# Chapter 2
# Mechanism of Phosphate Solubilization and Physiological Functions of Phosphate-Solubilizing Microorganisms

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**Abstract** Phosphorus (P) is the second important key plant nutrient after nitrogen. An adequate supply of P is therefore required for proper functioning and various metabolisms of plants. Majority of P in soils is fixed, and hence, plant available P is scarcely available despite the abundance of both inorganic and organic P forms in soils. A group of soil microorganisms capable of transforming insoluble P into soluble and plant accessible forms across different genera, collectively called phosphate-solubilizing microorganisms (PSM), have been found as best eco-friendly option for providing inexpensive P to plants. These organisms in addition to supplying soluble P to plants also facilitate the growth of plants by several other mechanisms, for instance, improving the uptake of nutrients and stimulating the production of some phytohormones. Even though several bacterial, fungal and actinomycetal strains have been identified as PSM, the mechanism by which they make P available to plants is poorly understood. This chapter focuses on the mechanism of P-solubilization and physiological functions of phosphate solubilizers in order to better understand the ecophysiology of PSM and consequently to gather knowledge for managing a sustainable environmental system. Conclusively, PSM are likely to serve as an efficient bio-fertilizer especially in areas deficient in P to increase the overall performance of crops.

**Keywords** Phosphate solubilization • Mineralization • Organic acids • Plant growth regulators • ACC deaminase

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## 2.1 Introduction

Phosphorus (P) is one of the most essential plant nutrients which profoundly affect the overall growth of plants (Wang et al. 2009) by influencing various key metabolic processes such as cell division and development, energy transport, signal transduction, macromolecular biosynthesis, photosynthesis and respiration of plants (Shenoy and Kalagudi 2005; Ahemad et al. 2009; Khan et al. 2009). On the contrary, unlike N, the atmosphere does not provide soluble P to plants. And hence, the source of P is largely the primary and secondary minerals and/or organic compounds. In comparison to other nutrients, P concentration in soil solution is much lower and ranges from 0.001 to 1 mg/l (Brady and Weil 2002). Broadly, P compounds in soil can be placed into three categories: (i) inorganic compounds, (ii) organic compounds of the soil humus and (iii) organic and inorganic P compounds associated with the cells of living matter. Mineral compounds of P usually contain aluminium (Al), iron (Fe), manganese (Mn) and calcium (Ca) and vary from soils to soils. For example, P forms a complex with Al, Fe and Mn in acidic soils, while in alkaline soils it reacts very strongly with Ca. However, under all conditions, the types of soil P compounds are determined mainly by soil pH and by the type and concentrations of soil minerals. Some of the most common P minerals are presented in Table 2.1. Of the total soil P pool, about 50 % of P is in the organic forms (Richardson 1994), which varies between 4 and 90 % in most soils (Yadav and Verma 2012). The organic P in plants includes (i) inositol phosphate (10–50 % in soil) which represents a series of phosphate esters ranging from monophosphates up to hexaphosphates. Phytic acid (inositol hexakisphosphate) is the main compound that plants use to store P in seeds to support early seedling growth following germination. Phytin (a Ca–Mg salt of phytic acid) is the most abundant of the known organophosphorus compounds in soils. Other organic P in soils occur as sugar phosphates, nucleotides (0.2–2.5 %), phosphoprotein (trace), phosphonates (Tate 1984) and phospholipids (1–5 %) (Yadav and Verma 2012). Of the various forms of P, plants take up only negatively charged primary and secondary orthophosphate ions ($H_2PO_4^-$ and $HPO_4^{2-}$) as nutrient. Indeed, the amount of plant available P is very low relative to the total soil P. Moreover, majority of the soil P is fixed, and only a small fraction of P is available for uptake by plants. Therefore, P deficiency results in stunted growth, dark leaves, and inhibition of flowering and root system development. In most plants, these symptoms will appear when P concentration in the leaves goes below 0.2 %. And, hence, in many cases, phosphatic fertilizers which are quite soluble and manures that also contain P (soluble P, organic P and inorganic P) are applied to overcome P deficiency in soils and to provide adequate P to plants. The P of the phosphatic fertilizers or the manure reacts very strongly with soil constituents and becomes unavailable to plants. The insoluble and inaccessible forms of P are hydrolysed to soluble and available forms through the process of solubilization (inorganic P)/mineralization (organic P). The immobilization in contrast is the reverse reaction of mineralization. During immobilization, microorganisms convert inorganic forms to organic phosphate,

**Table 2.1** Common phosphorous (P) minerals found in acid, neutral and calcareous soils

| S.No. | Minerals | Chemical formula |
|---|---|---|
| | Acid soils | |
| | Strengite | $FePO_4.2H_2O$ |
| | Variscite | $AlPO_4.2H_2O$ |
| | Neutral and calcareous soils | |
| | B-tricalcium phosphate | $Ca_3(PO4)_2$ |
| | Dicalcium phosphate | $CaHPO_4$ |
| | Dicalcium phosphate dihydrate | $CaHPO_4{\cdot}2H_2O$ |
| | Fluorapatite | $Ca_5(PO4)_3$ F |
| | Hydroxyapatite | $Ca_5(PO4)_3$ OH |
| | Octacalcium phosphate | $Ca_4H(PO4)_3{\cdot}2–5\ H_2O$ |

Adapted from Yadav and Verma (2012)

which are then incorporated into their living cells. Mineralization and immobilization of P occur simultaneously and are influenced by structure and compositions of microbes and physico-chemical characteristics of soils besides the exudates of various plant genotypes.

## 2.2 Phosphate Solubilization by Microbes: Current Perspective

The insoluble forms of P such as tricalcium phosphate ($Ca_3PO_4)_2$, aluminium phosphate ($Al_3PO_4$), iron phosphate ($Fe_3PO_4$), etc. may be converted to soluble P by P-solubilizing organisms inhabiting different soil ecosystems (Gupta et al. 2007; Song et al. 2008; Khan et al. 2013; Sharma et al. 2013). Soil microorganisms in this regard have generally been found more effective in making P available to plants from both inorganic and organic sources by solubilizing (Toro 2007; Wani et al. 2007a) and mineralizing complex P compounds (Bishop et al. 1994; Ponmurugan and Gopi 2006), respectively. Several workers have documented their findings in order to better understand as to how the microbial populations cause the solubilization of insoluble P (Illmer and Schinner 1995; Khan et al. 2007, 2009; Buch et al. 2008). Of the various strategies adopted by microbes, the involvement of low molecular mass organic acids (OA) secreted by microorganisms has been a well-recognized and widely accepted theory as a principal means of P-solubilization, and various studies have identified and quantified organic acids and defined their role in the solubilization process (Maliha et al. 2004; Khan et al. 2010; Marra et al. 2012). The OA produced by many P-solubilizers, for example, bacterial cultures (Table 2.2) or fungi (Table 2.3), in the natural environment or under in vitro conditions chelate mineral ions or decrease the pH to bring P into solution (Pradhan and Shukla 2005). Consequently, the acidification of microbial cells and their surrounding leads to the release of P-ions from the P-mineral by $H^+$ substitution for $Ca^{2+}$ (Goldstein 1994; Mullen 2005; Trivedi and Sa 2008). The

