microorganisms at higher concentrations, and therefore, zinc solubilization might limit the bacterial growth. Variable effects on the growth and activities of different microorganisms were observed by supplementation of zinc in the medium. For example, 10 mM concentration of Zn^{2+} decreased the survival of *Escherichia coli* but enhanced the survival of *Bacillus cereus*, whereas it did not significantly affect the survival of *Pseudomonas aeruginosa* and *Norcardia coralline* (Babich and Stotzky 1985). Saravanan et al. (2003) studied zinc tolerance limit of bacterial isolates ZSB-O-1 and ZSB-S-2, and population reduction was reported even at 25 mg L^{-1} of $ZnSO_4$ within 24 h. Nweke et al. (2006) assessed toxicity of Zn^{2+} on four planktonic bacteria by measuring dehydrogenase activity after exposing bacterial strains to various zinc concentrations (0.2–2.0 mM). Dehydrogenase activity was progressively inhibited at concentrations greater than 0.2 mM, indicating that these bacterial strains are sensitive to Zn^{2+} stress. Rajkumar et al. (2008) isolated a metal-resistant bacterial strain SM3 from a serpentine soil, and the strain was characterized as *Bacillus weihenstephanensis*. This strain exhibited resistance to nickel and zinc even at a concentration of 700 mg L^{-1} and also exhibited the capability of solubilizing phosphate both in the absence and presence of nickel, copper and zinc metals.

8.3 Prevalence of Zinc in Soil and Factors Affecting Zinc Availability

Zinc is found in the Earth's crust at a concentration of 0.008%, and more than 50% of Indian soils exhibit deficiency of zinc (Katyal and Rattan 1993; Ramesh et al. 2014). The worldwide prevalence of Zn deficiency in crops is due to low solubility of Zn rather low Zn availability in soil (Iqbal et al. 2010). The soluble zinc sulphate ($ZnSO_4$) is added as fertilizer to improve plant growth and crop productivity, but constraints are faced in absorbing zinc from the soil, because only 1–10% of total available zinc is utilized by the crop and 90% of applied zinc is transformed into different mineral fractions (Zn-fixation), which are not available for plant absorption (crystalline iron oxide bound and residual zinc). Zinc fixation is closely related to cation exchange in acidic soils, whereas under alkaline conditions, Zn fixation occurs by means of chemisorptions of zinc on calcium carbonate, which formed a solid solution of $ZnCaCO_3$ and by complexation by organic ligands (Alloway 2008).

The content of zinc and capacity to supply Zn for optimal crop growth varies widely in agriculture soils (White and Zasoski 1999). Soils deficient in their ability to supply Zn to crops are widespread all over the world including Australia (Sillanpaa 1990), China (Lui 1991) and India (Takkar 1996; Singh 2008; Behera et al. 2009b). The zinc applied to agriculture fields as zinc sulphate (soluble) gets converted to different insoluble forms like $Zn(OH)_2$ at high soil pH, $ZnCO_3$ in calcium-rich alkali soils and zinc phosphate in near-neutral to alkaline soils (with large application of P fertilizers) and ZnS under reducing conditions particularly during flooding (Sarathambal et al. 2010). Several factors have been found to affect Zn availability depending on the soil conditions. For example, solubility of Zn has been reported to decrease with the increase in pH (Anderson and Christensen 1988), high organic matter and bicarbonate content, high magnesium-to-calcium ratio and high availability of P and Fe (Wissuwa et al. 2006). Usually, extractable Zn was found to decrease with an increase in soil pH due to increased adsorptive capacity, formation of hydrolysed forms of zinc, possible chemisorption on calcium carbonate and co-precipitation in iron oxides (Cox and Kamprath 1972; Alloway 2008).

Zn deficiency is usually more prevalent in calcareous soils with high pH (Liu et al. 1983; Katyal and Vlek 1985). The problem of Zn deficiency is also more acute in sandy acidic soils having low organic matter content and low level of available plant nutrients (Rautaray et al. 2003). The acidic soils in India cover about 49 million ha of area, whereas more than 800 million ha of acidic soils are found worldwide (Sharma and Singh 2002). Therefore, soil acidity is causing a huge problem by affecting food production across Asia, Africa and Latin America, and it is imposing heavy costs on farmers in Europe and North America. Excessive accumulation of phosphorus in the soil has also been found to interfere on zinc uptake by plants, and thus, it has been found to cause zinc-imposed deficiency in plants (Salimpour et al. 2010).

After 7 years of continuous cropping of wheat (*Triticum aestivum*)—rice (*Oryza sativa*), wheat and maize (*Zea mays*) and chickpea (*Cicer arietinum*)—bajra (*Pennisetum typhoides*) decrease of soil pH was reported in a sandy loam soil (Chandi and Takkar 1982). These crop rotations showed diverse effects on labile Zn fractions in soil due to their effect on soil pH. Moreover, differential uptake of Zn by the crops was observed from different soil Zn fractions. Behera et al. (2009a) reported decline in organic matter and carbonate-bound Zn in an inceptisol as a result of intensive cropping with maize and wheat for more than three decades. Soil organic matter content was also reported to affect the availability of Zn (Lindsay 1972; Moody et al. 1997). High levels of organic matter increased exchangeable and organic fractions of Zn and decreased the oxide fractions of Zn in soil because of reducing conditions to enhance Zn availability for uptake by the plants.

