

# Chapter 3

## Endophyte-Promoted Nutrient Acquisition: Phosphorus and Iron

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### 3.1 Introduction

Phosphorus (P) and iron (Fe) are essential nutrients required by plants; however the bio-availability of both these macro- and micronutrients is low in soil as both P and Fe form insoluble mineral complexes; for example, iron is generally present as a  $\text{Fe}^{3+}$  (ferric ion) complex with oxyhydroxide polymers in soil and is not bio-available under alkaline pH conditions [1], whereas phosphorus complexes with calcium, iron, or aluminum in soil under alkaline or acidic pH conditions and these are not directly available in the form of orthophosphate anions for plant uptake [2, 3]. Plants have adapted to low mineral nutrient environments by using several strategies to overcome nutrient deficiency and increase plant nutrient uptake. These include inducing morphological or physiological changes to the root-soil interface by changing plant root architecture such as extensive root branching [4–6]. Increase in length of root and root hairs and root angle can also increase the spatial access and availability of nutrients present in soil to plants [7–10]. The increase in physiological or biochemical activities such as phytosiderophore production, organic anion production, and excretion of protons and increase in hydrolytic enzymes, e.g., phosphatase or phytase activity, are all associated with an increase in nutrient acquisition of either Fe or P by plants [4, 6, 11].

The improved availability of plant nutrients has long been associated with plant microbial interactions, in particular, arbuscular mycorrhizal fungi (AMF) associations that are involved in the transport or acquisition of P and also Fe in plants [12, 13]. The presence of microorganisms other than AMF associated with plants such as

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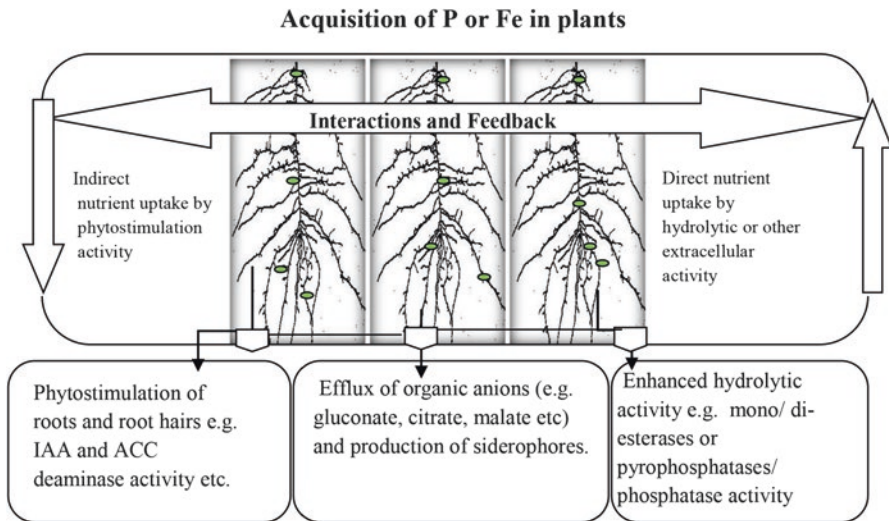
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bacteria and fungi present in rhizosphere soil or bacterial and fungal endophytes that occur asymptotically in plant organs and tissues has also been shown to provide benefits to plant health by nutrient acquisition [14–17]. In this chapter we focus on the importance of endophyte microorganisms with respect to their role in P and Fe nutrient acquisition in plants.

### 3.2 Microorganism Functions Implicated in Nutrient Fe and P Uptake in Plants

Plant-associated microorganisms (the plant microbiome) are commonly attributed with a range of plant growth promotion functions such as biological nitrogen fixation, phosphate solubilization, production of siderophores, ACC deaminase activity, production of phytohormones, and biocontrol activity [18, 19]. The plant and its associated microbiome have been termed the holobiont [20] and the plant microbiome is influenced by soil type and plant genome [21, 22]. Microorganisms can help increase nutrient Fe or P uptake and benefit plants directly due to microbial siderophore production or phosphate mineralization or solubilization activity. Other plant growth promotion traits such as plant hormone production or increasing plant stress tolerance by reducing plant ethylene levels by ACC deaminase activity or biocontrol functions may also help increase plant growth by increasing the soil root interface, thus indirectly increasing Fe and P nutrient uptake in the plant [23–25]; see Fig. 3.1.