**Table 2.2** Organic acid production and P-solubilization by PS bacteria

| PS bacteria | Organic acid produced | Initial pH | Final pH | Amount of P solubilized (μg/ml) | Time (h) | References |
|---|---|---|---|---|---|---|
| Enterobacter sp. Fs-11 | MA, GA | 7.0 | 4.5 | 43.5 | 240 | Shahid et al. (2012) |
| *Pseudomonas trivialis* (BIHB 769) | GA, 2-KGA, LA, SA, FA, MA | 7 ± 0.2 | 3.70 | 806.4 ± 2.3 | 120 | Vyas and Gulati (2009) |
| *P. poae* (BIHB 808) | GA, 2-KGA, SA, CA, MA | 7 ± 0.2 | 3.58 | 821.4 ± 1.7 | 120 | Vyas and Gulati (2009) |
| *Pseudomonas* spp. (BIHB 751) | OA, GA, 2-KGA, FA, MA | 7 ± 0.2 | 4.20 | 318.7 ± 2.0 | 120 | Vyas and Gulati (2009) |
| *Enterobacter* Hy-401 | OA, GA, MA, LA, CA, SA, FuA | 7–7.5 | 4.32 ± 0.02 | 623.6 ± 23.0 | 120 | Yi et al. (2008) |
| *Arthrobacter* Hy-505 | OA, GA, LA, CA | 7–7.5 | 5.50 ± 0.04 | 428.9 ± 15.3 | 120 | Yi et al. (2008) |
| *Azotobacter* Hy-510 | OA, GA, TA, LA, SA, FuA | 7–7.5 | 4.69 ± 0.05 | 229.03 ± 15.2 | 120 | Yi et al. (2008) |
| *Enterobacter* Hy-402 | OA, GA, TA, CA, SA, FuA | 7–7.5 | 4.51 ± 0.02 | 111.73 ± 8.07 | 120 | Yi et al. (2008) |
| *Rhodococcus erythropolis* (CC-BC11) | GA | 7–6.8 | 5.3 | 186.9 | 72 | Chen et al. (2006) |
| *Bacillus megaterium* (CC-BC10) | CA, LA, PA | 7–6.8 | 5.1 | 270.2 | 72 | Chen et al. (2006) |
| *Arthrobacter* sp. (CC-BC03) | CA, LA | 7–6.8 | 4.9 | 519.7 | 72 | Chen et al. (2006) |
| *A. ureafaciens* (CC-BC02) | CA | 7–6.8 | 5.0 | 316.1 | 72 | Chen et al. (2006) |
| *Serratia marcescens* (CC-BC14) | CA, GA, SA, LA | 7–6.8 | 4.9 | 421.8 | 72 | Chen et al. (2006) |
| *Delftia* (CC-BC21) | SA | 7–6.8 | 4.9 | 346.1 | 72 | Chen et al. (2006) |
| *Chryseobacterium* (CC-BC05) | CA | 7–6.8 | 6.0 | 298.9 | 72 | Chen et al. (2006) |
| *Phyllobacterium myrsinacearum* (CC-BC19) | GA | 7–6.8 | 5.2 | 201.2 | 72 | Chen et al. (2006) |

*GA* gluconic acid, *2-KGA* 2α-ketogluconic acid, *LA* lactic acid, *SA* succinic acid, *FA* formic acid, *MA* malic acid, *CA* citric acid, *OA* oxalic acid, *FuA* fumaric acid, *TA* tartaric acid, *PA* propionic acid, *AA* acetic acid, *IBA* isobutyric acid, *IVA* isovaleric acid, *VA* valeric acid, *ISA* isocaproic acid, *ND* not determined [modified from Khan et al. (2013)]

**Table 2.3** Some examples of organic acids produced by P-solubilizing fungi

| Organism | Predominant acids | References |
|---|---|---|
| *Aspergillus niger* FS1, *Penicillium canescens* FS23, *Eupenicillium ludwigii* FS27, *Penicillium islandicum* FS30 | Citric, gluconic, oxalic | Mendes et al. (2013) |
| *Aspergillus awamori* S19 | Oxalic, malic, citric, succinic, fumaric | Jain et al. (2012) |
| *T. flavus*, *T. helicus*, *P. purpurogenum*, *P. janthinellum* | Acetic, butyric, citric, fumaric, gluconic, glucuronic, lactic, oxalic, propionic, succinic, valeric | Scervino et al. (2010a, b) |
| *Aspergillus niger*, *Penicillium bilaiae*, *Penicillium* sp. | Oxalic, citric | Arwidsson et al. (2010) |
| *Aspergillus flavus*, *A. candidus*, *A. niger*, *A. terreus*, *A. wentii*, *Fusarium oxysporum*, *Penicillium* sp., *Trichoderma isridae*, *Trichoderma* sp. | Lactic, maleic, malic, acetic, tartaric, citric, fumaric, gluconic | Akintokun et al. (2007) |
| *A. flavus*, *A. candidus*, *Penicillium oxalicum* | Glutaric, malic, gluconic, oxalic | Shin et al. (2006) |
| *Aspergillus flavus*, *A. niger*, *P. canescens* | Oxalic, citric, gluconic, succinic | Maliha et al. (2004) |
| *Penicillium rugulosum* | Citric, gluconic | Reyes et al. (2001) |
| *A. niger* | Succinic | Vazquez et al. (2000) |

efficiency of solubilization, however, depends on the kind of organic acids released into the medium and their concentration. Furthermore, the quality of the acid is more important for P-solubilization than the total amount of acids produced by phosphate solubilizing (PS) organisms (Scervino et al. 2010a, b). Additionally, the simultaneous production of different organic acids by the PS strains may contribute to the greater potential for solubilization of insoluble inorganic phosphates (Marra et al. 2012).

There are also reports which suggest that insoluble P could be transformed into soluble forms of P without OA production by microbes (Asea et al. 1988; Illmer and Schinner 1992; Chen et al. 2006). For example, Altomare et al. (1999) while investigating the P-solubilizing ability of plant growth-promoting and biocontrol fungus *Trichoderma harzianum* T-22 did not produce OA under in vitro condition suggesting that the insoluble P could be solubilized by mechanisms other than acidification process also. The fungal-solubilizing activity was credited both to chelation and to reduction processes, which may be useful in the management of phytopathogens. Apart from the OA theory, some of the inorganic acids (Reyes et al. 2001; Richardson 2001) such as HCl (Kim et al. 1997), nitric acid and sulphuric acids (Dugan and Lundgren 1965) produced by chemoautotrophs and the $H^+$ pump, for example, in *Penicillium rugulosum*, have also been reported to solubilize the insoluble P (Reyes et al. 1999). The inorganic acids so released

convert TCP to di- and monobasic phosphates with the net result of an enhanced availability of the element to plants.

