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Review

Iron chelating bacteria: a carrier for biofortification and plant growth promotion

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Abstract

Biofortification is the process by which the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or modern biotechnology to focus on malnourishment in developing countries. Under iron restricted environment certain bacteria (iron chelating bacteria) produced iron chelating molecules called as siderophore. This review gives an overview of Need for biofortification, Plant growth promoting rhizobacteria, Plant growth promoting consortia, importance of iron for human health, uptake of iron in plants, iron chelating (siderophore producing) bacteria as plant growth promotor, siderophore, generalized mechanism for siderophore-mediated iron transport in bacteria and the possible approaches to enhancing iron content in plants by implementing iron chelating bacteria as biotechnological carrier for increasing plant nutrition, yield and quality.

Keywords: Biofortification, Iron, Siderophore, PGPR

1 Introduction

1.1 Need for Biofortification

Biofortification aims at either increase accumulation of micronutrients in edible plants or to increase their bioavailability and is considered a cost-effective strategy to focus on malnutrition in developing countries. Iron (Fe) is naturally occurring metalloid element, which is essential to human and other animal health in trace amounts. Fe is an essential micronutrient for most microorganisms, including plants and animals. It is an essential micronutrient for plants due to it plays a crucial role in transport of oxygen, oxidative metabolism, proliferation of cell and required in certain physiological processes like N2 fixation, photosynthesis (Nair and Iyengar, 2009). Iron

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is an important micronutrient in biological systems and is receiving growing attention worldwide because of increasing reports about iron deficiencies in human populations and crops. Mineral and vitamin deficiencies account for an approximately 7.3% of the worldwide disease burden. Iron deficiency is one of the most predominant micronutrient deficiencies and it is affecting an estimated two billion people. It is regarded as a major health problem for 39% of children younger than 5 years, 48% of children between 5 to 14 years, 42% of all women and 52% of pregnant women in developing countries are anaemic (Zimmermann and Hurrell, 2007); pregnant women, women of childbearing age, women with high menstrual losses (Bersamin et al., 2008). In developing countries legumes, vegetables and cereal grains are the primary source of nutrition. Nowadays, plant growth promoting rhizobacteria (PGPR) is used as alternatives for enhanced uptake of micronutrients in plants (Tariq et al., 2007). PGPR produce plant growth promoting compounds, which can play an important role in increase the growth of plant by changing its morphology, like enlarge root surface area for the uptake of nutrients within the soil and also protect crops against disease (Rana et al., 2012b).

1.2 Plant growth promoting rhizobacteria

Rhizosphere is the narrow zone of soil specifically influenced by the root system. It is populated by a varied range of microorganisms and the bacteria colonizing this habitat are called as rhizobacteria (Beneduzi et al., 2012). Various bacterial genera are dynamic components of soil. Soil bacteria are important in biogeochemical cycles which have been used for plant production. Plant growth promoting rhizobacteria (PGPR) represent a wide variety of soil bacteria which, when grown in association with a host plant, result in stimulation of growth of their host. The term Plant growth promoting rhizobacteria (PGPR) was coined over three decades ago (Kloepper and Schroth, 1978), they are strongly root colonizing bacteria on the plant root surface which increase yield of plant by direct and indirect mechanisms (Jha and Saraf, 2015). Direct mechanism includes nitrogen fixation, production of phytohormones, solubilization of phosphate and enhancing iron bioavailability. Indirect mechanism involves the ability of PGPR to decrease the harmful effects of plant pathogens on the plant growth. They are group of bacteria that directly or indirectly promote the plant growth and yield through various plant growth promoting substances (mechanisms) such as siderophore production, phosphate solubilization, indole acetic acid production, ammonia production, exopolysaccharide production, hydrogen cyanide production and ACC deaminase production that benefit to plant growth (Glick, 1995; Singh, 2013; Vejan et al., 2016). In general, PGPR can be divided into extracellular (ePGPR), present in the rhizosphere, on the rhizoplane, or in the spaces between cells of the root cortex; and intracellular (iPGPR), which exist inside root cells, usually in particular nodular structures (Jha and Saraf, 2015).

