Chapter 13
Microbial Biofortification: A Green Technology Through Plant Growth Promoting Microorganisms

Amir Khan, Jyoti Singh, Viabhav Kumar Upadhayay, Ajay Veer Singh, and Shachi Shah

Abstract The hidden hunger or malnutrition is considered to be the most dignified global challenge to human kind. Malnutrition afflicts approximately more than one billion of world's population in both developed and developing countries. Malnutrition includes diet related chronic diseases as well as overt nutrient deficiencies which leads to morbidity, reduced physical and mental growth. However, strategies to enhance supplementation of mineral elements and food fortification have not always been successful. Plant growth promoting microorganisms are known to fortify micro- and macro-nutrient contents in staple food crops through various mechanisms such as siderophore production, zinc solubilization, nitrogen fixation, phosphate solubilization, etc. Inoculation of potential microorganisms along with mineral fertilizers can increase the uptake of mineral elements, yield and growth. Therefore, biofortification of staple food crops by the implications of plant growth promoting microorganisms has an ability to attain mineral elements, is advocated as novel strategy not only to increase concentration of micronutrient in edible food crops but also to improve yields on less fertile soils.

Keywords Microbial biofortification · Plant growth promoting microorganisms · Malnutrition · Zinc · Micronutrient

---

A. Khan · J. Singh · V. K. Upadhayay · A. V. Singh (✉)
Department of Microbiology, College of Basic Sciences and Humanities, GBPUA&T, Pantnagar, Uttarakhand, India

S. Shah
School of Interdisciplinary and Transdisciplinary Studies, Indira Gandhi National Open University, New Delhi, India

S. Shah et al. (eds.), *Sustainable Green Technologies for Environmental Management*, https://doi.org/10.1007/978-981-13-2772-8_13

13.1 Introduction

For sustainable agriculture, the use of microbial based biofertilizers has prestigious role in enhancing level of crop productivity and in food safety. Microorganisms as invisible soil engineers maintain soil health, construct a hub for different biogeochemical cycles (Gadd 2010) and many soil microorganisms such as bacteria, actinomycetes, cyanobacteria and mycorrhiza present an eco-friendly approach for improved uptake of nutrients and enhanced plant growth. Microorganisms particularly plant growth promoting rhizobacteria (PGPR) are dwelled in rhizospheric region and efficiently colonize the roots of plants and confer tolerance in plants against several abiotic and biotic stresses (Prasad et al. 2015). Plant growth promoting microorganisms make nutrients available to plants by numerous mechanisms such as atmospheric nitrogen fixation, solubilizing the nutrients fixed in the soil matrix, and production of phytohormones. Such microorganisms make sure for further enhancement of micronutrients in plants, as they play a key role in organic material mineralization and as well as transforming inorganic nutrients. Microorganisms can also influence nutrient availability through presenting different characteristics such as chelation, solubilization and oxidation or reduction (Khan 2005) and also conferred resistance from pathogens causing diseases to the host plant by the secretions of antibiotics (Bonfante and Genre 2015). The aim of modern agriculture system, besides augmented crop yield, is also to produce nutritious safe food crops with improved level of micronutrients in the edible portion of crop plants. Human population is mainly dependent on crop based foods for the basic diet, and having foods with poor level of essential micronutrients creates serious health issues in humans. Deficiency of micronutrients (zinc, iron, selenium, copper, manganese and vitamins) in both humans and plants is narrated as 'hidden hunger' (Sharma et al. 2016), and bestows threat of malnutrition among world population. Therefore, implementation of biofortification strategy is an important mode for providing the preeminent solution for producing food crops with elevated level of necessary micronutrients. 'Biofortification' and 'standard fortification' are two different terms, where biofortification is related with consigning the nutrients aggregation inside plant cells whereas latter involves use of additives with the foods. Biofortification process deals with several approaches for enhancing bioavailability of key nutrients in crops.

13.2 Micronutrient Associated Malnutrition

Micronutrient deficiency occurs in humans, where populations of developing countries intake diet in the form of staple foods characterized by reduced bioavailability of essential micronutrients. Prevalence of micronutrient deficiency increase the risk of extensive disease burden in low and middle income countries (Black 2014), where populations of impecunious people cannot afford costlier nutrient rich foods and other nutrient supplements and suffers from wide varieties of micronutrients malnutrition associated ailments. According to the United Nations System Standing Committee on Nutrition (UNSSCN) (2004) micronutrients starvation are associated with more than 50% of all child mortality and also present the foremost risk factor for maternal mortality. Zinc (Zn), iron (Fe), and selenium (Se) are considered as important micronutrients and these are required in appropriate amount through routine diet for maintaining several life processes. Deficiency of one or more micronutrients creates negative impact on human health express in wide arrays of diseases (Fig. 13.1). The micronutrient zinc is most essential for all organisms including humans, and also has important structural roles in several proteins. Zinc deficiency is most prevalent micronutrient dearth and is associated with numerous human health related issues such as impairments of physical growth, greater risk of various infections, retarded growth, deferred wound healing, diarrhea, skeletal abnormalities and increased risk of abortion (Salgueiro et al. 2000).