The early interaction of microorganisms with land plants in the form of mycorrhizal fungal associations (AMF) is hypothesized to have evolved from fungal endophytes that developed external hyphae to provide plant nutrient support to plants in exchange for enriched carbon sources available from the host plant [26]. The AMF association with plants is the oldest and most widely represented on land [26, 27]. AMFs function by scavenging of P and Zn nutrients from soil but are also known to enhance acquisition of nutrients such as Fe, Ca, K, and S in plants [12]. Besides the AMF interactions, the other widely recognized group of fungi associated with plants are the non-clavicipitaceous group of Class 4 endophytes also known as dark septate endophytes (DSEs) [28]. The DSEs are known to be present in over 600 different plant species and are found worldwide [29]. The DSEs can help improve phosphorous supply in plants and in certain conditions appear to replace AMFs and ectomycorrhizal fungi at sites with extreme environmental conditions [28]. Among the other fungi, the basidiomycete fungus *Piriformospora indica*, a recently recognized endophyte, was shown to be distributed over a broad geographical area and interact with a number of angiosperms (around 145 or more) including the model plant *Arabidopsis thaliana* and with certain other members of the *Brassicaceae* family where AMF infections or associations are not detected. *P. indica* stimulates nutrient uptake in the roots [30, 31] and solubilizes insoluble phosphate in plants [32].



**Fig. 3.1** Microbial functional aspects in plants that impact nutrient P or Fe availability

The presence of large numbers of endophyte bacteria isolated from the plants' microbiota, for example, *Gluconacetobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Herbaspirillum*, *Pseudomonas*, *Achromobacter*, *Klebsiella*, *Chryseobacterium*, and *Pantoea* genera, has been observed to improve plant growth through stimulation of root development [33–35]. The microbial isolates such as *Pantoea*, *Pseudomonas*, *Citrobacter*, *Azotobacter*, *Streptomyces*, or other newly recognized groups of bacteria have also been identified as contributing to plant growth promotion by virtue of nutrient acquisition traits [33, 34, 36].

### 3.3 P and Fe Transport in Plants and Plant-Associated Microorganisms

The plant transports both Fe and P in response to nutrient deficiency conditions and there are several P or Fe transporters characterized both in plants and microorganisms [37–40]. Plant roots are the primary site for plant nutrient acquisition and under P- and Fe-depleted conditions undergo morphological changes in order to adapt to the changing nutrient condition or availability in soil [4, 11]. An increase in acidification of the rhizosphere environment such as by exudation of proton or carboxylate ions such as citrate, malate, or oxalate can greatly enhance mobilization of P in plants such as by chelation or ligand exchange of P bound or complexed to Ca, Fe, or Al present in soil [13]. Secretion of phosphatases or phytases can mobilize organic P through hydrolysis and has been shown to increase P availability in plants [6, 13].

The presence of microorganisms associated with plants is known to increase P availability in plants. Microorganisms such as bacteria and fungal endophytes isolated from plants have been shown under in vitro conditions to be involved in mineral phosphate solubilization activity by acidification of the extracellular environment, and by production of organic acid anions such as gluconic acid [41], malic acid [42], citric acid [43, 44], salicylic acid, and benzenoacetic acid [43]. Microorganisms are also hypothesized to be involved in other relevant activities such as proton extrusion and by ammonium ion assimilation that are linked with mineral phosphate solubilization activity [45]. The organic phosphate mineralization by microorganisms involves phosphatase activity, e.g., acid or alkaline phosphatase activity or phytase activity, which may contribute to availability of inorganic phosphate for plant uptake [46, 47].

The transport of P in both plants and microorganisms is mainly associated with two transporters, the high-affinity Pi transport systems and low-affinity Pi transport systems [13, 38]. The high-affinity Pi transporter in plants is the major transporter family responsible for transport of P in roots or in cells with close contact to the soil matrix. The low-affinity Pi transporters are mainly active in vascular tissues and involved in the internal distribution and re-mobilization of P [48]. Phosphate transport in plants by the high-affinity transporter system is H<sup>+</sup>/ATP dependent and is activated or expressed when the external P level in plants or cells in close contact to the soil matrix is low. The high-affinity phosphate transporter system is grouped within the major Pht1 family and has shared topology among fungi, yeast, plant, and animal Pht1 transporters [49, 50]. The high-affinity phosphate transporters have been characterized in a number of plant and fungal species; however, the role of phosphate transporters among certain AMFs has not been verified due to the lack of a stable transformation system [51]. A study on the P transporter of the fungal endophyte *Piriformospora indica* [51] identified a high-affinity phosphate transporter PiPT belonging to the major facilitator superfamily (MFS) found in bacteria which is also conserved in eukaryotes [52]. The study also recognized the structural/functional relationships of Pi/H<sup>+</sup> symporters and the proton motive force driving the translocation of Pi in the host plant by the basidiomycete fungus under the Pi limitation condition [51, 52].