Plant growth promoting rhizobacteria plays an important role in improving plant growth through a wide variety of mechanisms. There are several microorganisms reported as PGPR which include *Rhizobium, Bacillus*, *Pseudomonas*, *Paenibacillus*, *Azotobacter*, *Azospirillum*, *Burkholderia*, *Bradyrhizobium*, etc. to improve plant growth by various mechanisms (Bharucha et al., 2013). Among them Bacillus and Pseudomonas are the most abundant genus in the rhizosphere. (Kumar et al., 2018; Kumar et al., 2016).

1.3 Plant growth promoting consortia

Most approaches for plant growth promotion have used single bacterial species as biofertilizer while few had used a mixture of selected bacterial species as consortium. Combination of two or more microbial species which act together as a beneficially for a particular plant growth promotion called plant growth promoting consortia (PGPC) (Oluwambe and Kofoworola, 2016). Recently, application of two or more plant growth promoting rhizobacteria as consortium is gaining momentum in field application worldwide (Pandey et al., 2012). This act as multifarious approach of promoting plant growth and improve yield by uptake several nutrients from soil and synthesize certain compounds like siderophore production, phosphate solubilization, indole acetic acid, hydrogen cyanide production for the plant.

The application of plant growth promoting rhizobacteria as consortium is an emerging area of interest because these microbes have been found to enhance the growth and development of crops under both conventional and stressed environments in various production systems across varying ecological conditions. A variety of rhizospheric microorganisms, including *Bacillus* and *Pseudomonas* species, are commonly observed in the rhizosphere of leguminous and non-leguminous plants (Li and Alexander, 1988). Combination of some *Bacillus* strains with effective *Bradyrhizobium* resulted in enhanced nodulation and plant growth of green gram (*Vigna radiata* L.) (Sindhu et al., 2002).

1.4 Iron (Fe)

1.4.1 Importance of iron for human health

Iron is one of the essential elements for most organisms and for proper plant growth (Patel et al., 2018). Iron plays an importance role for the human body. One of the most significant roles of iron is to help red blood cells which transport oxygen to all parts of the human body and also produce the energy by DNA transport and DNA synthesis for human body. Iron malnutrition may be reduced by improving the bioavailable iron content through biofortification (Khalid et al., 2015). The recommended dietary allowances (RDAs) of iron for adult men are 8 mg and female are 18 mg, as below (Table 1.1). The RDAs for vegetarians are 1.8 times higher than the people who eat meat (NIH, 2018). This is because of the heme iron from meat is more bioavailable than nonheme iron from plant-based foods.

Table 1.1. Recommended dietary allowances (RDAs) for iron (NIH, 2018).

Age	Male	Female
Birth to 6 months	0.27 mg	0.27 mg
7 to 12 months	11 mg	11 mg
1 to 3 years	7 mg	7 mg
4 to 8 years	10 mg	10 mg
9 to 13 years	8 mg	8 mg
14 to 18 years	11 mg	15 mg 27 mg (Pregnancy) 10 mg (Lactation)
19 to 50 years	8 mg	18 mg 27 mg (Pregnancy) 9 mg (Lactation)
More than 51 years	8 mg	8 mg

1.4.2 Uptake of iron in plants

Iron is one of the essential elements for a plant due to it play important role in metabolic processes like photosynthesis (Rout, 2015). Depending on the soil properties, specific iron mobilization has been done in the plants. Mostly, there is a nearby geographical reports of soil iron deficiencies and human iron deficiencies, representing a high requirement of iron biofortification in food crops (Cakmak et al., 2010). In this regard, iron malnutrition can be reduced by food biofortification in edible crops. Iron accumulates in the edible parts of the plant like fruits and seeds which act as effective sinks for iron (Schuler and Bauer, 2012).