### 2.2.1 *Mineralization: Enzymatic Degradation of Complex Organic P Compounds*

Organic P compounds undergo mineralization, and the resulting P is taken up as nutrient by plants. In this regard, numerous soil microbes or rhizosphere microflora possess the ability to transform organic P into soluble forms of P (Tarafdar and Claassen 1988; Rodriguez et al. 2006). This mineralization process is mediated by the enzymes especially phosphatases (Tarafdar et al. 1988; Yadav and Tarafdar 2003; Aseri et al. 2009) and phytases (Maougal et al. 2014), released by the soil microbes. The enzyme phosphatases (e.g. acid and alkaline phosphatases) released exterior to the cell (exo-enzymes) are non-specific in nature and use organic P as a substrate to convert it into inorganic form (Beech et al. 2001). Of the two phosphatases, acid phosphatases (To-O et al. 2000), a widely distributed enzyme and commonly found in fungi (To-O et al. 1997; Abd-Alla and Omar 2001), for example, *Colletotrichum graminicola* (Schadeck et al. 1998a, b), are considered as the principal mechanism for mineralization of soil organic P (Hilda and Fraga 1999) where it catalyses the release of inorganic P from organic P compounds such as inositol hexaphosphate (Nozawa et al. 1998; Tarafdar and Gharu 2006; Yadav and Tarafdar 2007, 2011). However, the degradation of organic P mediated by phosphatases varies greatly among different fungi (Guimarães et al. 2006). Another attractive application of P-dissolving enzymes is the mineralization of soil organic P through phytate degradation mediated by the enzyme phytase, which specifically causes release of P from phytic acid. Phytate is a major component of organic P in soil. Though the ability of plants to obtain P directly from phytate is very limited, the growth and P nutrition of *Arabidopsis* plants supplied with phytate was improved significantly when they were genetically transformed with the phytase gene [phyA] derived from *Aspergillus niger* (Richardson 2001). This led to the increase in P nutrition to such an extent that the growth and P content of the plant was equivalent to control plants supplied with inorganic P. Similar increase in utilization of inositol P by plants in the presence of microbial communities including P-solubilizing fungus (*A. niger*) capable of producing phytase is reported (Richardson 2001; Vassilev et al. 2007). Phosphonatases (Kumar et al. 2013) and C–P lyases (Salimpour et al. 2010) are the other enzymes that cleave the C–P of organophosphonates. Once the inorganic or organic P compound is changed to soluble P, it can now easily be used up as P nutrient by plants, algae, cyanobacteria and autotrophic bacteria and thereafter could be immobilized into organic cellular macromolecules, for example, DNA, RNA and ATP. Considering the critical impact of such enzymes in dissolution of complex organic compounds into usable form of P, it is highly desirable to develop the bacterial/fungal inoculants with high

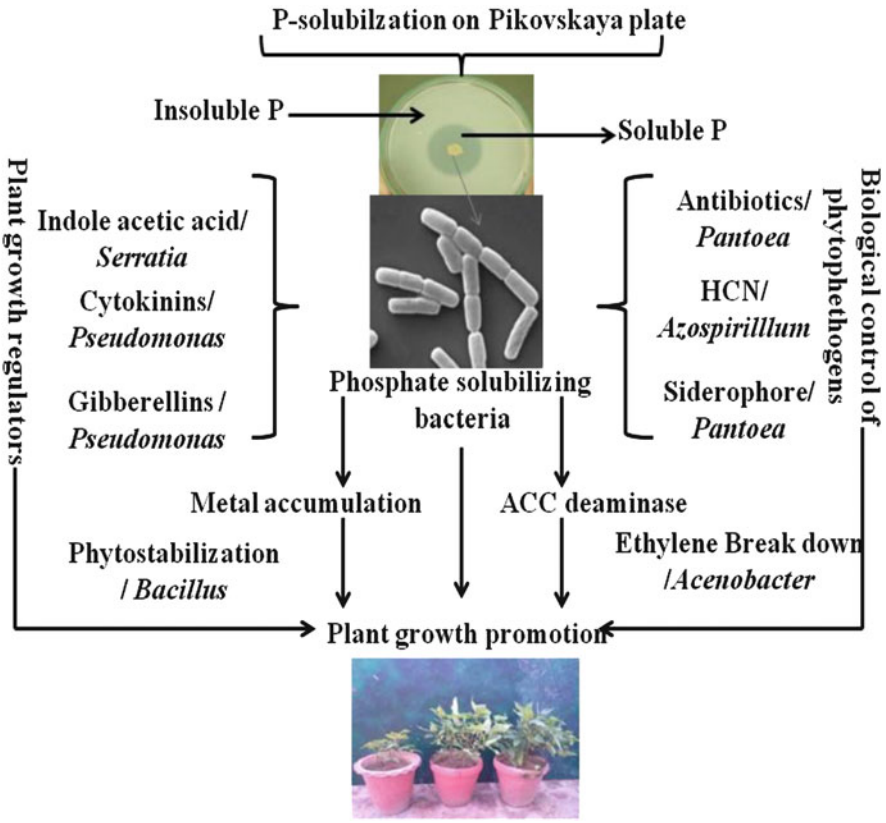


**Fig. 2.1** An illustration depicting functional diversity among PS bacteria [Adapted from Khan et al. (2013)]

phosphatase and phytase activity which in turn could possibly be of great practical value in sustainable crop production.

## 2.3 Physiological Functions of Phosphate-Solubilizing Microorganisms

Phosphate-solubilizing microorganisms increase the overall performance of plants by providing mainly soluble P to plants in different production systems. However, they also benefit plants by other mechanisms (Fig. 2.1). Indeed, PSM exhibit multifunctional properties (Vassileva et al. 2010; Yadav et al. 2011; Khan et al. 2013); for example, they are known to synthesize siderophores (Hamadali et al. 2008; Wani et al. 2008a; Tank and Saraf 2003; Viruel et al. 2011) and IAA and gibberellic acid (Sattar and Gaur 1987; Souchie et al. 2007; Viruel et al. 2011).

Phosphate-solubilizing bacteria such as Gram-negative *P. fluorescens*, *P. aeruginosa* and *Chromobacterium violaceum* also secrete antibiotics (Lipping et al. 2008; Taurian et al. 2010) and provide protection to plants against soilborne pathogens (biocontrol) (Khan et al. 2002; Vassilev et al. 2006; Singh et al. 2010). Other physiological traits of PSM involve the release of cyanide, a secondary metabolite which is ecologically important (Wani et al. 2007b) and gives a selective advantage to the producing strains (Rudrappa et al. 2008; Badawi et al. 2011). Besides strict P-solubilizers, a few genera of rhizobia, for example, *Bradyrhizobium* and *Rhizobium*, have also been found to solubilize P and secrete IAA (Pandey and Maheshwari 2007; Badawi et al. 2011). There are numerous PS bacteria that possess the ability to synthesize a key enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Glick et al. 2007), which hydrolyses ACC [the immediate precursor of plant hormone ethylene ($C_2H_4$)] to NH3 and α-ketobutyrate and thus mitigate the inhibitory effects of $C_2H_4$. Some of the compounds synthesized by PS bacteria with possible effect on plant growth are listed in Tables 2.4 and 2.5.

### 2.3.1 Some Examples of Positive Plant Growth Regulators Synthesized by PSM

Plant growth regulators (PGRs) are the substances that influence physiological processes of plants at very low concentrations and modify or control one or more specific metabolic events of a plant (Danova et al. 2012; Sane et al. 2012). According to the Environmental Protection Agency (EPA), the plant regulators have been defined as "any substance or mixture of substances intended, through physiological action, to accelerate or retard the rate of growth or maturation, or otherwise alter the behaviour of plants or their produce". Such compounds produced by the plant or by PGPR are called plant hormones (Davies 1995; Karadeniz et al. 2006). Broadly, on the basis of chemical structures and their subsequent effects on plants, plant growth-regulating substances have been divided into five general groups: (1) auxins, (2) gibberellins, (3) cytokinins, (4) ethylene and (5) a group called inhibitors, which includes abscisic acid (ABA), phenolics and alkaloids (Frankenberger and Arshad 1995; Ferguson and Lessenger 2006). The production of auxins (Glick 1995; Wani et al. 2007b, 2008b; Ahemad and Khan 2012) and ethylene (Sasek et al. 2012), for example, by PSM, is considered a common microbiological trait, while the synthesis of cytokinins by bacteria, for example, *Paenibacillus polymyxa* (Raza et al. 2008), is less common (Timmusk et al. 1999). The gibberellin secretion at high concentrations is, however, very rare (Solano et al. 2010). Generally, majority (>80 %) of the soil bacteria are capable of secreting auxins especially IAA, indole butyric acid or similar compounds via tryptophan metabolism (Solano et al. 2010; Legault et al. 2011). A few examples of the phytohormones secreted by PGPR including PS bacteria (Table 2.4) and PS