Deficiency of iron causes chlorosis in plants and results in reduced crop yield, and eventually affects human health through food-chain, specifically to people whose diets generally rely on plant resources. Iron deficiency engenders nutritional anemia and also associated with impaired immune functions in children and as well impaired neurocognitive development (Murray-Kolb 2013). Selenium is another instance of essential micronutrient possessing role in wide range of metabolic pathways such as antioxidant defense and thyroid hormone metabolism. Selenium (Se) deficiency may linked with numerous ailments including heart diseases, reduced male fertility, hypothyroidism, weakened immune system and high risk of infections, cancer, oxidative stress-related conditions and epilepsy (Hatfield et al. 2014). Deficiency of vitamins (Vitamin B6, Vitamin B12, Vitamin C, Vitamin E and folic acid), zinc and iron also

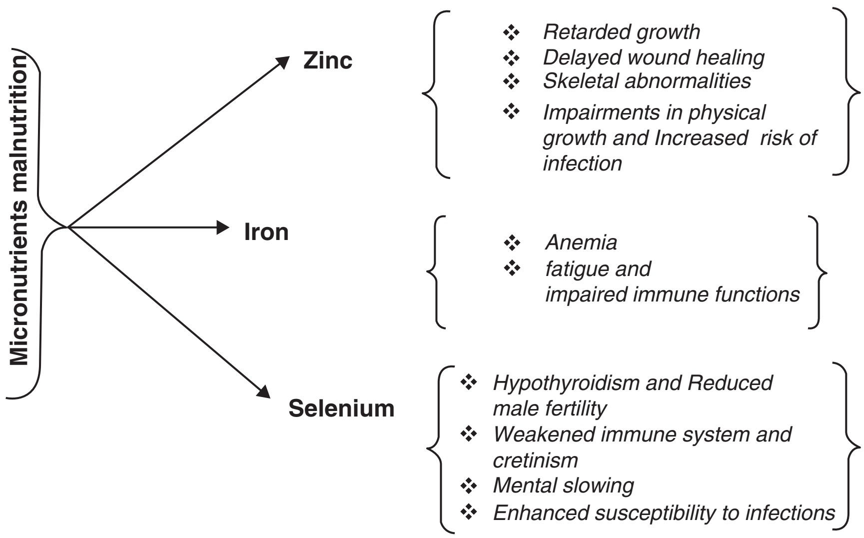

Fig. 13.1 Schematic representation of health effects of micronutrient (Zn, Fe and Se) malnutrition in humans

results in DNA damage through using similar strategies as radiation and various chemicals, and therefore considered a major factor to cause cancer and other disabilities. To circumvent problems associated with micronutrients malnutrition, investigation of those strategies are required which can improve the nutrient assimilation in plants.

13.3 Approaches for Biofortification

13.3.1 Biofortification Through Genetic Modification

Biofortification of vital food crops through genetic amendment and various biotechnological techniques is a sustainable solution for alleviating the micronutrient malnutrition. The techniques of genetic modification are being optimized for the development and production of healthy foods in addition to step up in the levels and activity of biologically active components in food crop system. Techniques of genetic modification have typically been targeted at escalating yields of staple food crops in developing countries. Though, the food crops with improved nutritional quality have gathered less consideration. An excellent example of a genetically modified biofortified crop is golden rice. Ordinary rice is not able to synthesise beta-carotene; however, due to genetic modification, golden rice can produce rice with beta-carotene in it. Moreover, stearidonic acid assimilation in soybean crop is also reported through genetic transformation (Singh et al. 2017).

13.3.2 Transgenic Approaches

Biotechnological techniques accredit the screening and selection of flourishing genotypes, isolation as well as cloning of favorable traits and formation of transgenic crops for sustainable agriculture system. Transgenic approaches can be used to increase the micronutrient content of staple food crops such as legumes and cereals, which can be achieved by insertion of specific genetic trait with the ability to produce the desired nutrients that are typically deficient in recipients. It involves the characterization, insertion or deletion of specific gene to improve the desired trait like nutritional quality from donor organisms. This may be achieved by the introduction of genes that code for trace element binding proteins, over expression of storage proteins already present or the expression of other proteins that are responsible for micronutrient uptake in plants**.** Furthermore, metabolic pathways from any microorganism and other organisms can also be applied into crops to utilize alternative pathways for metabolic engineering. Thus, these technologies provide a powerful tool that is unconstrained by the gene pool of the host**.** In addition, the transgenic approaches can be targeted to the edible portions of commercial crops (Hirschi 2009). As shown in Table 13.1, several crops have been genetically modified with traits of macronutrient and micronutrient that may provide reimbursement to consumers (Newell 2008).