Thus, Zn management in acidic soils is an emerging area of concern for obtaining higher crop yield. Soil surveys illustrating the geographic distribution of soil zinc availability will provide a better understanding of the nature and extent of zinc deficiencies and toxicities observed in plants, livestock and humans (White and Zasoski 1999). To evaluate the bioavailability of Zn in soils, several extractants are being used which include mineral acids, chelating agents, buffered salts and neutral salts. Diethylene triamine pentaacetic acid (DTPA) is the most widely used soil extractant for extraction of plant-available Zn in different soil types, but other extractants like ethylenediaminetetraacetic acid (EDTA), hydrochloric acid, ammonium bicarbonate-DTPA (ABDTPA), Mehlich 1 and Mehlich 3 are also widely used (Alloway 2008). The unavailability of zinc fertilizers at the time of need, poor quality of zinc fertilizers available in the market and lack of awareness of the farmers about effects of micronutrient on plant and human health are the major challenges faced by the farmers (Das and Green 2013).

8.4 Occurrence of Beneficial Microorganisms in the Rhizosphere

The plant–soil interface around living roots, termed as rhizosphere, is a narrow zone of soil that provides niche to various microorganisms including fungi, bacteria, actinomycetes, algae and nematodes (Prashar et al. 2014). Nearly 5–21% of all photosynthetically fixed carbon by plants is being transferred to the rhizosphere through root exudates (Marschner 1995; Flores et al. 1999). These root exudates support the growth of specific microbial populations and thereby markedly affect interactions between plants and the soil environment (Doornbos et al. 2012; Mendes et al. 2013). Phenolic metabolites released in root exudates attract particular rhizospheric and soil microbes and successfully manipulate the resident soil microbial population (Brimecombe et al. 2001).

Some plants shape their rhizosphere microbiome with the recruitment of beneficial bacteria or fungi (Berendsen et al. 2012), and host genotype also influences the overall composition of these microbial communities (Badri et al. 2013; Bulgarelli et al. 2015). In addition, edaphic and environmental factors also affect the composition of root microbiome (Hacquard et al. 2015). Legume plants release a specific kind of flavonoids in the root exudates, which interact with nodulation gene *nod*D of the host-specific rhizobia to establish symbiosis with legume plants (Bertin et al. 2003; Hassan and Mathesius 2011), which provide fixed nitrogen supply to the plant (Marschner et al. 2011; Oldroyd 2013). Some plant roots release strigolactones to attract mycorrhiza for improving phosphate supply (Akiyama et al. 2005). Recently, the changing climatic conditions were found to alter the rhizosphere biology by modifying rates of root exudation and biogeochemical cycling (Hawley et al. 2017). These rhizosphere bacteria improve plant growth by (1) supplying nutrients to crops; (2) producing plant hormones; (3) inhibiting the activity of plant pathogens; (4) improving soil structure; (5) reducing abiotic and biotic stress and (6) causing bioaccumulation or microbial leaching of inorganics and heavy metals (Ehrlich 1996; Sindhu et al. 2014).

Some beneficial rhizosphere microorganisms improve the plant growth and yield through nutrient cycling by providing mineralized nutrients (Bulgarelli et al. 2013; Sindhu et al. 2016, 2019). Beneficial plant growth-promoting rhizobacteria (termed as PGPR) include a wide range of genera, i.e. *Acinetobacter*, *Alcaligenes*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, *Rhizobium*, *Serratia*, etc.

(Sturz et al. 2000; Shoebitz et al. 2009). These rhizobacteria produce plant growth regulators/hormones, solubilize phosphorus and potassium, fix atmospheric inert nitrogen and act as elicitors for tolerance of abiotic and biotic stresses (Yang et al. 2008; Bhattacharyya and Jha 2012; Pérez-Montaño et al. 2014). Some bacteria produce phytohormones such as indole acetic acid (IAA), gibberellins (GA_3) and cytokinins, which alter root architecture and stimulate plant growth (Spaepen et al. 2007; Duca et al. 2014). Some species of *Pseudomonas* (e.g. *P. fluorescens*), *Streptomyces* and *Bacillus* have been found to inhibit the proliferation of the pathogens (Bhattacharyya and Jha 2012; Sharma et al. 2018b). Other PGPR strains have been reported to induce tolerance in plants to abiotic stresses. For instance, *Paenibacillus polymyxa*, *Achromobacter piechaudii* and *Rhizobium tropici* were found to ameliorate the drought stress in *Arabidopsis*, tomato (*Solanum lycopersicum*) and common bean (*Phaseolus vulgaris*), respectively, by accumulation of abscisic acid and due to degradation of reactive oxygen species and ACC (1-aminocyclopropane-1-carboxylate) (Mayak et al. 2004b; Yang et al. 2008). Salinity tolerance in plants was improved by inoculation of *Achromobacter piechaudii* and *B. subtilis* (Mayak et al. 2004a; Zhang et al. 2008; Choudhary and Sindhu 2016). Endophytic bacteria isolated from wild rice (*Oryza alta*) plants were found to supply fixed nitrogen to their host plants (Baldani et al. 2000; Chaudhary et al. 2012).

Infestation of plants with a pathogen has been reported to alter the soil microbiome composition through shifts in root exudation profile (Chaparro et al. 2013). For example, the presence of the pathogenic fungus *Fusarium graminearum* in the rhizosphere of barley triggered the exudation of many phenolic compounds that prevented fungal spore germination (Lanoue et al. 2009). The rhizobacterium Pst DC3000 was chemoattracted by secretion of L-malic acid by roots in response to infection of foliage. The interaction of the *B. subtilis* strain FB17 with the *Arabidopsis* plants altered the expression of host plant genes, which are involved in regulation of auxin production, metabolism, defence and stress responses and also caused modifications in cell wall (Lakshmanan et al. 2012). The hormones involved in plant immunity, i.e. salicylic acid and jasmonic acid, were also found to affect the root microbiome (Lebeis et al. 2015). Therefore, further understanding of the rhizosphere biology is required for promoting beneficial plant–microbe interactions as a low-input biotechnology for sustainable agriculture (Ryan et al. 2009; Dubey et al. 2016).