**Table 2.4** Growth-promoting substances produced by plant growth-promoting rhizobacteria

| Organisms | Growth regulators | References |
|---|---|---|
| *Pseudomonas putida* | ACC deaminase, IAA, siderophore, ammonia, HCN, P-solubilization | |
| *Pseudomonas* sp., *Pseudomonas fluorescens*, *Burkholderia glumae* | ACC deaminase, IAA, siderophore, ammonia, HCN, P-solubilization | Rashid et al. (2012) |
| *Bacillus* | ACC deaminase, IAA, siderophore, P-solubilization, lytic enzyme, HCN | Kumar et al. (2012) |
| *Azotobacter* | IAA, siderophore, P-solubilization | Farajzadeh et al. (2012) |
| *Klebsiella* | IAA, siderophore, P-solubilization, HCN | |
| *Azotobacter*, Fluorescent *Pseudomonas*, and *Bacillus* | IAA, siderophore, ammonia, HCN, P-solubilization | |
| *Pantoea dispersa* strain 1A | P-solubilization, IAA, siderophore, HCN | Selvakumar et al. (2008a, b) |
| *Bacillus* spp. | IAA, siderophore, HCN | Wani et al. (2007d) |
| *Pseudomonas*, *Bacillus* | Siderophore, IAA, P-solubilization | Rajkumar et al. (2006) |
| *Brevibacillus* sp. | IAA | Vivas et al. (2006) |
| *Xanthomonas* sp. RJ3, *Azomonas* sp. RJ4, *Pseudomonas* sp. RJ10, *Bacillus* sp. RJ31 | IAA | Sheng and Xia (2006) |
| *Bacillus* sp. | P-solubilization | Canbolat et al. (2006) |
| *Brevibacterium* sp. | Siderophore | Noordman et al. (2006) |
| *Bacillus subtilis* | IAA and P-solubilization | Zaidi and Khan (2006) |
| *Variovorax paradoxus*, *Rhodococcus* sp. and *Flavobacterium* (Cd tolerant) | IAA and siderophore | Belimov et al. (2005) |
| *Pseudomonas fluorescens* | IAA, siderophore, P-solubilization | Gupta et al. (2005) |
| *Pseudomonas putida* | Siderophore | Tripathi et al. (2005) |
| *Azotobacter*, fluorescent *Pseudomonas* | IAA | |

rhizobia (Table 2.5) and other compounds and their direct or indirect impact on plant growth and development are reviewed and discussed briefly in the following section.

**Table 2.5** Examples of plant growth-promoting substances synthesized by symbiotic nitrogen fixers

| Symbiotic $N_2$ fixer | Crop enhancer | References |
|---|---|---|
| *Rhizobium leguminosarum* RP2 | ACC deaminase, IAA, HCN, siderophore, ammonia, EPS | |
| *Bradyrhizobium* MRM6 | IAA, HCN, siderophore, ammonia, EPS | |
| *Rhizobium* MRL3 | IAA, HCN, siderophore, ammonia | |
| *Sinorhizobium* strain | Chitinase | Qing-xia et al. (2011) |
| *Rhizobium leguminosarum* var. *Phaseoli* | IAA | Stajković et al. (2011) |
| *Rhizobium* spp. | IAA, siderophore | Mehboob et al. (2010) |
| *Sinorhizobium meliloti* | IAA, P-solubilization | Bianco and Defez (2010) |
| *Bradyrhizobium* | IAA, gibberellic acid | Afzal et al. (2010) |
| *Mesorhizobium* | IAA | |
| *Rhizobium* spp. | IAA | Chakraborty et al. (2009) |
| *Rhizobium leguminosarum* | IAA, siderophore | |
| *Mesorhizobium* | IAA, HCN, siderophore, ammonia, P-solubilization | |
| *Rhizobium* strain TAL 1145 | ACC deaminase | Tittabutr et al. (2008) |
| *Rhizobium* spp. | IAA, gibberellic acid, zeatin | Boiero et al. (2007) |
| *Mesorhizobium loti* MP6 | IAA, HCN, siderophore, P-solubilization | Chandra et al. (2007) |
| *Rhizobium etli* USDA9032 | Phenazine, antibiotic | Krishnan et al. (2007) |

#### 2.3.1.1 Synthesis and Physiological Functions of Phytohormones

Synthesis of IAA

The synthesis of IAA by microbes (Fig. 2.2) involves one of the three pathways: (1) Indoleacetic acid formation via indole-3-pyruvic acid and indole-3-acetic aldehyde is found in the majority of bacteria like *Erwinia herbicola*; saprophytic species of the genera *Agrobacterium* and *Pseudomonas*; and certain representatives of *Bradyrhizobium*, *Rhizobium*, *Azospirillum*, *Klebsiella* and *Enterobacter*. (2) The conversion of tryptophan into indole-3-acetic aldehyde may involve an alternative pathway in which tryptamine is formed. This pathway is believed to operate in pseudomonads and azospirilla. (3) IAA biosynthesis via indole-3-acetamide formation is reported for phytopathogenic bacteria *Agrobacterium tumefaciens*, *Pseudomonas syringae* and *E. herbicola* and saprophytic pseudomonads like *Pseudomonas putida* and *P. fluorescens*. The genes controlling IAA synthesis via this pathway are also reported in symbiotic bacteria like *Rhizobium* spp.,

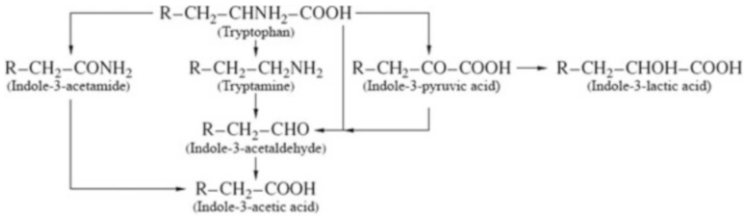


**Fig. 2.2** Biosynthetic pathways of IAA in bacteria [Adapted from Patten and Glick (1996)]

*Bradyrhizobium* spp. and *Azospirillum* spp., although the activity of the corresponding enzymes is either negligible or not detectable. Indoleacetic acid biosynthesis that involves tryptophan conversion into indole-3-acetonitrile is found in plants, *Alcaligenes faecalis*, and possibly the cyanobacterium *Synechocystis* sp., and the tryptophan-independent pathway, more common in plants, is also found in microorganisms (azospirilla and cyanobacteria). However, the synthesis of IAA using this pathway is reported to be insignificant, and the mechanisms are largely unknown. Many bacteria are known to synthesize auxins using such pathways and help the plants to grow better. Bacteria in general form maximum amount of IAA during the steady-state stage of their growth while ammonium ions and glutamine inhibit IAA biosynthesis (Tsavkelova et al. 2006). The genes involved in IAA synthesis in bacterial strains may be plasmid or chromosomal borne. For example, pathogenic bacteria contain Ti plasmids that control the formation of the phytohormone, whereas in saprophytic microorganisms, auxin biosynthesis is governed by chromosomal genes (Tsavkelova et al. 2006). It is reported that 80 % of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as secondary metabolites (Loper and Schroth 1986). Of the various PGPR strains, bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas* and *Rhizobium* as well as *Alcaligenes*, *Enterobacter*, *Acetobacter* and *Bradyrhizobium* have been shown to produce auxins which help in stimulating plant growth (Egamberdieva et al. 2007; Wani et al. 2007c; Kumar et al. 2008; Poonguzhali et al. 2008). However, the extent of IAA production by bacterial strains could be different due in part to the involvement of biosynthetic pathways, location of the genes, regulatory sequences and the presence of enzymes to convert active free IAA into conjugated forms. Moreover, the synthesis of IAA is also influenced by environmental factors (Patten and Glick 1996). Synthesis of IAA by *Rhizobium* spp. in the presence and absence of tryptophan has also been demonstrated (Wani et al. 2007c). In a similar study, Bent et al. (2001) reported that the concentration of indole compounds by three different strains, *Paenibacillus polymyxa* (L6), *P. poly*myxa (Pw-2) and *Pseudomonas fluorescens* (M20), increased with increasing rate of tryptophan (0–200 mg/ml) at different incubation interval.