Table 13.1 Genetically modified crops with description of macronutrient and micronutrient assimilation

Characteristics	Crop (details of characteristics)
Protein and amino acids	
Quality of protein and level	Maize (amino acid composition; protein↑)
	Potato (amino acid composition; protein↑)
	Rice (amino acid composition; protein↑)
	Soybean (amino acid balance)
	Sweet potato (protein↑)
Essential amino acid	
	Maize (Lys↑, Met↑, Trp↑)
	Potato (Met ↑)
	Sorghum (Lys↑)
	Soybean (Lys↑, Trp↑, Cys↑, Met↑)
Oils and fatty acids	
	Canola (lauric acid↑; + ω-3 fatty acids; 8:0 and 10:0 fatty acids↑; lauric and myristic acids↑; oleic acid↑; γ-Linolenic acid)
	Cotton (oleic acid↑, oleic + stearic acids↑)
	Grass, legumes (↓trans-fatty acids)
	Linseed (+ ω-3 and ω-6 fatty acids)
	Maize (oil↑)
	Oil palm (oleic acid↑ or stearic acids↑, oleic acid↑,+palmitic acid↓)
	Rice (α-linolenic acid↑)
	Soybean (oleic acid↑, α-linolenic acid↑, stearidonic acid↑, Arachidonic acid↑)
Carbohydrates	
Fructans	Maize (fructan↑)
	Potato (fructan↑)
Starch	Rice (amylase↑)
	Wheat (amylose↑)
Micronutrients and functional metabolites (Vitamins and carotenoids)	
	Canola (vitamin E↑)
	Maize (vitamin E↑, vitamin C↑, provitamin A)
	Mustard (+β-carotene)
	Soyabean (Vitamin E)
	Potato (β-carotene and lutein↑)
	Rice (+β-carotene, Vitamin B9↑)
	Wheat (provitamin A↑)
	Strawberry (vitamin C↑)
	Tomato (folate↑, phytoene and β-carotene↑, lycopene↑)

(continued)

Table 13.1 (continued)

Characteristics	Crop (details of characteristics)
Mineral availabilities	
	Alfalfa (phytase↑)
	Carrot (calcium↑)
	Lettuce (iron↑)
	Rice (iron↑, zinc↑)
	Maize (phytase↑, ferritin↑)
	Soybean (phytase↑)
	Wheat (phytase↑, iron↑, zinc↑)
	Alfalfa (phytase↑)
	Carrot (calcium↑)
	Lettuce (iron↑)
	Barley (zinc↑, phytase↑)

Modified from Singh et al. (2017)

13.3.3 Agronomic Biofortification

Biofortification through agronomical approach can be achieved through the implication of nutrient-rich fertilizers to foliage or soil to increase the micronutrient concentration in edible crop parts and thus increase the intake of essential micronutrients by consumers. Interaction between micronutrient and macronutrient can influence the efficiency of agronomic biofortification. Good quantity of macro elements (N, P and K) in crop has a positive effect on development of root architecture and transportation of nutrients from vegetative tissues to the seeds. Consequently, there is increased concentration of micronutrients in edible parts of the food crop (Prasad et al. 2014). However, when food crops are grown where mineral elements become straight away unavailable in the soil system, targeted application of soluble chemical fertilizers to foliar parts and roots are practised**.**

13.3.4 Chemical Fertilizer

Effectiveness of chemical fertilizer on crop performance is influenced by type of fertilizer. The interaction between chemical fertilizer and different forms of nutrients can have positive, negative or neutral effect on food crop in yield and nutrient bioavailability. The implication of fertilizer with soil is often the most efficient manner**.** However, soils often contain huge amounts of iron, but only little amount of iron is phytoavailable. The implication of inorganic Fe fertilizers to such soils is more often futile as it rapidly becomes unavailable to plant root system through adsorption, oxidation reactions and precipitation. For this reason, Fe chelators are often used as soil Fe fertilizers (Rengel et al. 1999). Zinc is commonly applied to crops as $ZnSO_4$ or as synthetic chelators (Shuman 1998). The application of Zn fertilizers to the soil system is effective for increasing Zn concentrations in cereal grains, growing mostly in soils and foliar applications of either $ZnSO_4$ or Zn chelators can increase Zn concentrations in plant via ample Zn mobility in the phloem. Similarly, applications of Zn fertilizers in soil and foliar can increase Zn concentrations in leaf, tuber and fruit (Shuman 1998; Rengel et al. 1999).

13.3.5 Biofortification Through Plant Growth Promoting Microorganisms (PGPM)

Biofortification of crops through implications of PGPMs can be considered as a promising accompanying measure, which along with transgenic varieties, can lead to augmented micronutrient concentrations in food crop system, besides improving yield and soil fertility. Plant growth promoting microorganism's have been reported to biofortify the micronutrient contents in food crops besides improving the soil fertility and crop yield (Rana et al. 2012). In addition, plant growth promoting microorganisms also facilitate the plant growth through N_2 fixation, insoluble phosphorus solubilization, production of phytohormones, lowering of ethylene concentration, antibiotics and antifungal metabolites synthesis and induced systemic resistance. In this way, PGPM are also known to boost the soil fertility in return, the plant acquiesce by supplying essential nutrients, growth regulators and enhancing the ethylene mediated stress by 1-aminocyclopropane-1-carboxylate (ACC) deaminase production along with improved plant stress tolerance to drought, salinity, metal and toxicity of pesticide (Singh and Prasad 2014; Singh and Singh 2017). Moreover, the potentiality of PGPM in agriculture is progressively increased as it provides an attractive approach to replace the exploitation of chemical fertilizers, pesticides and other supplements. Subsequently, biofortification of crops through application of PGPMs can be therefore considered as a potential supplementary approach, which along with breeding varieties, can escort to augment the concentrations of micronutrient in wheat crop, besides improving yield and soil fertility (Singh et al. 2017).