8.5 Characterization of Zinc-Solubilizing Bacteria from Rhizosphere

The soluble form of zinc fertilizers are applied to the field soils to surmount the Zn deficiency. These chemical fertilizers are very costly and cause pollution in soil, air and water. Therefore, an eco-friendly and cost-effective approach is required to supplement the Zn deficiency by inoculation of Zn-solubilizing microorganisms.

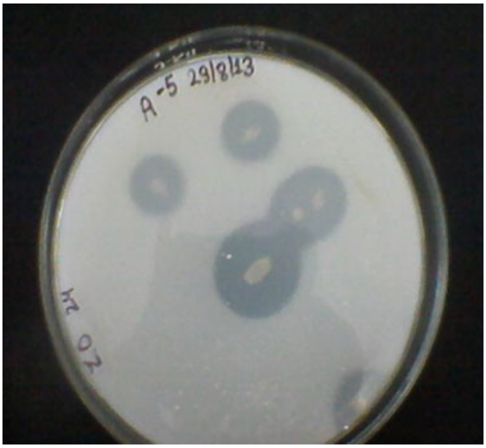

Fig. 8.1 Solubilization zone formed by zinc-solubilizing bacteria

Recently, the use of beneficial microorganisms is advocated for sustainable agriculture and restoration of soil fertility (Sindhu et al. 2019). For improving Zn availability in field soils, solubilization of insoluble Zn compounds [ZnO, $ZnCO_3$, $Zn_3(PO_4)_2$] by plant growth-promoting rhizobacteria has been reported (Saravanan et al. 2007a, b; Sharma et al. 2012; Krithika and Balachandar 2016; Gontia-Mishra et al. 2016) (Fig. 8.1). The inoculation of Zn-solubilizing bacteria (ZSB) has been found to increase the availability of soluble zinc for plant assimilation and eventually resulting in plant growth promotion.

Bacteria including *Thiobacillus thiooxidans*, *T. ferrooxidans* and facultative thermophilic iron oxidizers were reported to solubilize zinc from sulphide ore (Hutchins et al. 1986). Simine et al. (1998) isolated a zinc-solubilizing *Pseudomonas fluorescens* strain from forest soil. Zinc-solubilizing ability of *Bacillus* sp. (isolated from zinc ore) and *Pseudomonas* sp. (isolated from paddy soil) was assessed using zinc oxide, zinc sulphide and zinc carbonate in both plate and broth assays (Saravanan et al. 2003). A strain of *Gluconacetobacter diazotrophicus* was isolated that caused zinc solubilization and also showed anti-nematode activity against *Meloidogyne incognita* (Saravanan et al. 2007a, b). Sindhu (2014) obtained 38 bacterial isolates from rhizosphere soil of different crops and screened these isolates for solubilization of various insoluble zinc sources, i.e. zinc oxide, zinc sulphide and zinc carbonate. All the rhizobacterial isolates solubilized zinc oxide with solubilization index ranging from 1.56 to 36.00. Only three isolates solubilized zinc sulphide with the index varying from 1.96 to 4.00, and 33 isolates solubilized zinc carbonate with index 3.36 to 25.00. Fourteen rhizobacterial isolates showing zinc solubilization index more than 15.00 on zinc oxide-containing plates were also screened for phosphorus (P) solubilization and IAA production. All the 14 bacterial isolates solubilized P with an index ranging from 1.56 to 14.87, and only 11 isolates showed IAA production that varied in the range of 4.06–8.77 μg mL^{-1}.

Sharma et al. (2014) isolated 48 endophytic bacteria from soybean (43) and summer mungbean (5) rhizosphere. The zinc-solubilizing ability of these isolates was studied in Tris minimal medium separately amended with inorganic zinc compounds, viz. zinc oxide (ZnO) and zinc phosphate $Zn_3(PO_4)_2$ by plate assay method. Only two bacterial isolates solubilized ZnO, while other two isolates solubilized $Zn(PO_4)_2$ on Tris minimal medium. Due to their efficiency of phosphate solubilization, zinc solubilization and IAA production, endophytes 1J (*Klebsiella* spp.) and 19D (*Pseudomonas* spp.) were found to be the most promising bacterial isolates for stimulation of plant growth. Similarly, Gandhi et al. (2014) isolated 240 zinc-solubilizing bacterial strains from rhizosphere of rice, and of them, 15 isolates were found efficient zinc solubilizers. From eight different agricultural fields of Coimbatore district of Tamil Nadu, 35 zinc-solubilizing bacteria were isolated (Sunitha Kumari et al. 2016). Five bacterial isolates were selected as the best strains based on their solubilization efficacy and were identified using the 16S rRNA sequencing method. Of the five bacterial isolates, *Pseudomonas aeruginosa* showed maximum solubilization of zinc in the broth and also decreased the pH from 7 to 3.3.

Perumal et al. (2017) isolated six zinc-solubilizing bacterial strains from the rhizosphere of maize. Bacterial isolate ZSB SM-1 was found to be most effective in solubilization of insoluble zinc substances, viz. zinc oxide, zinc carbonate and Zn-EDTA. The insoluble Zn compounds were effectively solubilized at 0.1% concentration as compared to 0.2% concentration. Dhaked et al. (2017) isolated four potassium-solubilizing bacteria (KSB), eight zinc-solubilizing bacteria (ZnSB) and two zinc-solubilizing fungi (ZnSF) from rice, maize, cotton and sorghum rhizosphere soil. Screening of the KSB isolates for solubilization of insoluble zinc oxide showed that the solubilization zone for zinc oxide ranged from 6 to 16 mm. The isolate ZnSB-3 showed maximum solubilization zone of 16 mm, and the solubilization efficiency ranged from 150% to 333.33%. The isolate ZnSF-1 showed maximum solubilization zone of 85 mm followed by ZnSF-2 with 34 mm for ZnO. The solubilization zone ranged from 6 mm to 25 mm for ZnP. The isolate ZnSB-8 showed maximum solubilization zone of 25 mm for zinc phosphate, and solubilization efficiency ranged from 157.14% to 500%.