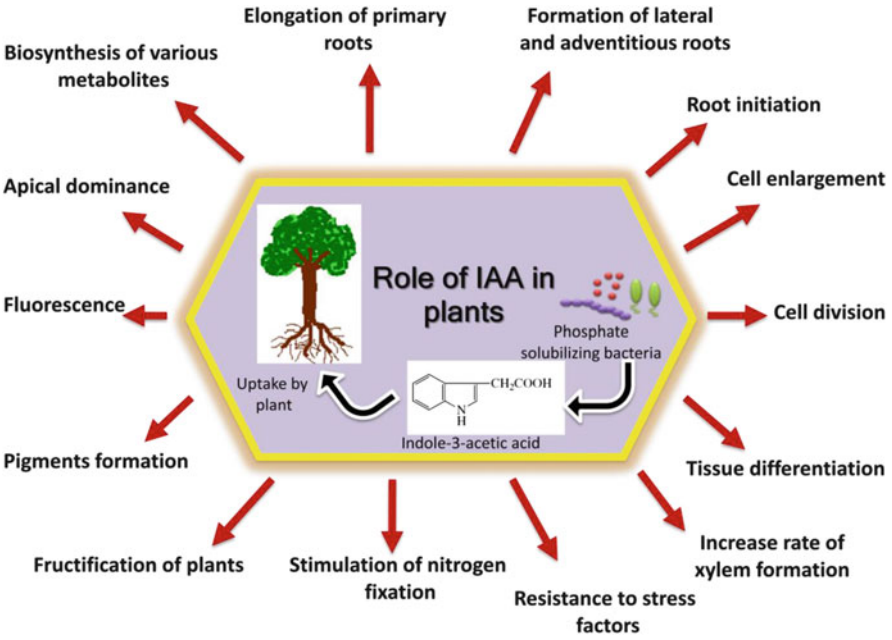


**Fig. 2.3** Indoleacetic acid affecting various stages of plant development

*Physiological Functions of IAA*

The production of phytohormones such as auxins by microbial communities has been reported by various workers over the last 20 years (Giordano et al. 1999a, b; Rajkumar and Freitas 2008; Singh 2008; Ahemad and Khan 2012). Among plant hormones, auxins are the major plant growth regulators produced by PSM (Oves et al. 2013) and exhibit many physiological functions as shown in Fig. 2.3. Apart from varying physiological functions of IAA, the role of IAA in legume–*Rhizobium* symbiosis is briefly discussed in the following section.

*Role of Indoleacetic Acid in Legume–*Rhizobium *Symbiosis*

Among nodule bacteria, rhizobial strains have been reported to produce auxins in variable amounts. For example, Vargas et al. (2009) in a study reported considerably lower frequency of auxin producers (23 %) among populations of clover nodulating *R. leguminosarum* bv. *trifolii*. The auxins so released by nodule bacteria are reported to affect nodulation, and accordingly, IAA synthesizing rhizobia have been found to produce more nodules than IAA-negative mutants (Boiero et al. 2007). The IAA produced by rhizobia may also induce root morphogenesis and consequently enhance its (1) size and weight, (2) branch numbers and patterns and (3) the surface area of roots as reported in non-legumes (Dazzo and Yanni

2006). Inoculation with auxin-producing bacteria may also result in the formation of adventitious roots (Solano et al. 2010). Furthermore, Noel et al. (1996) observed that the inoculation with IAA-producing strains of *R. leguminosarum* accelerated the germination of canola and lettuce. Similarly, Biswas et al. (2000) concluded that the inoculation of rice with *R. leguminosarum* bv. *trifolii* increased dry matter and grain production, besides an increment in N, P, K and Fe content in plant tissue. All these effects were ascribed due to the accumulation of IAA in the rhizosphere following rhizobial inoculation leading to some physiological changes in the root systems with consequent increase in nutrient uptake. In contrast, the overproduction of IAA in some cases by PGPR has been found to have deleterious impact on to plants (Schlindwein et al. 2008). For example, *R. leguminosarum* bv. *trifolii* strain TV-13 produced 171.1 mg/ml IAA in media enriched with tryptophan (Schlindwein et al. 2008), while strains of *Bradyrhizobium* sp. isolated from black wattle roots produced between 1.2 and 3.3 mg/ml IAA and increased the seedling vigour in relation to un-inoculated control plants. The variation in the amount of IAA produced by PGPR was, however, suggested due to differences in the composition of the growth medium and tryptophan concentration. In a follow-up study, Sridevi et al. (2008) observed that IAA production by rhizobia occurred only when tryptophan was added to YM and that the isolates produced the maximum amount of IAA in medium supplemented with 2.5 mg/ml tryptophan concentration.

### Other Phytohormones

Like auxins, cytokinins influence both cell division and cell enlargement and also affect seed dormancy, flowering, fruiting and plant senescence (Ferguson and Lessenger 2006). Cytokinin production by PGPR (Boiero et al. 2007) is, however, less obvious compared to the production of auxins. This is probably due to the lack of methods used for cytokinin detection, and hence, reports on cytokinin synthesis by PGPR in general are scarce. Gibberellin is yet another growth regulator which (1) affects seed germination (Miransari and Smith 2009), (2) stimulates growth of plants (Guo et al. 2011) and (3) delays ageing (Ferguson and Lessenger 2006). The production of gibberellins at high concentrations is considered very rare and has been reported for two strains of *Bacillus*, isolated from the *Alnus glutinosa* rhizosphere (Solano et al. 2010). The concentration of gibberellins in nodules is, however, generally higher than in nearby root tissue as supported by the fact that rhizobia have the capacity to produce some amount of gibberellin-like substances. However, it is not known whether bacteria contribute significantly to the amount of gibberellins within the nodule or it is just imported from some remote host plant tissue (Dobert et al. 1992; Hedden and Thomas 2012). Despite all these contrasting facts, the role of gibberellin in *Rhizobium*–legume symbiosis that may have important implications in the endophytic colonization of non-legumes by rhizobia is adequately described. For example, *A. caulinodans* infects the semi-aquatic legume *Sesbania rostrata* via the intercellular crack entry, a process mediated by gibberellins. Considering that crack entry is the main process of endophytic colonization

of non-legumes by rhizobia, the production of gibberellins by the bacterium is reported to facilitate this process (Lievens et al. 2005).

### 2.3.2 Negative Plant Growth Regulator

Abscisic acid is one of the strong inhibitor of growth and germination and promotes seed dormancy (Miransari and Smith 2009; Yang et al. 2009). Apart from these, ABA also helps plants to tolerate abiotic stresses. When plants are exposed to drought stress, the hormonal balance of plants changes and ABA content in the leaves increases, which reduce the level of cytokinin. This in turn elicits stomata closure (Yang et al. 2009). Cohen et al. (2009) in a similar study suggested that ABA produced along with gibberellins by PGPR strain significantly contributed to water stress alleviation of maize plants. Some rhizobial strains such as *B. japonicum* USDA110 also produce ABA (Boiero et al. 2007) and function in the same way as do the other PGPR (Zhang et al. 2012).