13.4 Mechanisms of Plant Growth Promoting Microorganisms

A variety of plant growth promoting microorganisms (PGPM) has been reported to enhance plant growth and productivity by means of various mechanisms. Illustration of some of the mechanisms is as follows.

13.4.1 Iron Chelation

Iron is a vital component for all forms of life including prokaryotes as well as eukaryotes. It is the component of electron transport carrier, cofactor of various enzymes and important part of various constituent such as hemoglobin. Due to the aerobic environment conditions, iron is present in its oxidized form (Fe^{3+}, insoluble at neutral pH) instead of reduced form (Fe^{2+}, soluble at neutral pH) which are taken up by plants. To sequester the iron, many fungi, bacteria and some plants have an unusual adaptation to produce low molecular weight compounds called as siderophores, a group of low molecular weight compounds (<10 KD) those have immense affinity towards Fe^{3+} ions. Siderophores are PGPM secreted compounds that are ultimately taken up by plants therefore transporting molecule of iron to the plants cells. Plant roots might be able of take up siderophore and use them as sources of iron. Therefore, microbial siderophore can enhance plant growth by improving iron uptake as well as by inhibiting the plant pathogen by means of competition ultimately leads to the iron biofortification in plants and their grains (Srivastava et al. 2013). Many researchers has been reported the siderophore production in wide range of bacterial species *viz. Bacillus, Pseudomonas, Azotobacter, Arthrobacter, Burkholderia, Enterobacter, Rhodospirrilum, Serratia, Azospirillum* and *Rhizobium* and fungal species *viz. Aspergillus Penicillium Rhizopus*, *Syncephalastrum* (Leong and Neilands 1982; Das et al. 2007; Duran et al. 2016; Srivastava et al. 2013). There are many types of siderophore such as hydroxamate, catacholate and carboxalate that are secreted by microbes varies from species to species. Furthermore, mixed type of siderophore has been secreted by many baceterial species (Wandersman and Delepelaire 2004). Hence we can say that use of siderophore producing PGPM is better approach over other conventional methods such as chemical fertilizers to enhance iron content in plants and grains.

13.4.2 Zinc Solubilizer

Zinc is one of the essential nutrients required for growth and metabolic activities. Zinc ions takes part in many physiological activities, it act as cofactor in various enzymes; take part in defense; play role in cell division and growth in prokaryotes as well as in eukaryotes. Zinc ions are highly reactive in nature and present in close interaction with soil constituents therefore soluble zinc is very low in soil**.** Generally, it is found in the form of oxides, phosphates and carbonates. Plant associated microorganisms adopt several mechanisms to solubilize zinc such as chelation (Whiting et al. 2001), reduction in soil pH (Subramanian et al. 2009), or through improving root growth and root absorptive area (Burkert and Robson 1994). Zinc chelation by microbes and making them available for plants roots is a well known phenomenon. Microbes produce chealating compounds, which forms complex upon binding with zinc. In addition, they releases chealated zinc at the root surface and enhance the zinc availability ultimately lead to the zinc biofotification in plants. Whiting et al. (2001) reported production of metallophores as the possible strategies used by bacteria to chelate Zn. Reduction in soil pH also enhance availability of zinc. Decline in pH has been reported, when *Pseudomonas* and *Bacillus* spp. solubilized zinc complex compounds (ZnS, ZnO and $ZnCO_3$) into zinc ions in a broth culture (Saravanan et al. 2004). Zinc solubilization methods differ from one microorganism to another to improve Zn availability in soil system and plant tissues. Many microbes including many bacterial and fungal species (*Pseudomonas, Microbacterium, Enterobacter, Bacillus, Arbuscular mycorrhizae*) have the incredible capability to solubilize Zn from complex compounds (Whiting et al. 2001; Fasim et al. 2002; Subramanian et al. 2009) and consequently take part in improvement of food quality and nutrient status of plants and grains.