8.6 Mechanisms Involved in Solubilization of Zinc by Zinc-Solubilizing Bacteria

Zinc-solubilizing bacteria increase the availability of zinc in the rhizosphere through different mechanisms, which ultimately improve the uptake of soluble zinc by the plant (Fig. 8.2). Different mechanisms employed by zinc-solubilizing bacteria to improve zinc bioavailability are discussed below.

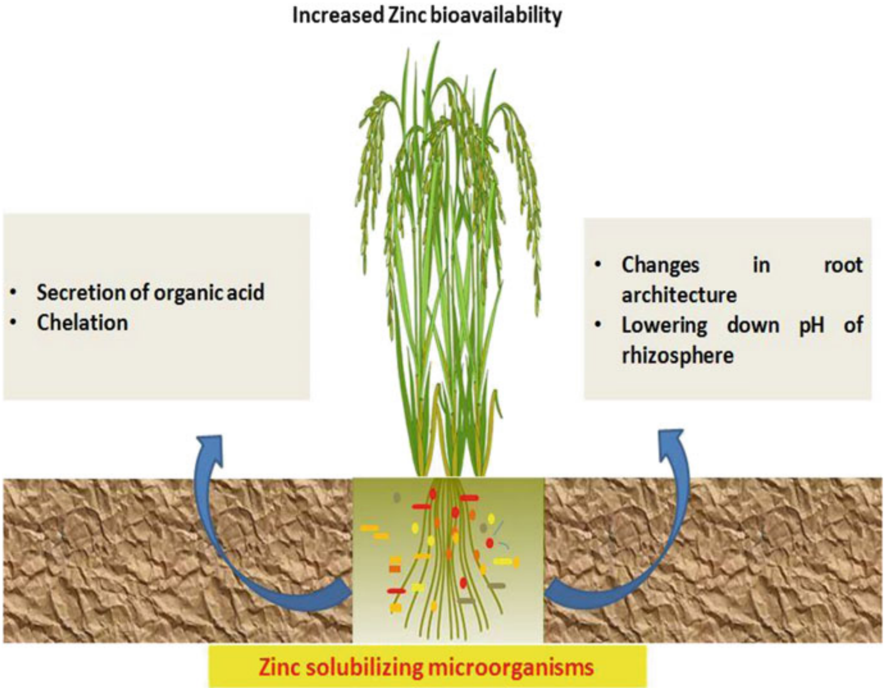

Fig. 8.2 Mechanisms involved in solubilization of zinc by microorganisms in the rhizosphere of crop plant

8.6.1 Lowering the pH of Rhizosphere

Plant growth-promoting bacteria have been reported to release organic acids and extrude protons, which lowers the pH of the rhizosphere (Fasim et al. 2002; Wu et al. 2006; Parmar and Sindhu 2018). For example, the secretion of 2-ketogluconic acid and gluconic acid by *Pseudomonas fluorescens* resulted in solubilization of zinc phosphate in the culture. Furthermore, coinoculation of *Pseudomonas* and *Bacillus* spp. in broth culture lowered down the pH, which solubilized zinc sulphide, zinc oxide and zinc carbonate (Saravanan et al. 2004). The availability of micronutrients in soil is also influenced by the pH of the soil, and it has been reported that decrease in one unit of pH resulted in 100 times increase in the availability of Zn in the soil (Havlin et al. 2005). The role of low pH has also been correlated with potassium solubilization in efficient potassium-solubilizing strains, i.e. *Bacillus subtilis* ANctcri 3 and *Bacillus megaterium* ANctcri 7 isolated from rocks in Kerala (Anjanadevi et al. 2016). Similarly, inoculation of arbuscular mycorrhizae (AM) was found to lower the soil pH in the rhizosphere, and it contributed to release of zinc from mineral fraction (Subramanian et al. 2009). However, the reduction in rhizosphere pH varied among different microorganisms (Giri et al. 2005). Wu et al. (2006) observed a decrease in pH up to 0.47 units with bacterial inoculation due to the release of organic acids and H^+, which ultimately improved the Zn solubilization and uptake by plants.

8.6.2 Zinc Chelation

Chelation of zinc by soil/rhizosphere microorganisms is another dominant mechanism to improve Zn bioavailability and uptake by plant roots. Usually, the plant-available Zn fraction in the soil is less due to low persistency and high reactivity of Zn in soil solution. Zn-chelating compounds have been found to increase the bioavailability of zinc in the rhizosphere (Obrador et al. 2003). These chelating compounds are released by the plant roots and microorganisms present in the rhizosphere, which chelate the Zn and increase its availability in root zone of the plants. Various metabolites secreted by the rhizosphere microorganisms form complexes with Zn^{2+} (Tarkalson et al. 1998) and thereby reduce their reaction with the soil. Some bacteria, e.g. *Pseudomonas monteilii*, *Microbacterium saperdae* and *Enterobacter cancerogenesis*, have been found to synthesize Zn-chelating metallophores for enhancing water-soluble Zn, which is bioavailable in soil for plant uptake (Whiting et al. 2001). Tariq et al. (2007) reported release of fixed insoluble zinc by the biofertilizer strains containing *Pseudomonas* sp. (96-51), *Azospirillum lipoferum* (JCM-1270, ER-20) and *Agrobacterium* sp. (Ca-18) due to production of chelating agent ethylenediaminetetraacetic acid and made the zinc available for longer period to rice. Inoculation of *Penicillium bilaji* was found to enhance the bioavailability of zinc to plants through chelating mechanism (Kucey 1987).