The capability of plants to respond to iron availability eventually related to human nutrition, in terms of both iron content of edible parts and plant yield (Morrissey and Guerinot, 2009). Although iron is one of the ample metals in the earth's crust, its bioavailability to plant roots is very low which depended on the soil pH and redox potential. In soils of higher pH, iron is readily oxidized and pre-dominantly in the form of insoluble ferric oxides. Whereas, at low pH, the ferric iron is free from the oxide which becomes more biologically available for uptake by roots (Morrissey and Guerinot, 2009).

Rhizosphere is a microecological zone in direct vicinity of plant roots. It is the small region of soil that is directly influenced by root secretions and associated soil microbes (McNear, 2013). In cultivated soils, iron is generally oxidized and precipitated as ferric oxides with low accessibility for plants (Colombo et al., 2014). Uptake of micronutrients from the rhizosphere is the first step for accumulating micronutrients into the crop (Waters and Sankaran, 2011). Uptake of Fe from the rhizosphere of plant is an active process, it is dependent on the plant's ability to reduce Fe+3 iron to Fe+2 iron. Chemically iron fertilization of crop is not very effective because of insolubility of iron in soil. An alternative solution is iron biofortification by plant growth promoting rhizobacteria which efficiently mobilize and uptake Fe to the edible parts of plants through iron chelating bacteria. (Sperotto et al., 2012).

1.4.3 Iron chelating (siderophore producing) bacteria as plant growth promoter

Plant growth promoting rhizobacteria (PGPR) can biofortify iron concentration in edible parts of plant by enhancing its bioavailability via certain mechanisms. Plant growth promoting bacteria can synthesize siderophores and release it into surrounding environment, followed by mineralization of the iron by iron siderophore complex (Khalid et al., 2015). These iron siderophores complexes are taken up by plant via transporter proteins called outer membrane receptor protein (OMRP), which are located on plasma membrane of root (Boukhalfa and Crumbliss, 2002).

Bacteria and Fungi are the vital siderophore producing microbes. Certain important siderophore producing bacteria includes *Escherichia coli*, *Salmonella*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Aerobacter aerogenes*, *Enterobacter* sp., *Yersinia* sp. and *Mycobacterium* sp. whereas, fungi include *Aspergillus nidulans*, *Penicillium chrysogenum*, *Mucor*, *Rhizopus* and *Saccharomyces cerevisiae* (Kannahi and Senbagam, 2014). Application of *Pseudomonas* sp., *Azospirillum* sp. as plant growth promoting bacteria could enhance the iron content in rice (Sharma et al., 2013). Biofortification of plant through plant growth promoting bacteria is considered as safe practice to enhance the iron content in different edible parts of plant and to alleviate its malnutrition (Khalid et al., 2015). Siderophore producing bacteria have the capability to inhibit the different types of phytopathogens and can be used as a potential bio-control agent (Pahari et al., 2017).

1.4.4 Siderophore

Siderophores (Greek word "Iron carrier") are small molecule with low molecular weight, and high affinity iron-chelating compounds which is produced by microorganisms like bacteria, fungi in iron deficient environment and work as transporter for iron across cell membranes (Hider and Kong, 2010). Iron is an essential microelement for plants and microorganisms, both have the ability to produce siderophores.

Phyto-siderophores are similar to siderophores but synthesized by plants under iron deficient conditions. Siderophore are important in certain metabolic activities such as electron transport system, act as cofactors for enzymes, synthesis of chlorophyll and formation of heme (Seneviratne and Vithanage, 2015). Microbial siderophores were reported to increase Fe content in certain plant species (Bar-Ness et al., 1992; Seneviratne and Vithanage, 2015).