13.4.3 Biofertilizers

In the present era, use of chemical fertilizers to enhance plant growth, productivity and to replenish soil nutrient status is very common. But several problems coincide with the use of chemical fertilizers such as high cost, unavailability of large portion of nutrients, toxic and non-degradable nature, leading to enhancement of environmental pollution and making land unsuitable for cultivation. Therefore as an alternative strategy, application of biofertilizers can be used to enhance crop productivity and biofortification of nutrients in grains. Biofertilizers which are the fertilizers based on source of biological origin such as microbes (including bacteria and fungi) are used instead of synthetic compounds. Use of biofertilizers is an ecofriendly approach as they are of biological origin and keeps environment healthy due to its low persistence. The objective of biofertilizers is to increase the soil organic contents improve soil structure and reduce loss of nutrients such as nitrogen, iron, zinc, calcium and phosphorus (Lal and Greenland 1979). Biofertilizers serves as source of all nutrients due to their ability to solubilize complex form of nutrients into soluble form (Singh et al. 2010, 2013, 2018). Plants utilize phosphorus in the form of orthophosphate (Pi). Plant growth promoting microorganisms possess various mechanisms to enhance phosphate solubilization (Fig. 13.2). Jones et al. (1998) reported 3.1–4.7 times more efficacy in mycorrhizal associated plants for phosphorus uptake than nonmycorrhizal plants. Commercially biofertilizers are developed by coating of various bacteria (*Azotobacter, Azospirillum, Rhizobium, Pseudomonas* and *Bacillus*) on seeds, a process called bacterization. *Azotobacter chrococcum* secretes azotobacterin, *Bacillus megaterium* secretes phosphobacterin, which is used for preparation of biofertilizers (Kumar and Bohra 2006). These bacteria may or may not form symbiotic association but enhances lateral root hairs of plants to increase mineral and water absorption, also increases nitrogen availability, secretes plant growth stimulating substances such as vitamin, auxins, gibberellic acid, cytokinins which leads to increase in photosynthesis capacity ultimately enhancing nutrient status in plants. Rhizobial biofertilizers are able to fix 50–150 N/ha/annum.

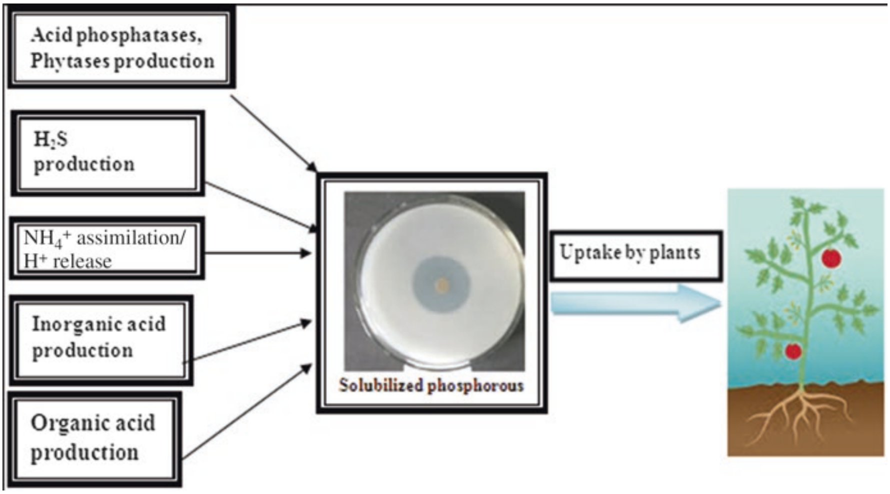

Fig. 13.2 Phosphate solubilization mechanisms of plant growth promoting microorganisms

It has been well known, that application of biofertilizers with plants, significantly increases plant growth, high nutrient status, low level of pathogen attack (Gupta et al. 2003; Yadav et al. 2016). Therefore, such free living and symbiotic microorganisms are promoted to reduce the dependence on chemical fertilizers. Some biofertilizers are listed in Table 13.2.

13.4.4 Biocontrol Agents

Under natural environmental conditions, plants are continuously exposed to various pathogenic bacteria and fungi, which causes disease in plants leading to the reduction in crop production or death of the plants. Therefore, plant diseases need to be controlled to maintain the quality and nutritional status of plants. Different approaches may be used to prevent or control plant diseases. In present circumstances, many pesticides are used to prevent disease but the disadvantage is that the pest may adapted towards pesticide and it is also cost intensive as well as act as environmental pollutant due to its persistence. Therefore, there is a novel approach i.e. application of biocontrol agent to suppress or demise pathogen growth. Biocontrol agents are microorganisms, which controls the fungi, insect, pest and any other pathogen (Beattie 2006). Generally bacteria, fungi, virus and protozoans are used as biocontrol agents. Generally plant growth promoting microorganisms produces various substances that protect plants against pathogens by direct interactions i.e. antagonistic activity or indirectly by inducing host resistance (Induce systemic resistance or systemic acquired resistance). Plant growth promoting microorganisms that indirectly act on pathogens may have some mechanisms to control plant pathogen such as the following (Table 13.3).

Table 13.2 Biofertilizers, their mode of action and crops benefitted

Organisms		Action	Crop	References
Bacteria	*Rhizobium leguminosarum*	Symbiotic nitrogen fixation	All leguminous crops	Bagali (2012)
	Bacillus megaterium	Phosphate solubilization	Mustard	Kang et al. (2014)
	Bacillus subtilis	Micronutrient solubilizer	Cotton	Yao et al. (2006)
	Pseudomonas fluorescens	Micronutrient solubilizer	Bean	Alemu (2013)
	Azotobacter sp.	Free living nitrogen fixation	Leguminous crops	Bagali (2012)
	Azospirillum sp.	Associated Symbiotic nitrogen fixation	Leguminous crops	Bagali (2012)
Fungi	*Penicillium bilaiae*	Phosphate solubilization	Coffee, Casurina	Malhi et al. (2013)
	Glomex sp.	Phosphate solubilization	Coffee, Casurina	Malhi et al. (2013)
Cyanobacteria	*Nostoc*	Free living nitrogen fixation	Rice	Vaishampayan et al. (2001)
	Anabana	Free living nitrogen fixation	Rice	Vaishampayan et al. (2001)
	Anabana- Azolla	Symbiotic nitrogen fixation	Rice	Vaishampayan et al. (2001)