Iron transport or acquisition in plants involves two strategies and is dependent on the plant type under iron-deficient conditions. The acidification of the extracellular soil environment by proton extrusion and reduction of chelated Fe<sup>3+</sup> by ferric chelate reductase at the plant root surface enhance bio-availability of Fe as ferrous (Fe<sup>2+</sup>) ion in nongraminaceous monocotyledonous and in dicotyledonous plants. However, production of mugineic acid dependent phytosiderophores is an important mechanism for Fe chelation and availability as ferrous iron (Fe<sup>2+</sup>) for transport or acquisition of Fe in monocotyledons, especially among grasses [53]. The uptake of Fe by microorganisms involves a similar strategy to that of plants and involves chelation of unavailable Fe by specific or a range of siderophores and/or the use of reductases which help to increase available extracellular Fe for uptake by the microorganism [54]. Iron uptake in both plants and microorganisms involves Fe transporters and there is considerable similarity in certain cases between some microorganism and plant Fe transporters such as in the case of yeast and specific

plant Fe transporters [55, 56]. In conclusion there are a number of key plant growth promotion functional traits that are associated with microorganisms involved in P and Fe availability and these are summarized in Table 3.1.

### 3.4 Functional Role of P and Fe Bacterial and Fungal Endophytes

#### 3.4.1 P Endophytes and their Role (Fungal and Bacterial)

The P endophytes or other associated P microorganisms increase Pi availability in soils by mineral phosphate solubilization or by organic phosphate mineralization activity. The mechanistic basis or direct involvement of the P endophyte to increase plant growth or biomass has been demonstrated in studies under P limitation that is discussed below and summarized in Table 3.2.

Mineral phosphate solubilization is an effective strategy for the provision of P to the plant. In a study by Crespo et al. [70] they identified that the ability to solubilize inorganic phosphate was associated with acidification of the plant root environment by plant root-associated bacteria. The root of wheat and tomato was colonized by the bacterial endophyte *Gluconacetobacter diazotrophicus* previously isolated from sugarcane [82] that efficiently enhanced acidification of the plant root by production of gluconic acid. In contrast, the gluconic acid biosynthesis gene mutant (PQQ-GDH) of *G. diazotrophicus* lacked the ability to acidify in a test medium with plants, thus underlying the functional role of this trait.

The direct role of P endophytes in plant growth promotion have also been described in other studies, for example, Kumar et al. [74] in a study on maize colonized by the fungal endophyte *P. indica* reported a higher increase in biomass of plants under P limiting conditions. The difference in biomass between colonized and non-colonized plants was a 2.5-fold increase at limiting P and 1.2-fold increase at non-limiting P conditions respectively, thus underlying the function of the endophyte to be more effective under the P deficient condition. Li et al. [75, 76] in a study on perennial grass *Achnatherum sibiricum* infected by the fungal species *Neotyphodium* sp. recognized a significant increase in acid phosphatase activity under P deficient and N non-limiting conditions. The biomass of the endophyte infected plant was not affected by P limitation and was similar to plants grown under non-limiting P or N conditions. Malinowski et al. [80] found that an infected *Festuca arundinacea* (tall fescue) with the endophyte *Neotyphodium coenophialum* under P limiting conditions expressed an increased root absorption area through reduced root diameter and increased root hair length compared with the endophyte free counterpart. Altered root diameter and root hair length in this study was associated with the functional role by the endophyte present in tall fescue.