### 2.3.3 Growth Modulation Enzyme ACC Deaminase

Ethylene is a plant hormone which under normal conditions regulates many physiological processes, such as (1) seed germination, (2) root hair development and root elongation, (3) leaf and organ senescence, (4) leaf and petal abscission, (5) epinasty and (6) fruit ripening (Abeles et al. 1992; Frankenberger and Arshad 1995; Arshad and Frankenberger 2002; Siddikee et al. 2011). Also, ethylene regulates nod factor signalling and nodule formation and has primary functions in plant defence systems. Besides its physiological role in different developmental processes of plants, ethylene is also considered as a stress hormone, whose synthesis in plants is increased substantially by a number of biotic and abiotic stresses. At higher concentrations, ethylene, however, inhibits growth and development of plants (Grichko and Glick 2001). The enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (E.C. 4.1.99.4) which however mitigates the ethylene stress was first purified to homogeneity from *Pseudomonas* sp. strain ACP (Honma and Shimomura 1978), later on partially purified from *P. chlororaphis* 6G5 (Klee et al. 1991) and *P. putida* GR12-2 (Jacobson et al. 1994) and then purified to homogeneity from *P. putida* UW4 (Hontzeas et al. 2004). Enzyme ACC deaminase (a multimeric enzyme) has been found thereafter to be synthesized by a variety of PGPR (Belimov et al. 2005; Rajkumar et al. 2006; Madhaiyan et al. 2007; Mellado et al. 2007).

#### 2.3.3.1 How the Bacterial ACC Deaminase Works

Mechanistically, the ACC deaminase-producing plant growth-promoting bacteria first bind to the surface of a plant (usually seeds or roots), although these bacteria may also be found on leaves and flowers or within a plant's internal tissues, i.e. as an endophyte (Glick et al. 1998). Along with other small molecular components of root exudates, some of the plant ACC (a non-ribosomal amino acid) is exuded from seeds, roots or leaves (Penrose et al. 2001) and may be taken up by the bacteria associated with these tissues and subsequently cleaved by ACC deaminase (Penrose and Glick 2003). The ACC, the immediate precursor of $C_2H_4$, when hydrolysed by ACC deaminase results in $NH_3$ and α-ketobutyrate formation (Glick et al. 1998; Penrose and Glick 2003; Reed et al. 2005; Safronova et al. 2006), and hence, it strongly alleviates the stress induced by ethylene-mediated impact on plants by lowering the $C_2H_4$ levels in plants (Glick et al. 2007; Sessitsch et al. 2005; Sun et al. 2009). The bacteria utilize the $NH_3$ so evolved from ACC as a source of N and thereby restrict the accumulation of $C_2H_4$ within the plant, which otherwise inhibits plant growth (Belimov et al. 2002). Thus, the decreased levels of $C_2H_4$ in turn allow the plants to grow better (Zahir et al. 2008). A model to explain how ACC deaminase promotes plant growth is depicted in Fig. 2.4. It has been observed that plants inoculated with PGPR containing ACC deaminase were dramatically more resistant to the deleterious effects of stress ethylene, synthesized under stressful conditions such as flooding (Grichko and Glick 2001), heavy metals (Burd et al. 1998; Grichko et al. 2000), presence of phytopathogens (Wang et al. 2000), drought and high saline conditions (Mayak et al. 2004). The net result of the cleavage of exuded ACC by bacterial ACC deaminase is that the bacterium is de facto acting as a sink for ACC. Additionally, plants growing in association with ACC deaminase-containing plant growth-promoting bacteria generally have longer roots and shoots and are more resistant to growth inhibition by a variety of ethylene-inducing stresses. Furthermore, the reduction of ethylene levels in plant tissues following ACC deaminase activity can cause significant morphological changes in root tissue, such as changes in root hair length and increases in root mass, accompanied by the consequent improvement in nutrient uptake. The morphological changes in plants are further increased when ACC deaminase action is coupled with the production of auxins by PGPR. The question arises, how bacterial ACC deaminase selectively reduces the deleterious ethylene levels (the second ethylene peak) without affecting the small first peak of ethylene that is thought to activate plant defence responses. In this regard, ACC deaminase is generally present in bacteria at a relatively low level until it is induced, and the induction of enzyme activity is a rather slow and complex process. Immediately following an abiotic or biotic stress, the pool of ACC in the plant is low as is the level of ACC deaminase in the associated bacterium. Stress induces the induction of ACC oxidase in the plant so that there is an increased flux through ACC oxidase resulting in the first (small) peak of ethylene that in turn induces the transcription of protective/defensive genes in the plant. At the same time, bacterial ACC deaminase is induced by the

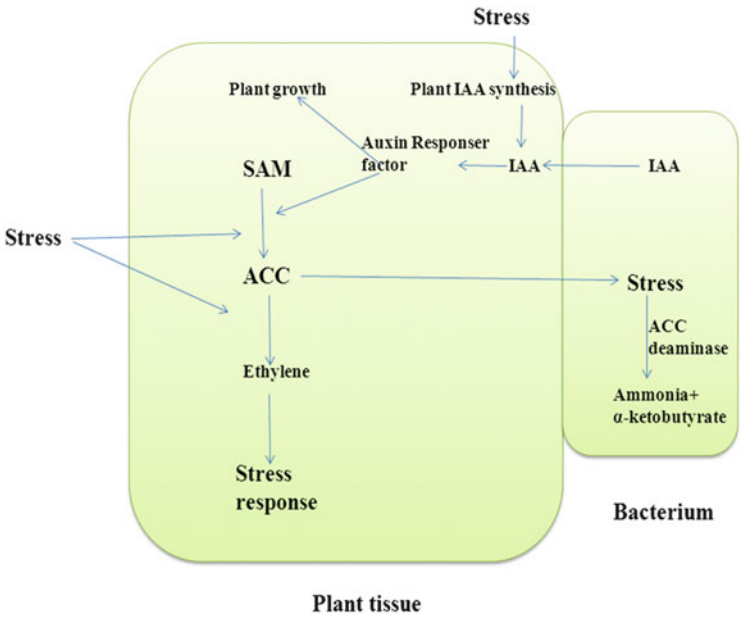


**Fig. 2.4** A schematic model of how plant growth-promoting bacteria that both produce ACC deaminase and synthesize IAA may facilitate plant growth. The only enzyme shown in this scheme is ACC deaminase. SAM is converted to ACC by the enzyme ACC synthase; ACC is converted to ethylene by ACC oxidase. IAA biosynthesis, both in bacteria and in plants, is a complex multi-enzyme/protein process as is IAA signal transduction. *ACC* 1-aminocyclopropane-1-carboxylate, *IAA* indole-3-acetic acid, *SAM* S-adenosyl methionine [Adapted from Glick (2014)]

increasing amounts of ACC that ensue from the induction of ACC synthase in the plant so that the magnitude of the second, deleterious, ethylene peak is decreased significantly (typically by 50–90 %). Because ACC oxidase has a greater affinity for ACC than does ACC deaminase, when ACC deaminase-producing bacteria are present, plant ethylene levels are dependent upon the ratio of ACC oxidase to ACC deaminase. That is, to effectively reduce plant ethylene levels, ACC deaminase must function before any significant amount of ACC oxidase is induced. Thus, in the absence of some other mechanism, IAA-producing bacteria might all be expected to ultimately be inhibitory to plant growth. However, this is in fact not the case because as plant ethylene levels increase, the ethylene that is produced through feedback mechanism inhibits IAA signal transduction thereby limiting the extent that IAA can activate ACC synthase transcription (Pierik et al. 2006; Prayitno et al. 2006; Czarny et al. 2007; Stearns et al. 2012). In plants inoculated with PGPR that secrete both IAA and ACC deaminase, the level of ethylene does not increase compared to the plants inoculated only with IAA-secreting bacteria. In the presence of ACC deaminase, there is much less ethylene and subsequent ethylene feedback inhibition of IAA signals transduction so that the bacterial IAA can continue to promote both plant growth and increase ACC synthase

transcription. However, in this case, a large portion of the additional ACC which is synthesized is hydrolysed by the bacterial ACC deaminase. Therefore, the use of such plant growth-promoting bacteria containing ACC deaminase may prove useful in developing strategies to facilitate plant growth in stressed soil environments.