Table 13.3 Major biocontrol agents, their target pathogen and mechanism of action

Biocontrol agent	Target pathogen	Crop	Action	References
Bacillus thuringiensis	*R. solani,* All phytopathogen	Cotton	Lytic enzymes	Shaikh and Sayyed (2015)
Pseudomonas fluorescence	*Erwinia carotovora,Puccinia ultimum, Fusarium glycinia*	Potato, wheat, Sugar beat	Siderophor, Antibiotics production	Shaikh and Sayyed (2015)
Strepromyces sp.	*S. sclerotiorum*	Potato, Tomato	Lytic enzymes	Shaikh and Sayyed (2015)
Trichoderma harzianum	*Botrytis cinerea, Meloidogyne javanica*	Bean, Tomato	Lytic enzymes, Competition	Woo et al. (1999), Sahebani and Hadavi (2008) and Puyam (2016)
Trichoderma viride	*Sclerotium rolfsii*	Groundnut	Lytic enzymes, Competition	Hirpara et al. (2017) and Puyam (2016)
Pseudomonas cepacia	*Bipolaris maydis*	Maize	Antibiotics production	Shaikh and Sayyed (2015)

1. The PGPR may have ability to produce siderophore that chelates iron, which makes iron unavailable for plant pathogens (Singh et al. 2017)
2. The PGPR may possess capacity to secrete some anti-pathogenic metabolites such as antibiotics, cell wall degrading hydrolytic enzyme (Glucanases, chitinases, proteases, lipase, pectinases) or hydrogen cyanide (HCN), which suppresses pathogen growth (Maksimov et al. 2011).
3. The PGPR may compete for nutrients and niche with pathogen (Kamilov et al. 2005).
4. The PGPR may stimulate Induced Systemic Resistance (ISR) or Systemic Acquired Resistance (SAR) (Van loon et al. 1998).

A wide range of microorganisms are reported to act as biocontrol agents, as they possess one or more than one mechanisms to suppress pathogen attack or growth. Due to less persistence in environment, specificity for target pest, cost effectiveness and ecofriendly nature, biocontrol agents are good alternative to pesticides.

13.5 Conclusion

Development of crops with elevated concentration of micronutrients is immensely and urgently needed to combat the problem of micronutrient deficiency. Plant growth promoting microorganisms (PGPMs) make interaction with plants and exert plant growth promoting activities and enhance the capability of the plant for uptake of micronutrients from surrounding soils. Zinc solubilization and siderophore secreting microorganisms enhance the level of zinc and iron in the various edible portions of crop plants and provide an alternative strategy to fortify micronutrients and produce micronutrients rich foods. Application of such microorganisms inoculants reduces the dependency on costly biofortification approaches i.e. agronomic and genetic approaches. In future, formulation of microorganisms possessing multiple beneficial traits can be applied for biofortification strategies to tackle the problem of hidden hunger. Using plant growth promoting microorganisms for biofortification as part of green technology approach can be a better strategy to achieve environmental sustainability.

References

Alemu, F. (2013). Isolation of *Pseudomonas fluorescens* from rhizospheric soil of faba bean and assessment of their phosphate solubility: In vitro study, Ethiopia. *Scholars Academic Journal of Biosciences, 1*(7), 346–351.

Bagali, S. S. (2012). Review: Nitrogen fixing microorganisms. *International Journal of Microbiology, 3*(1), 46–52.

Beattie, G. A. (2006). Plant-associated bacteria: Survey, molecular phylogeny, genomics and recent advances. In S. S. Gnanamanickam (Ed.), *Plant-associated bacteria* (pp. 1–56). Dordrecht: Springer.

Black, R. E. (2014). Global distribution and disease burden related to micronutrient deficiencies. *Nestle Nutrition Institute Workshop Series, 78*, 21–28.

Bonfante, P., & Genre, A. (2015). Arbuscular mycorrhizal dialogues: Do you speak 'plantish' or 'fungish'? *Trends in Plant Science, 20*, 150–154.

Burkert, B., & Robson, A. (1994). Zn uptake in subterranean clover (*Trifolium subterraneum* L.) by three vesicular-arbuscular mycorrhizal fungi in a root-free sandy soil. *Soil Biology and Biochemistry, 26*, 1117–1124.

Das, A., Prasad, R., Srivastava, A., Giang, P. H., Bhatnagar, K., & Varma, A. (2007). Fungal siderophores: Structure, functions and regulations. In A. Varma & S. B. Chincholkar (Eds.), *Microbial siderophores* (pp. 1–42). Berlin/Heidelberg: Springer.