The role of fungal P endophytes to increase plant growth and to enhance phosphorous efficiency was also demonstrated by studies involving dark septate fungi (DSEs) present in the plant. Barrow and Osuna [79] reported an increase in shoot and root

**Table 3.1** Examples of plant-associated endophyte microorganisms implicated in nutrient acquisition and other plant functions

Isolation source plant	Endophyte microorganism	Functional traits	Reference
<i>Arachis hypogaea</i> Peanut	<i>Pantoea agglomerans</i>	Mineral phosphate solubilization, siderophore production	[57]
<i>Calophyllum brasiliense</i> Guanandi	<i>Trichoderma</i> sp.	Mineral phosphate solubilization	[58]
<i>Glycine max</i> Soybean	<i>Enterobacter sakazakii</i> , <i>Pseudomonas straminea</i> , <i>Acinetobacter calcoaceticus</i> , <i>Pseudomonas</i> sp.	Mineral phosphate solubilization, IAA, biological nitrogen fixation	[59]
<i>Glycine max</i> Soybean	<i>Rhizoctonia</i> sp. <i>Fusarium verticillioides</i>	Phytase	[60]
<i>Lippia sidoides</i> Pepper-rosmarin	<i>Lactococcus lactis</i>	Calcium phosphate, phosphate mineralization activity-calcium phytate, solubilize/mineralize phosphate from poultry litter	[61]
<i>Mammillaria fraileana</i> Wild cactus	<i>Pseudomonas putida</i> M5TSA, <i>Enterobacter sakazakii</i> M2PFe, <i>Bacillus megaterium</i> M1PCa	Mineral phosphate solubilization	[62]
<i>Manihot esculenta</i> Cassava	<i>Pantoea dispersa</i>	Mineral phosphate solubilization, biological nitrogen fixation	[43]
<i>Miscanthus giganteus</i> Miscanthus	<i>Pseudomonas fluorescens</i>	Mineral phosphate solubilization	[41]
<i>Moringa peregrine</i> Moringa	<i>Sphingomonas</i> sp. LK18, <i>Methylobacterium radiotolerans</i> LK17, <i>Bacillus subtilis</i> LK14, <i>Bacillus subtilis</i> LK15, <i>Sphingomonas</i> sp. LK16	Mineral phosphate solubilization, acid phosphatase, IAA	[63]
<i>Oryza sativa</i> var. Japonica c.v. Rice	<i>Paenibacillus kribbensi</i> , <i>Bacillus aryabhattai</i> , <i>Klebsiella pneumoniae</i> , <i>Bacillus subtilis</i> , <i>Microbacterium trichotecenolyticum</i>	Biological nitrogen fixation, mineral phosphate solubilization, IAA	[64]
<i>Pachycereus pringlei</i> Giant cardon cactus	<i>Bacillus pumilus</i> var.2, <i>B. subtilis</i> var.2, <i>Actinomadura oligospora</i> , <i>Citrobacter</i> sp.	Mineral phosphate solubilization	[65]

(continued)

**Table 3.1** (continued)

Isolation source plant	Endophyte microorganism	Functional traits	Reference
<i>Panax ginseng</i> Ginseng	<i>Lysinibacillus fusiformis</i> , <i>Bacillus cereus</i> , <i>B.</i> <i>megaterium</i> , <i>Micrococcus</i> <i>luteus</i>	Mineral phosphate solubilization IAA, siderophore production	[117]
<i>Phaseolus vulgaris</i> Common Bean/French bean	<i>Rhizobium endophyticum</i> sp. Nov	Phytate	[66]
<i>Phaseolus vulgaris</i> Common bean/French bean	<i>Pseudomonas</i> sp.	Mineral phosphate solubilization	[67]
<i>Piper nigrum</i> Black pepper	<i>Klebsiella</i> sp., <i>Enterobacter</i> sp.	Mineral phosphate solubilization siderophore production, ACC deaminase, IAA production	[68]
<i>Pseudotsuga menziesii</i> Douglas-fir	<i>Rhodotorula graminis</i> , <i>Acinetobacter</i> <i>calcoaceticus</i> , <i>Rhizobium</i> <i>tropici</i> bv <i>populus</i> , <i>Sphingomonas</i> <i>yanoikuyae</i> , <i>Pseudomonas</i> <i>putida</i> , <i>Rahnella</i> sp., <i>Burkholderia</i> sp., <i>Sphingomonas</i> sp.	Mineral phosphate solubilization, Siderophores production, biological nitrogen fixation	[69]
<i>Saccharum officinarum</i> Sugarcane	<i>Gluconacetobacter</i> <i>diazotrophicus</i>	Biological nitrogen fixation, mineral phosphate solubilization	[70]
<i>Shorea leprosula</i> and <i>Shorea selanica</i> Meranti	<i>Trichoderma spirale</i>	Mineral phosphate solubilization and inhibition of fungal pathogen ( <i>fusarium</i> )	[71]
<i>Triticum aestivum</i> Wheat	<i>Streptomyces tricolor</i> <i>mhce0811</i>	Mineral phosphate solubilization phytase, siderophores, IAA, chitinase	[42]