#### 2.3.3.2 Role of ACC Deaminase in Nodulation

ACC deaminase-containing bacteria are relatively common in soil and have been found in a wide range of environments across the world. Indeed, the ability of bacteria to hydrolyse ACC has a competitive advantage over other soil inhabitants because it can use ACC as an N source (Jacobson et al. 1994). This hypothesis suggests that ACC may act as a unique/novel source of N for some soil bacteria. While searching for ACC deaminase positive rhizobial strains, it was found that amongst 13 different rhizobial strains, five strains displayed enzyme activity while seven strains had the *acdS* gene (Ma et al. 2003). Conclusively, it was reported that the *Mesorhizobium* strain only expressed this activity when the bacterium was present within a root nodule. In other investigation conducted in southern Saskatchewan, Canada, of the total 233 rhizobial strains isolated from soil samples collected from 30 different sites, nearly 12 % (27 strains) displayed the ACC deaminase activity (Duan et al. 2009). Similarly, ACC deaminase genes have been reported in chickpea *Mesorhizobium* isolates (Nascimento et al. 2012), *B. japonicum* E109, USDA110 and SEMIA5080 (Boiero et al. 2007). Rhizobial strains that express ACC deaminase are up to 40 % more efficient at forming nitrogen-fixing nodules than strains that lack this activity (Ma et al. 2003, 2004). However, strains of rhizobia that express ACC deaminase have only a low level of enzyme activity compared with free-living plant growth-promoting bacteria, i.e. typically around 2–10 %. Thus, free-living bacteria bind relatively non-specifically to plant tissues (mainly roots) and have a high level of ACC deaminase activity that can protect plants from different abiotic and biotic stresses by lowering ethylene levels throughout the plant. On the other hand, (symbiotic) rhizobia that generally bind tightly only to the roots of specific plants have a low level of enzyme activity which facilitates nodulation by locally lowering ethylene levels. It is not known whether the large differences in enzyme activity that are observed when comparing free-living bacteria with rhizobia are a consequence of differences in the amount of enzyme synthesized by one type of bacteria versus the other or of differences in the specific catalytic activity of the enzymes from the different types of bacteria. It has also been observed that some rhizobia reduces the plant ethylene levels mediated by ACC deaminase activity and enhances nodulation in host legumes (Zahir et al. 2008; Belimov et al. 2009) or modifies root system of non-legumes. For instance, strains of *R. leguminosarum* bv. *viciae* and *Mesorhizobium loti* increased the number of lateral roots in *Arabidopsis thaliana* because of this plant growth-promoting mechanism (Contesto et al. 2008). In addition to the more common mode of *acdS* transcriptional regulation, *acdS* genes from various strains of *M. loti* have been found to be under the transcriptional control of the *nifA* promoter that is

normally responsible for activating the transcription of nif, nitrogen fixation genes (Kaneko et al. 2000; Sullivan et al. 2002; Uchiumi et al. 2004; Nukui et al. 2006; Nascimento et al. 2012). The consequence of this somewhat unusual mode of regulation is that, unlike ACC deaminases from other rhizobia, the *M. loti* ACC deaminase does not facilitate nodulation but, rather, is expressed within nodules. The result of this unusual regulation is, in *M. loti*, ACC deaminase may act to decrease the rate of nodule senescence. This is particularly important because of the fact that nitrogen fixation, a process that utilizes a very high level of energy in the form of ATP, could (perhaps inadvertently) activate stress ethylene synthesis resulting in premature nodule senescence.

### 2.3.4 Physiological Functions of Siderophores

Iron is essential for almost all life for processes such as respiration and DNA synthesis. Despite being one of the most abundant elements in the Earth's crust, the bioavailability of iron in many environments such as the soil is limited by the very low solubility of the $Fe^{3+}$ ion. In the aerobic environment, iron accumulates in common mineral phases such as iron oxides and hydroxides and hence becomes inaccessible to organisms. Microbes (e.g. bacteria and fungi) have, therefore, evolved a strategy to acquire iron by releasing siderophores (Greek: "iron carrier"), small (generally less than 1,000 molecular weight) high-affinity iron-chelating compounds, which scavenge iron from the mineral phases by forming soluble $Fe^{3+}$ complexes that can be taken up by active transport mechanisms. Broadly, siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron starvation (Miethke and Marahiel 2007; Indiragandhi et al. 2008). There are more than 500 different siderophores which are produced mainly by Gram-positive and Gram-negative bacteria. Siderophores are highly electronegative and bind Fe (III), preferentially forming a hexacoordinated complex. The iron ligation groups have been tentatively classified into three main chemical types: (1) hydroxamate (e.g. aerobactin and ferrichrome), (2) catecholates/phenolates (e.g. enterobactin) and (3) hydroxyl acids/carboxylates (e.g. pyochelin). Some siderophores contain more than one of the three iron-chelating groups (Table 2.6). Siderophores are, however, usually classified by the ligands used to chelate the ferric iron. Citric acid can also act as a siderophore. The wide variety of siderophores may be due to evolutionary pressures placed on microbes to produce structurally different siderophores (Fig. 2.5). Siderophores are important for some pathogenic bacteria for their acquisition of iron. The strict homeostasis of iron leads to a free concentration of about $10^{-24}$ mol/l, and hence, there are great evolutionary pressures put on pathogenic bacteria to obtain this metal. For example, the anthrax pathogen *Bacillus anthracis* releases two siderophores, bacillibactin and petrobactin, to scavenge ferric iron from iron proteins.

**Table 2.6** Some examples of siderophores produced by various bacteria and fungi

| S.No. | Siderophores | Producing organisms |
|---|---|---|
| 1 | Hydroxamate | |
| A | Ferrichrome | *Ustilago sphaerogena* |
| B | Desferrioxamine B (deferoxamine) | *Streptomyces pilosus*, *Streptomyces coelicolor* |
| C | Desferrioxamine E | *Streptomyces coelicolor* |
| D | Fusarinine C | *Fusarium roseum* |
| E | Ornibactin | *Burkholderia cepacia* |
| 2 | Catecholate | |
| A | Enterobactin | *Escherichia coli* |
| B | Bacillibactin | *Bacillus subtilis*, *Bacillus anthracis* |
| C | Vibriobactin | *Vibrio cholera* |
| 3 | Mixed ligands | |
| A | Azotobactin | *Azotobacter vinelandii* |
| B | Pyoverdine | *Pseudomonas aeruginosa* |
| C | Yersiniabactin | *Yersinia pestis* |