Durán, P., Acuña, J. J., Armada, E., López-Castillo, O. M., Cornejo, P., Mora, M. L., & Azcón, R. (2016). Inoculation with selenobacteria and arbuscular mycorrhizal fungi to enhance selenium content in lettuce plants and improve tolerance against drought stress. *Journal of Soil Science and Plant Nutrition, 16*(1), 211–225.

Fasim, F., Ahmed, N., Parsons, R., & Gadd, G. M. (2002). Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery. *FEMS Microbiology Letters, 213*(1), 1–6.

Gadd, G. M. (2010). Metals, minerals and microbes: Geo-microbiology and bioremediation. *Microbiology, 156*(Pt 3), 609–643.

Gupta, R. K., Kaushik, S., Sharma, P., & Jain, V. K. (2003). Biofertilizers: An ecofriendly alternative to chemical fertilizers. In A. Kumar (Ed.), *Environmental challenge of 21th century* (pp. 275–287). New Delhi: APH Publication Corporation.

Hatfield, D. L., Tsuji, P. A., Carlson, B. A., & Gladyshev, V. N. (2014). Selenium and selenocysteine: Roles in cancer, health, and development. *Trends in Biochemical Sciences, 39*(3), 112–120.

Hirpara, D. G., Gajera, H. P., Hirpara, H. Z., & Golakiya, B. A. (2017). Antipathy of *Trichoderma* against *Sclerotium rolfsii* Sacc.: Evaluation of cell wall-degrading enzymatic activities and molecular diversity analysis of antagonists. *Journal of Molecular Microbiology and Biotechnology, 27*, 22–28.

Hirschi, K. D. (2009). Nutrient biofortification of food crops. *Annual Review of Nutrition, 29*, 401–421.

Jones, M. D., Durall, D. M., & Tinker, P. B. (1998). A comparison of arbuscular and ectomycorrhizal *Eucalyptus coccifera*: Growth response, phosphorus uptake efficiency and external hyphal production. *New Phytologist, 140*(1), 125–134.

Kamilova, F., Validov, S., Azarova, T., Mulders, I., & Lugtenberg, B. (2005). Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. *Environmental Microbiology, 7*, 1809–1817.

Kang, S. M., Radhakrishnan, R., You, Y. H., Joo, G. J., Lee, I. J., Lee, K. E., & Kim, J. H. (2014). Phosphate solubilizing Bacillus megaterium mj1212 regulates endogenous plant carbohydrates and amino acids contents to promote mustard plant growth. *Indian Journal of Microbiology*, *54*(4), 427–433.

Khan, A. G. (2005). Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *Journal of Trace Elements in Medicine and Biology, 18*(4), 355–364.

Kumar, A., & Bohra, C. (2006). Green technology in relation to sustainable agriculture. In *Green technologies for sustainable agriculture*. New Delhi: Daya Publishing House.

Lal, R., & Greenland, D. J. (1979). *Soil physical properties and crop production*. Chichester: Wiley.

Leong, S. A., & Neilands, J. B. (1982). Siderophore production by phytopathogenic microbial species. *Archives of Biochemistry and Biophysics, 281*, 351–359.

Maksimov, I. V., Abizgil'dina, R. R., & Pusenkova, L. I. (2011). Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens. *Applied Biochemistry and Microbiology, 47*, 333–345.

Malhi, S. S., Vera, C. L., & Brandt, S. A. (2013). Relative effectiveness of organic and inorganic nutrient sources in improving yield, seed quality and nutrient uptake of canola. *Agricultural Sciences, 4*(12), 1–18.

Murray-Kolb, L. E. (2013). Iron and brain functions. *Current Opinion in Clinical Nutrition and Metabolic Care, 16*(6), 703–707.

Newell, M. M. (2008). Nutritionally improved agricultural crops. *Plant Physiology, 147*, 939–953.

Prasad, R., Shivay, Y. S., & Kumar, D. (2014). Agronomic biofortification in cereal of cereal grains with iron and zinc. *Advances in Agronomy, 125*, 55–91.

Prasad, R., Kumar, M., & Varma, A. (2015). Role of PGPR in soil fertility and plant health. In D. Egamberdieva, S. Shrivastava, & A. Varma (Eds.), *Plant growth-promoting rhizobacteria (PGPR) and medicinal plants* (pp. 247–260). Cham: Springer.

Puyam, A. (2016). Advent of *Trichoderma* as a bio-control agent – A review. *Journal of Applied and Natural Sciences, 8*(2), 1100–1109.

Rana, A., Joshi, M., Prasanna, R., Shivay, Y. S., & Nain, L. (2012). Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. *The European Journal of Soil Biology, 50*, 118–126.

Rengel, Z., Batten, G. D., & Crowley, D. E. (1999). Agronomic approaches for improving the micronutrient density in edible portions of field crops. *Field Crops Research, 60*, 27–40.

Sahebani, N., & Hadavi, N. (2008). Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. *Soil Biology and Biochemistry, 40*, 2016–2020.

Salgueiro, M. J., Zubillaga, M., Lysionek, A., Cremaschi, G., Goldman, C. G., Caro, R., De Paoli, T., Hager, A., Weill, R., & Boccio, J. (2000). Zinc status and immune system relationship: A review. *Biological Trace Element Research, 76*(3), 193–205.