8.6.3 Organic Acid Production

The production of organic acids like citric, oxalic and tartaric acids and the production of capsular polysaccharides by microorganisms were found to cause dissolution of the minerals illite and feldspar to release potassium (Vyas and Gulati 2009; Qureshi et al. 2017; Parmar and Sindhu 2018). The pH of the medium decreased from 7.0 to 2.05 after growth of bacterial and fungal cultures during bioextraction of potassium using feldspar. Species of *Bacillus* and *Pseudomonas* were found to produce organic acids, which decreased the pH in the root zone, and Zn was made available to plants (Saravanan et al. 2004). Some PGPR strains were reported to produce gluconic acids (Saravanan et al. 2011) or its derivatives such as 2-ketogluconic acid (Fasim et al. 2002), 5-ketogluconic acid (Saravanan et al. 2007a, b) and various other organic acids (Tariq et al. 2007) for solubilization of zinc. Zinc phosphate solubilization was studied by a strain of *Pseudomonas fluorescens* and gluconic acids produced in culture medium was found to help in solubilization of zinc salts (Simine et al. 1998). Similarly, *Bacillus* sp. AZ6 was found to solubilize insoluble zinc compounds by releasing organic acids like cinnamic acid, ferulic acid, caffeic acid, chlorogenic acid, syringic acid and gallic acid in a liquid medium (Hussain et al. 2011). Martino et al. (2003) found that mycorrhizal fungi secreted organic acids to solubilize zinc from insoluble $Zn_3(PO4)_2$ and ZnO.

Enhanced production of organic acids was found to improve the available zinc in the culture broth. Desai et al. (2012) reported that higher availability of Zn is directly proportional to acidic pH of the culture broth. Solubilization of zinc phosphate occurred by both an increase in the H^+ concentration of the medium and the production of gluconic acid. Perumal et al. (2017) studied solubilization of insoluble zinc substances, viz. zinc oxide, zinc carbonate and Zn-EDTA using six bacterial strains isolated from the rhizosphere of maize. They concluded that solubilization of zinc from insoluble zinc substances might be due to production of acids by the culture, since the pH of the broth decreased from 7.0–7.3 to 3.0–4.8 after 10 days of inoculation.

8.7 Inoculation Effect of Zinc-Solubilizing Bacteria on Crop Growth and Yield

Micronutrient deficiencies in the soil have been found to reduce the quality and yield of the agriculture produce. It has been reported that more than 3 billion people worldwide experience micronutrient deficiency (Hennessy et al. 2014). Zn deficiency is reported as a global nutritional problem, and this deficiency is more severe in developing countries (Zamana et al. 2018). The Zn deficiency has been attributed to consumption of cereal grains having very low grain Zn concentrations, which are usually grown in Zn-deficient soils. Zinc deficiency can be minimized by nutritional diversification, food enrichment and biofortification. Zinc biofortification is a viable choice to augment the bioavailable concentrations of vital micronutrients in edible portions of crop plants through agronomic practices or genetic methods (Zamana et al. 2018). The quality of crop produce biofortification has been found to depend on the chemical properties of the soil, crop genotypes, agricultural management practices and climatic factors (Schulin et al. 2009). Attempts are being made worldwide to improve the genetic potential of crop plants for enhancing the micronutrient bioavailability in common staple food crops such as wheat, rice, maize, beans and oilseeds (Cakmak et al. 2010). Plant breeding approaches are being used to enhance the amount of a number of minerals concurrently available in edible tissues of food, whereas transgenic approaches are used to improve nutrient mobilization from the soil, transport to the shoot and leaf and build-up of mineral elements in bioavailable forms in edible tissues (Borrill et al. 2014). The plant breeding approach to increase micronutrient uptake by plant roots is tedious, and results take a long time, whereas the transgenic approach is costly.

Another eco-friendly alternative approach is the application of potential plant growth promoting microorganisms (PGPMs) to increase micronutrient uptake by roots. These PGPMs could facilitate the growth of crop plants by modulating of root architecture resulting in growth of deep root systems in nutrient-deficient soils and

Table 8.1 Effect of various zinc-solubilizing bacterial isolates on plant growth parameters

Zinc-solubilizing bacterial isolates	Effects on plant growth	Crop plant	Reference
Pseudomonas sp. strain ZSB-S-I	Improved the zinc content in plant tissues	Soybean	Saravanan et al. (2004)
Pseudomonas strain BA-8 and *Bacillus* strain M-3	Increased fruit yield per plant, i.e. 91.73% and 81.58% when treated with BA-8+M-3 and M-3, respectively	Strawberry	Esitken et al. (2009)
P. aeruginosa strain CMG860	Increase in root (144%) and shoot length (120%)	Rye	Shahab et al. (2009)
Bacillus isolates	Increase zinc accumulation in seeds	Soybean	Sharma et al. (2012)
Pseudomonas strains B_1 and B_2	Increased grain Zn concentration (31%)	Rice	Deepak et al. (2013)
Burkholderia strain BC and *Acinetobacter* strains AB and AX	Increased mean number of productive tillers (21.1%), number of grains per year (5.7%), thousand grain weight (10.1%), grain yield (18.1%) and straw yield (3.1%) and reduced phytic acid concentration (17.6%)	Wheat	Vaid et al. (2013)
Bacillus aryabhattai strains MDSR7, MDSR11 and MDSR14	Strains MDSR7 and MDSR14 substantially influenced mobilization of zinc and its concentration in edible portion, yield of soybean and wheat	Soybean and wheat	Ramesh et al. (2014)
Bradyrhizobium japonicum	Phosphorus supplementation caused increase in micronutrients uptake; but decrease in Zn content was observed in few organs	Cowpea	Nyoki and Ndakidemi (2014)
Bacillus strain AZ6	Increased shoot length (59%) and photosynthetic rate (90%)	Maize	Hussain et al. (2015)
Bacillus sp. and *Bacillus cereus*	Suppressed *Pyricularia oryzae* and *Fusarium moniliforme*, and enhanced Zn translocation toward grains and increased yield of basmati-385 (22–49%) and super basmati rice varieties (18–47%)	Rice	Shakeel et al. (2015)