Hydroxamate and catecholate are two main types of siderophore produced by bacteria (Storey, 2005). Hydroxamate-type siderophores contain a carboxyl group attached to nitrogen, which chelates ferric iron (Storey, 2005). They are usually more complex structurally and considered hydrophilic in nature. The desferrioxamine-E and ferrichrome are typical hydroxamate siderophores. Catecholate type siderophores bind ferric iron with hydroxyls of catechol rings, and are mostly derived from 2, 3 - dihydroxybenzoic acid (DHBA) (Crosa and Walsh, 2002; Storey, 2005). Enterobactin and chrysobactin are the best example of catecholate siderophores. Hydroxamates are produced in high iron environment; catecholates are formed in low iron environment. Detection of siderophores is mostly achieved in iron limited media (Neilands, 1995). Structure of hydroxamate and catecholate type of siderophore are as blow (Figure 1.1).

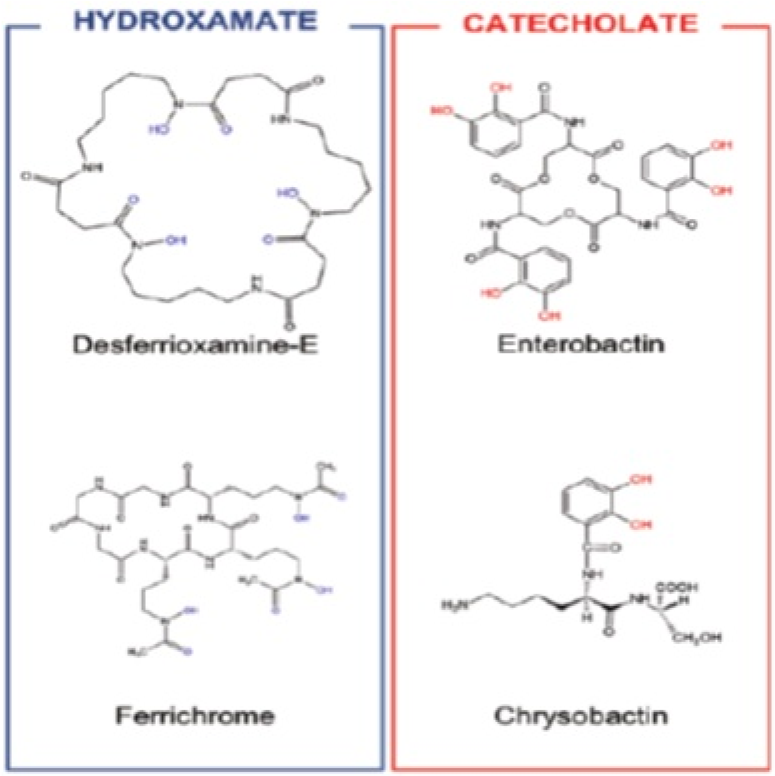

Figure 1.1. Structure of hydroxamate and catecholate siderophore (Aznar and Dellagi, 2015).

1.4.5 Generalized mechanism for siderophore-mediated iron transport in bacteria Siderophores are part of a multi-component system for transporting ferric iron into a cell (Storey, 2005). The other components include a specific outer membrane receptor protein (OMRP), a periplasmic binding protein, an inner membrane ATP-dependent ABC-type transporter, and the TonB-ExbBExbD protein complex in the inner membrane, (Chakraborty et al., 2003). Generalized mechanism for siderophore-mediated iron transport is given below (Figure 1.2).

Inside the cell there are higher concentrations of iron, whereas outside the cell there is extremely low concentration of bioavailable iron. Thus, under iron limited conditions gene induced for active transport of ferric siderophore complexes across the outer membrane of Gram-negative bacteria through a specific outer membrane receptor protein (OMRP) (Earhart and McIntosh, 1977; Storey, 2005).

Once periplasmic binding proteins (PBP) have been recognized inside the periplasm that binds the ferric siderophore complexes (Storey, 2005). These proteins are usually synthesized at a low level as compared to periplasmic binding proteins that bind sugars, amino acids and have a lower affinity for ferric siderophore complexes as compared with OMRPs (Sprencel et al., 2000).