biomass, and phosphorus use efficiency in *Atriplex canescens* by the fungal endophyte *Aspergillus ustus*. Jumpponen et al. [77] reported increased foliar P concentration and an increase in plant biomass of more than 50% following fungal inoculation and N amendment in endophyte-infected *Pinus contorta* by the fungal endophyte *Phialocephala fortini*. Newsham [78] recognized increased root, shoot, total P content, and total biomass and an increase in the number of tillers in endophyte-infected *Vulpia ciliata* by the fungal endophyte *Phialophora graminicola*.

Studies defining the mechanistic basis of P transport by endophytic microorganisms present in plants under P-deficient conditions also demonstrate the essential role of the endophyte. Hiruma et al. [73] in a study on an ascomycete fungal endophyte *Colletotrichum tofieldiae* (*Ct*) in *Arabidopsis* identified the role of this endophyte in

**Table 3.2** Mechanisms of microbial phosphorous solubilization/transfer in endophyte-plant interactions

Endophyte	Original plant host	characteristics Or functional trait	Plant host/study	Effects	Reference	Comments
<i>Gluconacetobacter diazotrophicus</i>	<i>Saccharum officinarum</i> (sugarcane)	Transposon mutants in <i>pqq BC</i> and <i>E</i> and also <i>gdhA</i>	Not applicable In vitro study	A transposon mutant library identified gluconic acid synthesis as a required pathway for mineral phosphate (P) and zinc solubilization	[72]	However no direct link with the plant's regulatory network and P uptake in plants has been recognized to date
<i>Gluconacetobacter diazotrophicus</i>	<i>Saccharum officinarum</i> (sugarcane)	PQQ-GDH mutant MF105strain of <i>G. diazotrophicus</i>	<i>Lycopersicon esculentum</i> (tomato) <i>Triticum aestivum</i> (wheat)	Seedlings inoculated with a PQQ-GDH mutant MF105strain of <i>G. diazotrophicus</i> showed no acidification compared to WT	[70]	<i>G. diazotrophicus</i> produced solubilization only when aldoses were used as the C-source
<i>Colletotrichum tofieldia</i> Ct	<i>Arabidopsis thaliana</i>	<sup>32</sup> P translocation experiments and transcriptional analysis of P transporter genes in <i>Arabidopsis</i>	<i>Arabidopsis thaliana</i>	<i>C. tofieldiae</i> (Ct) root Fungal endophyte transfers the macronutrient phosphorus to shoots, promotes plant growth, and increases fertility under phosphorus-deficient conditions The host's phosphate starvation response (PSR) system controls Ct root colonization and is needed for plant growth promotion (PGP)	[73]	Nine transporter genes were significantly upregulated in phosphate-starved roots in the absence of Ct while two, Pht1.2 and Pht1.3, were induced at higher levels only in the presence of Ct under Pi-limiting conditions, similar to mycorrhizal associations



<i>Piriformospora indica</i>	<i>Zea mays</i> (maize)	Knockdown gene encoding a phosphate transporter (PiPt) in <i>P. indica</i>	<i>Zea mays</i> (maize)	Higher amounts of phosphate were found in plants colonized with WT <i>P. indica</i> than that of non-colonized plants or plants with knockdown gene encoding a phosphate transporter (PiPt) in <i>P. indica</i>	[51]	Observation suggests that PiPt is actively involved in <i>P. indica</i> and helps improve the nutritional status of the status of the host plant
<i>Piriformospora indica</i>	<i>Zea mays</i> (maize)	<sup>32</sup> P translocation experiments	<i>Zea mays</i> (maize)	Increase in biomass between colonized and non-colonized plants was 2.5 times that at limiting <i>P. indica</i> enhances growth more effectively at low P. Considerable amount of <sup>32</sup> P was measured in maize plants, suggesting that hyphae of <i>P. indica</i> were transporting P to host plant under P-limiting conditions	[74]	Speculated that phosphocholine effluxed by the fungus to the plant and pi would then be taken up by the plant via an H+ co-transporter. A phosphate transporter PiPT gene was not expressed in P-rich conditions, only under P-deprived condition