the excretion of ligands/siderophores or acids/alkalis to mobilize micronutrients. Microbial transformation of unavailable forms of soil zinc to plant-available zinc by zinc-solubilizing bacteria could influence the mobilization and uptake of zinc in edible portion and may improve the yield of different cereals, legumes and horticulture plants (Table 8.1).

8.7.1 Zinc Uptake by PGPR and ZnSB

Saravanan et al. (2004) isolated zinc-solubilizing bacterial cultures from soil and ore (sphalerite) sources both by direct plating and by enrichment technique in the modified Bunt and Rovira medium incorporated with 0.1% zinc. Among these, ZSB-O-1 and ZSB-S-4 were characterized as *Bacillus* sp. and ZSB-S-2 as *Pseudomonas* sp. The results revealed that *Pseudomonas* sp. (ZSB-S-1) was able to correct the zinc deficiency in soybean plants when used along with 1% (w/w) zinc oxide. Tariq et al. (2007) inoculated plant growth-promoting rhizobacteria for mobilizing indigenous soil zinc in rice (*Oryza sativa* L.) and compared it with the available form of chemical Zn source as Zn-EDTA. Application of PGPR decreased the zinc deficiency symptoms and increased the total biomass (23%), grain yield (65%) and zinc concentration in the grains invariably. Positive effects on root length (54%), root weight (74%), root volume (62%), root area (75%), shoot weight (23%), panicle emergence index (96%) and higher Zn mobilization efficiency were observed in inoculated plants in comparison to the uninoculated control. Li et al. (2007) investigated the effects of *Burkholderia cepacia* on metal uptake by the hyperaccumulating plant *Sedum alfredii* with different concentrations of cadmium and zinc. Inoculation with bacteria significantly enhanced plant growth (up to 110% with zinc treatment), phosphorus uptake (up to 56.1% with cadmium treatment), and metal uptake (up to 243% and 96.3% with cadmium and zinc treatment, respectively) in shoots, the tolerance index (up to 134% with zinc treatment) and translocation of metals (up to 296% and 135% with cadmium and zinc treatment, respectively) from root to shoot.

Kuffner et al. (2008) obtained ten rhizospheric isolates (*Pseudomonas*, *Janthinobacterium*, *Serratia*, *Flavobacterium*, *Streptomyces* and *Agromyces*) from heavy-metal-accumulating willows. These isolates were analysed for plant growth promotion and zinc and cadmium uptake in *Salix caprea* plantlets grown in sterilized, zinc–cadmium–lead-contaminated soil. *Agromyces* strain AR33 was found to increase plant growth and also enhanced the total amount of zinc and cadmium extracted from soil. Iqbal et al. (2010) studied the inoculation effects of five bacterial isolates (U, 8M, 36, 102 and 111) on the growth of *Vigna radiata*. Bacterial isolates were applied alone or together with zinc phosphate [$Zn_3(PO_4)_2 \cdot 4H_2O$]. The maximum increase in root and shoot length was observed as a result of inoculation with the isolate 102. The fresh and dry weight of seedlings was also enhanced in comparison to control. Bacterial isolate 36 with amendment of 1 mM zinc phosphate resulted in a maximum increase of almost 1.7 times in the seedling length (35.1 cm) in comparison to control (19.3 cm), indicating that bacteria can be used as a biofertilizer for improving the growth of mungbean plants in presence of water-insoluble zinc phosphate.

Sharma et al. (2012) isolated 134 *Bacillus* isolates from soybean rhizosphere soils to select effective zinc solubilizers for increased assimilation of Zn in soybean seeds. Inoculation of *Bacillus* isolates significantly increased the Zn concentration in soybean as compared with uninoculated control (47.14 μg/g). Goteti et al. (2013)

screened ten zinc solubilizing strains on maize crop in a short-term pot culture experiment. Seed bacterization with zinc-solubilizing *Pseudomonas* sp. strain P29 significantly enhanced the concentrations of macronutrients and micronutrients such as manganese (60 ppm) and zinc (278.8 ppm) in comparison to uninoculated control. In similar studies, Vaid et al. (2014) assessed the capacity of three bacterial strains, i.e. *Burkholderia* strain BC and *Acinetobacter* strains AB and AX, isolated from a zinc-deficient rice–wheat field to improve Zn nutrition in Zn-responsive (NDR359) and Zn non-responsive (PD16) varieties of rice. Bacterial inoculation significantly enhanced the total zinc uptake per pot (52.5%) as well as grain methionine concentration (38.8%). Inoculation with bacteria either singly or in combination significantly increased the mean dry matter yield/pot (12.9%), productive tillers/plant (15.1%), grain yield (17.0%) and straw yield (12.4%) over the control and Zn fertilizer treatment. The phytate-to-zinc ratio in grains was also reduced by 38.4% in treatments with bacterial inoculations.