Ferric siderophores bound to periplasmic binding proteins are transported to a specific ATP-dependent ABC-type transporter located in the internal membrane (Storey, 2005). There are four subunits of ABC-type transporter. The two subunits are hydrophobic and span the membrane multiple times, whereas other two bind nucleotides and are expressed to the cytoplasm. Interaction between ligand-bound periplasmic binding protein and ABCtype transporter stimulates the ATPase activity of the transporter (Locher, 2004).

The TonB-ExbB-ExbD protein complex is supposed to combine cytoplasmic membrane energy to energy-dependent processes held at the outer membrane. In gram negative microbes, the TonB protein complex is common to all siderophore intermediated iron transport systems (Storey, 2005).

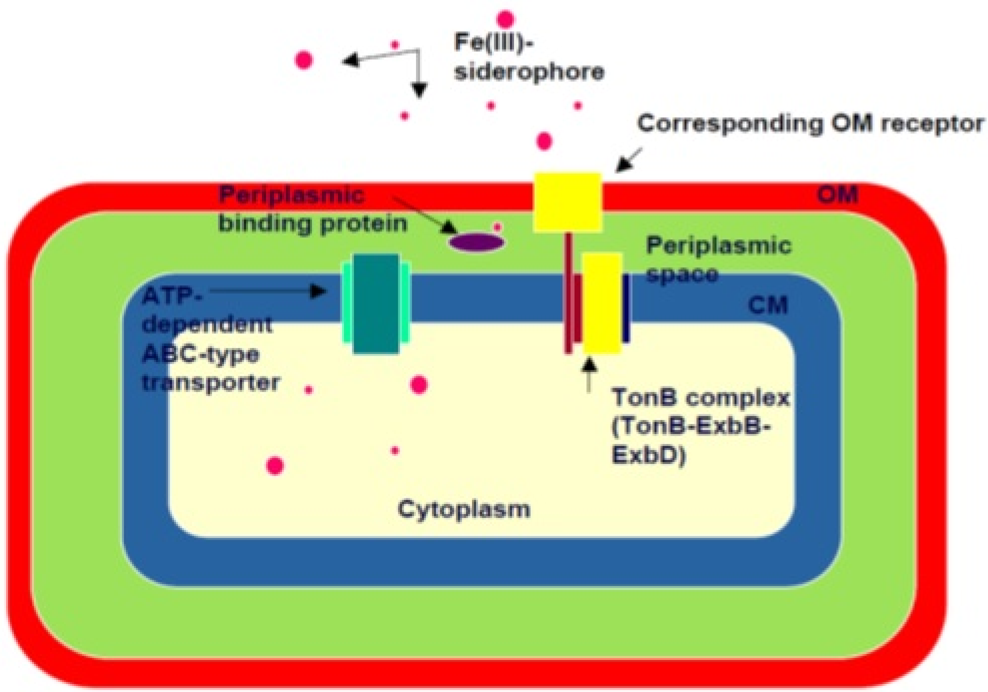

Figure 1.2. Generalized mechanism of siderophore mediated iron transport (Storey, 2005).

Once transported into the cytoplasm, there are two probable ways that iron is released from the siderophore. One of these is that ferric reductases reduce ferric iron to ferrous iron, due to the ferrous state have reduced binding affinity (Wandersman and Delepelaire, 2004). Second is that the siderophore brokes down and releases the iron as ferrous form (Storey, 2005).

2 Conclusion

Application of iron chelating bacteria in soil or the rhizosphere will stimulate natural processes of plant growth promotion, nutrient uptake and plant productivity which can be considered as a safe and cost-effective approach. Iron biofortified plants can be good source of iron to meet the daily necessity of human populations and prevent the diseases associated iron deficiencies. Potential biotechnological implementation of iron chelating bacteria is an effective strategy for iron biofortification.

Conflict of interests

Authors declare that they have no conflict of interest.

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