(continued)

Table 3.2 (continued)

Endophyte	Original plant host	characteristics Or functional trait	Plant host/study	Effects	Reference	Comments
<i>Neotyphodium</i> sp.	<i>Achnatherum sibiricum</i>		<i>Achnatherum sibiricum</i>	Under P deficiency and N availability, endophyte infection significantly increased acid phosphatase activity of endophyte-infected plants and the biomass of endophyte-infected plants was not affected under P deficiency, with similar growth as under available P or available N conditions	[75, 76]	Under both N- and P-deficient conditions, plant had greater P concentrations in roots and no significant difference in biomass was found between endophyte-infected and endophyte-free plants
<i>Phialocephala fortini</i>	<i>Pinus contorta</i>	Inoculated vs. non-inoculated plants	<i>Pinus contorta</i>	Increased foliar P concentration. A combination of N amendment and fungal inoculation increased host biomass by more than 50% beyond that obtained via N amendment alone	[77]	
<i>Phialophora graminicola</i>	<i>Vulpia ciliata</i> ssp. <i>ambigua</i>	Inoculated vs non-inoculated plants	<i>Vulpia ciliata</i> ssp. <i>ambigua</i>	Increased tillers, increased root, shoot, and total biomass. Increased root N content plus increased root, shoot, and total P, reduced shoot N	[78]	

<i>Aspergillus ustus</i>	<i>Atriplex canescens</i>	Experimental system to allow access to P only through the endophytic fungus	<i>Atriplex canescens</i>	A. <i>ustus</i> obtained plant carbon, increased shoot and root biomass, and phosphorus use efficiency [79]	
<i>Neotyphodium coenophialum</i>	<i>Festuca arundinacea</i> (tall fescue)	<i>Neotyphodium</i> infected vs. non-infected plant	<i>Festuca arundinacea</i> (tall fescue)	<i>N. coenophialum</i> under P-limiting conditions expressed an increased root absorption area through reduced root diameter and increased root hair length compared with the endophyte-free counterpart [80]	
<i>Dark septate fungal</i> sp. (unknown)	<i>Carex firma</i> and <i>Carex sempervirens</i>		<i>Carex firma</i> and <i>Carex sempervirens</i>	Significant increase in shoot P content in both the host plants. Increase in dry matter production detected in <i>Carex firma</i> [81]	

transfer of phosphorus to *Arabidopsis* shoots. This study showed that the host's phosphate starvation response (PSR) system controls *Ct* root colonization and is needed for plant growth promotion, and also the role of *Ct*-mediated plant growth promotion was recognized to be mediated by the plant innate immune system. This study hypothesized that the *Ct* association in the host root of *A. thaliana* and other *Brassicaceae* members has essential components important for developing these associations that are usually absent in mycorrhizal symbiosis. The importance of the P endophyte and P transport function in plants has also been demonstrated by *P. indica* in maize plants. Yadav et al. [51] reported that higher amounts of phosphate were found in plants colonized with wild-type *P. indica* than that of non-colonized plants or plants with a knockdown phosphate transporter (PiPT). It was suggested that PiPT of *P. indica* was actively involved in phosphate transfer *in planta* and can improve the nutritional status of the host plant.

### 3.4.2 *Fe Endophytes and their Role (Fungal and Bacterial)*

The role of endophyte and other microorganisms in iron acquisition by plants is associated with siderophore production. There are over 500 different types of siderophores produced by microorganisms [37] and purified bacterial siderophore has been recently shown to restore growth to iron-limited and stunted tomato plants [83]. Siderophores produced by microorganisms not only directly improve Fe availability to microorganisms and plants by direct chelation from soil but can also increase iron availability based on their competition for Fe with other microorganisms and pathogens. Studies defining the Fe availability and plant growth or biocontrol function by Fe endophytes are summarized in Table 3.3.