8.7.2 Inoculation Effect of AM Fungi on Zinc Uptake

Root colonization by arbuscular mycorrhizal (AM) fungi was found to increase the uptake of metal micronutrients, such as copper in white clover (Li et al. 1991), copper, zinc, manganese and iron in *Zea mays* (Liu et al. 2000) and zinc in field pea crops (Ryan and Angus 2003). Similarly, higher uptake of iron, manganese, zinc and copper was reported in wheat by inoculation of *Azospirillum* and mycorrhizae in comparison with uninoculated control plants (Ardakani et al. 2011). Inoculation of rice roots with arbuscular mycorrhizal fungi was found to increase zinc uptake and mobilization and showed enhanced growth of rice (Purakayastha and Chhonkar 2001). Higher Zn uptake and increase in wheat and maize growth was observed by inoculation of AM fungi in zinc-deficient soils after addition of zinc as a fertilizer (Kothari et al. 1990).

8.7.3 Application of ZnSB Along with Manure and Fertilizers

Strains of *Bacillus cereus* (N_2 fixing), *Brevibacillus reuszeri* (phosphorus solubilizing) and *Rhizobium rubi* (both N_2 fixing and phosphorus solubilizing) were inoculated on broccoli to evaluate their effect on plant growth, nutrient uptake and yield in comparison with manure (control) and mineral fertilizer application under field conditions (Yildirim et al. 2011). Bacterial inoculations with manure significantly increased yield, plant weight, head diameter, chlorophyll content and nitrogen, potassium, calcium, sulphur, phosphorus, magnesium, iron, manganese, zinc and copper content of broccoli in comparison to control treatment. Senthilkumar et al. (2014) reported that the combination of fertigation and a consortium of biofertilizers in banana significantly enhanced accumulation of secondary nutrients and micronutrients (Fe, Zn, V and Mn) in the leaves, pseudostem and fruits at harvest. Senthil et al. (2004) conducted a field study to assess the effect of Zn-enriched organic manures and Zn-solubilizing bacteria on the yield, curcumin content of turmeric and nutrient status of the soil. When treated with farm yard manure (FYM) along with zinc-solubilizing bacteria, higher turmeric rhizome yield (21.6%) was observed in comparison with those treated with FYM alone (9.1%) and without manure (control). The dry rhizome yield showed the promising effect of Zn- and Fe-enriched coir pith or FYM. The highest values for available N, P and K contents in the soil were observed by use of FYM along with Zn-solubilizing bacteria. Significant effect on the availability of N, P and K was observed in treatment with inoculation of Zn-solubilizing *Bacillus* sp. The application of $ZnSO_4$, $FeSO_4$ and fortified FYM along with Zn and Fe and their foliar spray showed synergistic effect and enhanced the bioavailability of micronutrients as well as potassium.

The effect of micronutrients and inoculation of zinc-solubilizing bacteria was studied on the yield and quality of grape variety Thompson seedless (Subramoniam et al. 2006). Recommended doses of N, P and K fertilizers were applied along with foliar sprays of $ZnSO_4$ (0.2%) + boric acid (0.2%) + $FeSO_4$ (0.2%) + $MnSO_4$ (0.2%) + $MgSO_4$ (0.5%) + $CaCl_2$ (0.5%) + KNO_3 (0.5%) + urea (1%) at blooming and 15 days after blooming stages. Both the inoculation of zinc-solubilizing bacteria along with application of fertilizers and foliar sprays were recommended as cost-effective technology for increasing the grape yield. The fruits' quality such as juice content, TSS, titratable acidity, specific gravity, total sugar and TSS/acidity ratio were also higher in the treatment having inoculation of zinc-solubilizing bacteria along with fertilizers in comparison to control uninoculated treatment.

8.7.4 Coinoculation of Phosphorus- and Zinc-Solubilizing Bacteria

Phosphorus is the second major plant nutrient required for the proper growth and metabolic activities of a plant (Sindhu et al. 2014). Hu et al. (2006) isolated two phosphate- and potassium-solubilizing *Paenibacillus mucilaginosus* strains KNP413 and KNP414 from the soil of Tianmu Mountain. Both the strains effectively dissolved mineral phosphate and potassium, while strain KNP414 showed higher dissolution capacity. In a similar way, it is desired that coinoculation of phosphorus or potassium-solubilizing bacteria having zinc solubilizing activity may show synergistic effects leading to significant stimulation of the plant growth. Woo et al. (2010) isolated phosphate-solubilizing bacterial isolates from the rhizosphere of Chinese cabbage and found that 10 strains having higher phosphorus-solubilization potential also solubilized insoluble ZnO. Recently, Zeng et al. (2017) reported that production of organic acids by *Pseudomonas frederiksbergensis* strain JW-SD2 is correlated with phosphorus-solubilizing activity, and its effects on plant growth promotion of poplar seedlings were greater in the non-sterilized than sterilized soil.