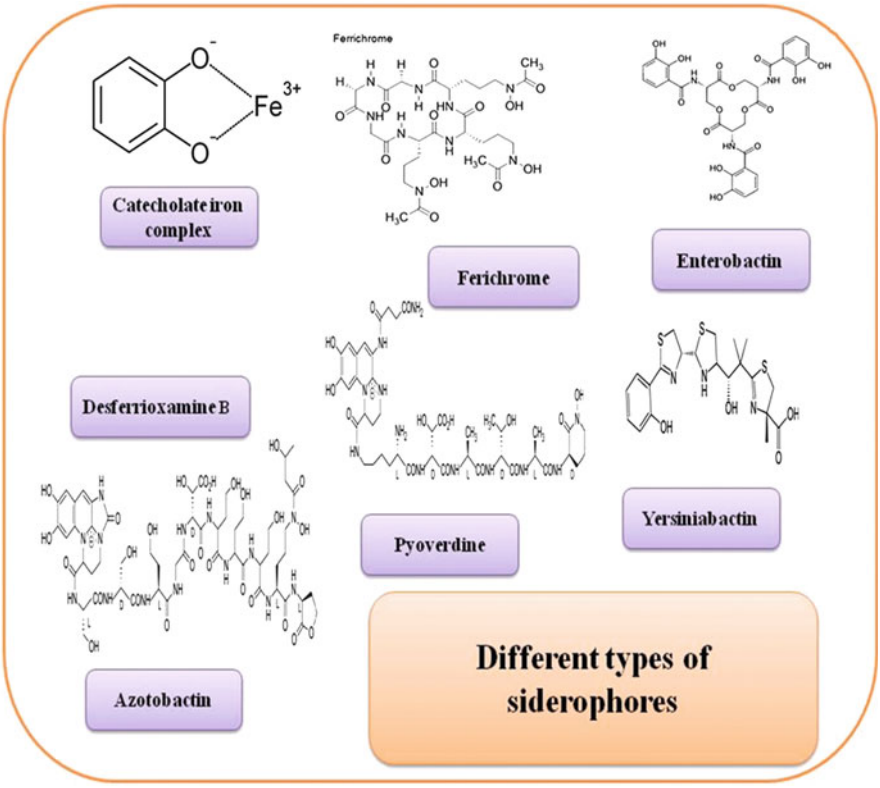


**Fig. 2.5** Different types of siderophores

#### 2.3.4.1 Role of Siderophores in Biological Nitrogen Fixation

Siderophore produced by majority of PGPR (Rajkumar et al. 2010) including rhizobia (Ahemad and Khan 2012) has been suggested as one of the modes of growth promotion of nodulated legumes under field conditions wherein siderophores facilitate the uptake of iron (assimilation) from the environment (Kloepper and Schroth 1978; Katiyar and Goel 2004). The iron enzymes involved include nitrogenase, leghemoglobin, ferredoxin and hydrogenase with nitrogenase and leghemoglobin constituting up to 12 % and 30 % of total protein in the bacterial and infected plant cells, respectively (Verma and Long 1983). A nodulated legume has been found to have an increased demand for iron compared to that of a non-nodulated plant (Derylo and Skorupska 1993). For example, *Pseudomonas* sp. strain 267 enhanced symbiotic $N_2$ fixation in clover under gnotobiotic conditions, produced fluorescent siderophores under low-iron conditions and secreted B group vitamins (Marek-Kozaczuk and Skorupska 2001). However, Tn5 insertion mutants of strain 267 defective in siderophore production did not differ from the wild type in promoting the growth of clover suggesting that the siderophore production had no effect on stimulating nodulation. In contrast, Gill et al. (1991) demonstrated that mutants of *R. melioti* that were unable to produce siderophores were able to nodulate the plants, but the efficiency of $N_2$ fixation was less compared to the wild type, indicating the importance of iron in $N_2$ fixation. In a similar study, *Kluyvera ascorbata*, a siderophore-producing PGPR, was able to protect plants from heavy metal toxicity (Burd et al. 1998).

### *2.3.5 Cyanogenic Compounds*

Cyanide is yet another secondary metabolite produced during the early stationary growth phase (Knowles and Bunch 1986) by several PGPR, notably *Pseudomonas* spp. and *Bacillus* (Wani et al. 2007d), *Chromobacterium* (Faramarzi and Brand 2006) and *Rhizobium* spp. (Wani et al. 2008a, b) by oxidative decarboxylation pathway using glycine, glutamate or methionine as precursors (Curl and Truelove 1986). The cyanide so released by microbial communities in solution acts as a secondary metabolite and confers a selective advantage onto the producer strains (Vining 1990). Although cyanide is a phytotoxic agent capable of disrupting enzyme activity involved in major metabolic processes, its role as a biocontrol substance is overwhelming (Devi et al. 2007; Voisard et al. 1989). Hydrogen cyanide (HCN) among cyanogenic compounds effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms at picomolar concentrations. However, producer microbes, mainly pseudomonads, are reported to be resistant (Bashan and de-Bashan 2005).

### 2.3.6 *Production of Lytic Enzymes*

In high input modem agricultural practices, pesticides are frequently and inappropriately used to protect crop plants from damage by insects, disease and so on which today still destroy almost 33 % of all food crops. The use of pesticides in this respect is considered effective if they provide the desired biological results and are inexpensive. However, the indiscriminate use of pesticides has resulted in the adverse impact on soil fertility, human health and the environment. Therefore, one of the safest strategies involving microorganisms for controlling/managing plant pests often called as "biocontrol" holds great promise as an alternative to the use of synthetic agrichemicals. Biological control agents are generally considered more environmentally sound than the pesticides and other antimicrobial treatments. In this context, the antagonistic potential of microbes in particular has formed the base for effective applications of such organisms as an alternative to the chemical control measures against a range of fungal and bacterial plant pathogens. And hence, a variety of microbial compounds have been identified/extracted that have been found to inhibit/suppress the phytopathogenic growth leading thereby to the reduction in damage to plants (Helbig 2001; Yang et al. 2005; Raza et al. 2008). These microbially synthesized compounds include defence enzymes, such as chitinase, β-1,3-glucanase, peroxidase, protease and lipase (Bashan and de-Bashan 2005; Karthikeyan et al. 2006). Chitinase and β-1,3-glucanase degrade the fungal cell wall and cause lysis of fungal cell. Furthermore, chitin and glucan oligomers released during degradation of the fungal cell wall by the action of lytic enzymes act as elicitors that elicit various defence mechanisms in plants. Such enzymes produced by *Pseudomonas stutzeri* have demonstrated the lysis of the pathogen *Fusarium* sp. (Bashan and de-Bashan 2005). Peroxidase (PO) represents another component of an early response in plants to pathogen attack and plays a key role in the biosynthesis of lignin which limits the extent of pathogen spread (Bruce and West 1989). In bean, rhizosphere colonized by various bacteria induced PO activity (Zdor and Anderson 1992). In a study, a rapid increase in PO activity was recorded in coconut (*Cocos nucifera* L.) treated with a mixture of *P. fluorescens*, *T. viride* and chitin which contributed to induced resistance against invasion by *Ganoderma lucidum*, the causal agent of *Ganoderma* disease (Karthikeyan et al. 2006). These findings suggest that PGPR possessing the ability to synthesize hydrolytic enzymes can effectively be utilized for managing the plant diseases and can help to reduce the pesticide usage.

## 2.4 Conclusion

In intensive agricultural practices, P is supplied to plants through synthetic phosphatic fertilizers, which indeed is expensive and environment disruptive. Application of phosphate-solubilizing microorganisms as an alternative to chemically

synthesized phosphatic fertilizer is therefore an urgent requirement in crop production systems. The success of this microbiological approach, however, depends on identification, preparation and delivery of multifunctional phosphate solubilizers to farm practitioners. Understanding the mechanistic basis of phosphate solubilization by soil dwellers is critical and requires multifaceted approach to uncover the hidden phosphate-solubilizing potentials of functionally diverse yet naturally abundant soil microflora. Moreover, the functional variations among phosphate solubilizers need to be identified. Once identified and physiologically characterized, phosphate-solubilizing microbes are likely to provide benefits to crops in sustainable agriculture.

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