The importance of the siderophore-producing trait by Fe endophytes is demonstrated by its direct role in increasing plant growth or by improvement of host fitness. Rungin et al. [89] in a study using a bacterial endophyte *Streptomyces* sp. previously isolated from jasmine rice (*Oryza sativa* L. cv. KDML105) and its siderophore mutant (*desD*) showed an enhancement of plant growth with a significant increase in plant biomass in rice (*Oryza sativa*) and mungbean (*Vigna radiata*) by the siderophore-producing endophyte. The increase in plant growth or biomass was higher in *Streptomyces*-treated plants producing siderophore compared to a siderophore-deficient *desD* mutant and untreated control plants, thus underlying the functional importance of siderophore in enhancement of growth in plants. Rosconi et al. [86] in a study on a serobactin-producing bacterial endophyte *Herbaspirillum seropedicae* responsible for Fe acquisition by the microorganism and with its uptake mutant (Hsero\_2345 gene) in an experiment on rice (*Oryza sativa*) showed that serobactin-mediated iron acquisition contributes to competitive fitness in the host plant.

The role of siderophore produced by endophytic bacteria has also been demonstrated through its biocontrol function or synergistic role in plant growth promotion and colonization in certain studies. Verma et al. [88] in a study on bacterial

**Table 3.3** Mechanisms of microbial iron solubilization/transfer in endophyte-plant interactions

Endophyte	Original plant host	Characteristics	Plant host	Effects	Reference	Comments
<i>Epichloë festucae</i>	<i>Festuca trachyphylla</i> ( <i>Festuca longifolia</i> )	Epichloenin A (ferrichrome siderophore) <i>sidN</i> mutant defective in synthesis	<i>Lolium perenne</i> (ryegrass)	Fungal siderophore was detected by HRMS and NMR in plant guttation fluid (xylem sap) inoculated with fungus but not in mutant inoculated plants	[84]; [85]	Indicates fungal siderophore is produced in planta. Role in maintaining mutualistic nature of symbiosis
Herbaspirillum seropedicae Z67	<i>Oryza sativa</i> (rice)	Serobactin uptake mutant Hsero_2345 gene	<i>Oryza sativa</i> (rice)	Contributes to competitive fitness of endophyte within plant. WT is more competitive after 8 days	[86]	
<i>Pseudomonas fluorescens</i> PICF7	<i>Olea europaea</i> (olive)	Pyoverdine (pseudobactin) mutant ( <i>pvdI</i> ). Siderophore negative	<i>Olea europaea</i> (olive)	Does not affect external or internal root colonization. Does not affect biological control of olive (VWO) ( <i>Verticillium dahliae</i> )	[87]	Uptake of pseudobactin would not be affected. Did not test for competition with WT

(continued)

Table 3.3 (continued)

Endophyte	Original plant host	Characteristics	Plant host	Effects	Reference	Comments
<i>Streptomyces</i> azR-051	<i>Azadirachta indica</i>	Wt high-expressing siderophore and IAA strains	<i>Lycopersicon esculentum</i> (tomato)	PGPR and enhanced biocontrol of early blight disease caused by <i>Alternaria alternata</i> compared to strains with lower levels of IAA and siderophore	[88]	Not clear if IAA or siderophore is the key factor
<i>Streptomyces</i> sp. GMKU3100	<i>Oryza Sativa</i> (Thai jasmine rice)	High siderophore producer but no P sol or IAA production. <i>desD</i> mutant-siderophore negative	<i>Vigna radiata</i> (mung bean) <i>Oryza sativa</i> (rice)	Increase in plant biomass parameters with WT inoculation compared to siderophore mutant or uninoculated controls	[89]	
<i>Chryseobacterium</i> C138	<i>Oryza sativa</i> (Rice)	Siderophore extracted from this strain	<i>Lycopersicon esculentum</i> var. <i>Marglobe</i> (tomato)	Iron-starved tomato plants when treated with siderophore C138 were restored with respect to growth parameters to full-iron Hoagland solution	[83]	Purified bacterial siderophore C138 could supply Fe to iron-starved plants

*Streptomyces* endophytes recovered from *Azadirachta indica* reported significant plant growth promotion in tomato and biocontrol against *Alternaria alternata*, causal agent of early blight disease in tomato plants. A significant antagonistic activity by *Streptomyces* endophytes against the pathogen was linked to the high Fe complexing capacity of isolates (i.e., siderophore production). All isolates of *Streptomyces* prolifically produced IAA and siderophores demonstrating that both IAA and siderophores play a vital role in promotion of plant growth and in suppression of the pathogen. Koulman et al. [84] in a study using the fungal endophyte *Epichloë festucae* isolated from *Festuca trachyphylla* and its siderophore *sidN* mutant in an experiment on *Lolium perenne* reported the role of siderophore in colonization in xylem sap of *L. perenne*. Further work by Johnson et al. [85] demonstrated that this gene (*sidN*) played a key role in maintaining the mutualistic interaction with its host plant, highlighting the importance of iron homeostasis for the symbiotic interaction.