To assess the impacts of *B. japonicum* inoculation and phosphorus supplementation on the uptake of micronutrients in cowpea, a field and pot house experiment was conducted (Nyoki and Ndakidemi 2014). Significant improvement in micronutrients uptake was observed in the *B. japonicum*-inoculated treatments over the control. Phosphorus supplementation (40 kg P/ha) also resulted in significant increase in the uptake of some micronutrients, while it caused decrease in Zn uptake in few plant organs. Significant interaction between *B. japonicum* inoculation and addition of phosphorus was observed with the root uptake of Zn for the field experiment. Sindhu (2014) tested three bacterial isolates MR1, CR2 and OR1 for zinc solubilization, and their inoculation effect was studied on growth and yield of mungbean crop under pot house conditions. The inoculation of isolate MR1 caused 72.6% increase in shoot dry weight in comparison to uninoculated control. Inoculation of mungbean with bacterial isolates MR1 and CR2 showed 104.8% and 72.0% increase in seed yield, respectively, as compared to uninoculated control. Treatment with $ZnSO_4$ at 25 kg ha^{-1} along with inoculation of isolate OR1 was found significantly superior to all other treatments and caused 184% and 92.6% increase in seed yield and shoot dry weight in comparison to uninoculated control. The selected two strains, CR2 (highest zinc solubilizer) and OR1 (highest plant growth promoter), were identified as *Bacillus stratosphericus* and *Bacillus altitudinis* by 16S rRNA gene sequence analysis. It was concluded that the *Bacillus altitudinis* isolate OR1 showing maximum plant growth promotion effect under pot house conditions could be exploited as a Zn-solubilizing biofertilizer for plant growth promotion of mungbean under field conditions.

8.7.5 ZnSB Role in Disease Control

Global crop yields are reduced by 20–40% annually due to pests and diseases (Strange and Scott 2005). Sustainable agricultural practices are revitalizing the interest of scientists in characterization of plant beneficial microorganisms having both nutrient mobilization and control of plant diseases by biological control agents. Recently, some of the microbial strains were isolated for solubilization/mobilization of phosphorous, potassium or zinc, and these strains also inhibited the growth of pathogenic fungi resulting in suppression of plant diseases (Sharma et al. 2018a, b; Parmar and Sindhu 2018). Zinc-solubilizing bacteria *Gluconacetobacter diazotrophicus* was found to possess antagonistic activities, and therefore, it was also used as a biocontrol agent against root nematodes and various fungal phytopathogens (Saravanan et al. 2007a, b). Shakeel et al. (2015) isolated *Bacillus* sp. and *Bacillus cereus*, which suppressed the growth of *Pyricularia oryzae* and *Fusarium moniliforme* (22%–29%), and their inoculation increased the yield of basmati rice variety 385 by 22–49% and super basmati rice varieties by 18–47%. Inoculation of zinc-solubilizing bacteria and their consortium in wheat along with $ZnSO_4.7H_2O$ at 5 mM significantly enhanced the plant height, chlorophyll content and grain number of wheat plants (Deepak et al. 2013).

8.7.6 Auxin Production by Zinc-Solubilizing Bacteria

Phytohormones have been found to affect the physiological processes of plants. Production of indole acetic acid (IAA) is more frequent among rhizosphere bacteria than other hormones such as gibberllic acid and cytokinins (Spaepen and Vanderleyden 2011). About 80% of rhizosphere bacteria have been reported to possess IAA production ability (Patten and Glick 1996; Jangu and Sindhu 2011). Skoog (1940) reported relationship between zinc solubilization and auxin production, which resulted in improvement of growth in higher plants. Shahab et al. (2009) tested efficient zinc phosphate-solubilizing bacteria for auxin production. These bacteria exhibited positive effects on the growth of root and shoot elongation of mung bean (*Vigna radiata*). Sindhu (2014) isolated 38 zinc-solubilizing bacteria from rhizosphere soil of different crops. Fourteen rhizobacterial isolates showing zinc solubilization index more than 15.00 on zinc oxide-containing plates were also screened for phosphorus solubilization and IAA production. All the 14 bacterial isolates solubilized P with an index ranging from 1.56 to 14.87, and only 11 isolates showed IAA production in the range of 4.06–8.77 μg mL^{-1}.

8.8 Conclusion

The widespread incidences of zinc deficiency in crop plants are correlated with low solubility of zinc compounds (Cakmak 2009). The chemical fertilizers are applied in the soil to improve crop productivity, which results in high costs to farmers, and excessive use of fertilizers is also responsible for environmental pollution. The development of sustainable agriculture system requires new eco-friendly technologies to minimize the use of chemical fertilizers while maintaining proper crop yields. Generally, a major part of added fertilizers gets converted to insoluble fractions and becomes unavailable to plants. Therefore, the application of PGPR having nutrient solubilization potential in agriculture will not only reduce the cost expenditure by minimizing the use of expensive agro-chemicals but also provide safe and healthy environment (Herrera et al. 1993; Glick 1995; Requena et al. 1997; Vessey 2003). Keeping in view the importance of zinc in various crops and role of Zn-solubilizing bacteria in making it available to the plants, identification of zinc-solubilizing bacteria is necessary to solubilize zinc in the soil. Recently, zinc-solubilizing bacteria have been isolated from the rhizospheric soil of different crops (Sunitha Kumari et al. 2016; Dhaked et al. 2017; Zamana et al. 2018). Inoculation of ZnSB ensures proper functioning and plant growth and presents a viable, self-sustainable, low input and eco-friendly alternative to chemical fertilizers for use in agro-ecosystems. These microbial strains capable of solubilizing zinc minerals can conserve our existing resources and avoid environmental pollution hazards caused by excessive use of chemical fertilizers. Thus, inoculation of microbial consortium possessing the capability of N, P, K and Zn mineralization is a cost-effective and eco-friendly approach for enhancing crop yields in sustainable agriculture (Badr et al. 2006; Zhang et al. 2013: Dhaked et al. 2017; Sindhu et al. 2019). On the applied side, the coinoculation of zinc-solubilizing bacteria with growth-promoting rhizosphere bacteria or the inoculation of microbial consortia is preferable because these microorganisms might express beneficial functions more continually in a soil or rhizosphere system, even under ecologically different and/or variable conditions.

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