Saravanan, V. S., Subramoniam, S. R., & Raj, S. A. (2004). Assessing in vitro solubilization potential of different zinc solubilizing bacterial (zsb) isolates. *Brazilian Journal of Microbiology, 35*(1–2), 121–125.

Shaikh, S. S., & Sayyed, R. Z. (2015). Role of plant growth-promoting rhizobacteria and their formulation in biocontrol of plant diseases. In *Plant microbes symbiosis: Applied facets* (pp. 337–351). New Delhi: Springer.

Sharma, P., Aggarwal, P., & Kaur, A. (2016). Biofortification: A new approach to eradicate hidden hunger. *Food Reviews International, 33*(1), 1–21.

Shuman, L. M. (1998). Micronutrient fertilizers. *Journal of Crop Production, 1*, 165–195.

Singh, A. V., & Prasad, B. (2014). Enhancement of plant growth, nodulation and seed yield through plant growth promoting rhizobacteria in lentil (*Lens culinaris Medik* cv. VL125). *International Journal of Current Microbiology and Applied Sciences, 3*(6), 614–622.

Singh, J., & Singh, A. V. (2017). Microbial strategies for enhanced phytoremediation of heavy metals contaminated soils. In R. N. Bharagava (Ed.), *Environmental pollutants and their bioremediation approaches* (Vol. 9, pp. 249–264). Boca Raton: Taylor & Francis.

Singh, A. V., Shah, S., & Prasad, B. (2010). Effect of phosphate solubilizing bacteria on plant growth promotion and nodulation in soybean (*Glycine max* (L.) Merr). *Journal of Hill Agriculture, 1*(1), 35–39.

Singh, A. V., Chandra, R., & Reeta, G. (2013). Phosphate solubilization by *Chryseobacterium* sp. and their combined effect with N and P fertilizers on plant growth promotion. *Archives of Agronomy and Soil Science, 59*(5), 641–651.

Singh, J., Singh, A. V., Prasad, & Shah, S. (2017). Sustainable agriculture strategies of wheat biofortification through microorganisms. In A. Kumar, A. Kumar, & B. Prasad (Eds.), *Wheat a premier food crop*. New Delhi: Kalyani Publishers.

Singh, A. V., Prasad, B., & Goel, R. (2018). Plant growth promoting efficiency of phosphate solubilizing *Chryseobacterium* sp. PSR 10 with different doses of N and P fertilizers on Lentil (*Lens culinaris* var. PL-5) growth and yield. *International Journal of Current Microbiology and Applied Sciences, 7*(05), 2280–2289.

Srivastava, M. P., Tewari, R., & Sharma, N. (2013). Effect of different cultural variables on siderophores produced by *Trichoderma* spp. *International Journal of Advanced Research, 1*, 1–6.

Subramanian, K. S., Tenshia, V., Jayalakshmi, K., & Ramachandran, V. (2009). Role of arbuscular mycorrhizal fungus (*Glomus intraradices*)–(fungus aided) in zinc nutrition of maize. *Journal of Agricultural Biotechnology and Sustainable Development, 1*, 29–38.

United Nations System Standing Committee on Nutrition (UNSSCN). (2004). *5th report on the world nutrition situation nutrition for improved development outcomes*. Geneva: SCN.

Vaishampayan, A., Sinha, R. P., Hader, D. P., Dey, T., Gupta, A. K., Bhan, U., & Rao, A. L. (2001). Cyanobacterial biofertilizers in rice agriculture. *The Botanical Review, 67*(4), 453–516.

Van Loon, L. C., Bakker, P. A. H. M., & Pieterse, C. M. J. (1998). Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology, 36*, 453–483.

Wandersman, C., & Delepelaire, P. (2004). Bacterial iron sources: From siderophores to hemophores. *Annual Review of Microbiology, 58*, 611–647.

Whiting, S. N., de Souza, M. P., & Terry, N. (2001). Rhizosphere bacteria mobilize Zn for hyperaccumulation by Thlaspi caerulescens. *Environmental Science & Technology, 35*, 3144–3150.

Woo, S. L., Donzelli, B., Scala, F., Mach, R. L., Harman, G. E., Kubicek, C. P., Del Sorbo, G., & Lorito, M. (1999). Disruption of the ech42 (endochitinase-encoding) gene affects biocontrol activity in *Trichoderma harzianum* P1. *Molecular Plant-Microbe Interactions, 12*, 419–429.

Yadav, R., Singh, A. V., Kumar, M., & Yadav, S. (2016). Phytochemical analysis andplant growth promoting properties of endophytic fungi isolated from tulsi and *Aloe vera*. *International Journal of Agricultural and Statistics Sciences, 12*(1), 239–248.

Yao, A. V., Bochow, H., Karimov, S., Boturov, U., Sanginboy, S., & Sharipov, A. K. (2006). Effect of FZB 24® *Bacillus subtilis* as a biofertilizer on cotton yields in field tests. *Archives of Phytopathology and Plant Protection, 39*(4), 323–328.