### 3.5 Perspectives on the Role of Endophytes in Fe and P Nutrient Acquisition: Potential Application for Agriculture and Future Prospects

The unavailability of both Fe and P in many soils had been recognized as a major growth-limiting factor in many agricultural systems [36, 90]. The inoculation of crops with specific microorganisms has the potential to reduce application rates of phosphate and can also improve iron uptake by plants [91–93]. Endophytes are able to enhance the growth of many plant species with or without concomitant nutrient uptake both directly and indirectly (Table 3.1). However, the impact of endophyte colonization on nutrient uptake in planta can be variable among strains and is considered to be dependent on host species/cultivars, endophyte taxa, and environmental conditions [94]. Although a broad range of endophytes are described with nutrient acquisition traits as reported in this study few endophytes have been studied in detail to conclusively demonstrate the mechanism(s) of nutrient transfer/acquisition of nutrient in planta. The basidiomycete fungal endophyte *Piriformospora indica* has gained substantial interest as a potential growth-promoting agent [95]. *P. indica* may serve as a model system to elucidate the mechanisms of host growth or fitness, as it has the capability in mobilizing plant unavailable P by production of extracellular phosphatases and in translocation of P in plants [95, 96]. *P. indica* stimulates plant growth as well as seed production of many plants and possesses a broad host range specificity [96, 97]. An increasing number of studies on this fungus provide a scientific basis for agricultural application, and also importantly that this fungal endophyte can easily be grown axenically [98–100]. The P acquisition potential of this endophyte was tested in maize, barley, and *Arabidopsis* [51, 101, 102]; further testing its P acquisition

potential in a range of plant hosts will validate the mechanistic basis and may identify if P transport or acquisition is a generalized mechanism of nutrient transfer among crops.

Besides *P. indica*, a number of dark septate endophyte (DSE) fungi (the non-clavicipitaceous group of fungi) have been recognized in plants from a range of ecosystems [103]. The DSEs can help increase plant growth by increasing acquisition of plant nutrients such as N and P in certain plants [77–79]. It has been proposed that DSE symbioses, like mycorrhizas, are multifunctional and not limited to nutritional acquisition and host growth response [28]. However the overall functional potential of this class of fungi or its inoculation potential in plants needs to be verified in order to utilize it for plant growth promotion [104]. The clavicipitaceous endophytes are another group of fungi whose role remains elusive, and work is mostly focused on two related genera *Epichloë* and their anamorphic *Neotyphodium* relatives [105]. These fungi are recognized as increasing P nutrients and in certain cases are also known to be involved in other functions such as abiotic stress tolerance [106], remediation of metal contamination [107], and biocontrol activity [105]. This group of fungi mostly inhabit grasses (family Poaceae) and may have potential in plant growth improvement [105].

Bacteria have also been shown to be important with respect to P and Fe acquisition in plants and are involved in P solubilization or mineralization activity and siderophore production as discussed in previous sections in this chapter. The commercialization of endophyte bacteria such as *Gluconacetobacter diazotrophicus* has gained substantial attention, as N-Fix® (Azotic Technologies, UK) or NITROFIX™-AD (AgriLife, India) for biological nitrogen fixation in plants. *G. diazotrophicus* is also recognized for other traits such as phosphate solubilization activity and this function could be synergistic in plants [72]. Strains of *G. diazotrophicus* are isolated in many areas of world and are utilized commercially to enhance plant production [108].

The biofertilizer potential of siderophore-producing endophytes is also of importance in agriculture not only in terms of improving direct Fe availability in plants by siderophore production but also by biocontrol of pathogens indirectly by increasing Fe nutrient status of plant and depriving the pathogen of iron. The siderophore-producing endophyte may also have phytoremediation potential for remediation of contaminated soils [75, 76, 109] and may share functional similarity with phosphate-solubilizing endophytes which have the ability to produce organic acids and similar to siderophore production may assist in the remediation of contaminated soils [75, 76, 110].

The successful manipulation of the plant microbiome has the potential to increase agricultural production [111, 112] and reduce chemical inputs [113–115] and greenhouse gasses [116] which will result in more sustainable agricultural practices. However, this will require a more detailed exploration of the mechanisms involved in P- and Fe-facilitated mobilization by the plant microbiota.



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