Plant growth promotion by microbes[1]

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Abstract

Since the world's population is still growing, food production should be increased. This should be done without damaging the environment further and with a decreased input of chemical hormones and fertilizers. This realization has resulted in an increasing interest in the use of microbes as sustainable and inexpensive alternatives for agrochemicals. In this chapter, we will describe three classes of microbial alternatives for agrochemicals, namely a) general microbial plant growth promoters, b) microbial fertilizers for specific nutrients, and c) microbial plant growth regulators which act through a hormonal mechanism. The latter class includes microbial stress controllers.

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Introduction

The world's population is assumed to increase from 7 billion now to 8.3 billion in 2025. The world will need 70 to 100 percent more food by 2050 (Godfray et al., 2010). Therefore, the production of cereals, especially wheat, rice and maize, which accounts for half of the human's calorie intake, has to be increased. Currently, plant growth is enhanced by the input of chemicals which act as plant growth regulators (using a hormonal mechanism) and as nutrients. Of the nutrients added to the soil, nitrogen and phosphorous are the major ones. They are, together with potassium, applied as chemical fertilizers to improve grain yield. According to Roberts (2009) the present global annual use of chemical nitrogen, phosphorous, and potash fertilizer is 130, 40, and 35 million tonnes, respectively.

The high input of chemicals raises a number of concerns such as water contamination leading to euthrophication and health risks for humans. Moreover, it results in soil degradation and loss of biodiversity. In this chapter we will describe beneficial microbes which can act as environmentally friendly alternatives for agrochemicals. Their application will increase the sustainability of agriculture.

We will sub-divide these beneficial microbes in the following groups. A. General plant growth promoters. These microbes stimulate plant growth through a variety of known mechanisms or by one or more unknown mechanisms. B. Microbial fertilizers for specific nutrients, the most important ones being N, P and Fe^{3+}. C. Microbial plant growth regulators. These secrete hormones or hormone-like substances which stimulate plant growth in extremely low concentrations. This sub-division is not perfect since one microbe can combine several mechanisms.

The major global nutrition processes will be illustrated in Figures, whereas the PGP traits of some species will be listed in Table 1.

A. General microbial plant growth-promoters

Some microbes and molecules have a general plant growth promoting effect. They can stimulate for example plant establishment and enhance plant vigor. They will be treated in this section. Other microbes have a more specific effect for a certain nutrient. They will be discussed in section B**.**

A.1. Arbuscular Mycorrhizal Fungi

Approximately 90% of the land plants live in symbiosis with AMF (**Fig. 1** and **Table 1A**). AMF are not host-specific. Combinations of AMF and plant roots can form enormous

underground networks. Since exudates from fungal hyphae solubilise more P than root exudates alone, it was suggested that mycorrhizae contribute to the increase of P-uptake through P-solubilisation. AMF can enhance plant establishment and increase water and nutrient uptake, especially of P, Zn and Cu (Clark and Zeto, 2000; see **Fig. 1; Table 1A**). AMF also protect plants against biotic and abiotic stresses and can improve soil structure (Smith and Read, 2008). Since AMF perform similar functions as roots, they functionally extend the root system. Therefore, the area around roots with attached AMF is called mycorrhizosphere. Because of their smaller diameter, the fungal hyphae are able to reach places where roots cannot penetrate. AMFs are also beneficial for soil structure, because they cause aggregate formation.

SLs (strigolactones), the recently discovered class of shoot branching inhibiting hormones, are involved in early stages of the plant-AMF interaction (see **Fig. 1**). They

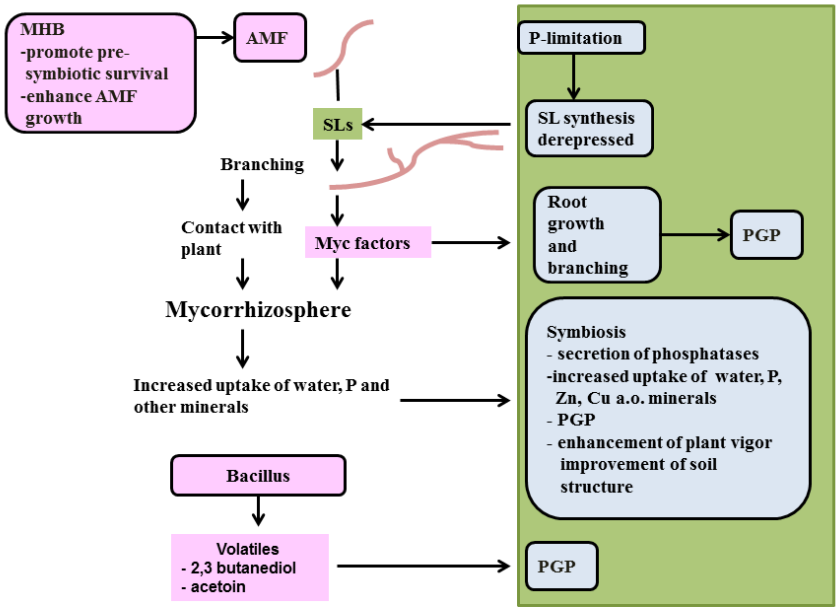

Fig. 1. Role of Arbuscular Mycorrhizal Fungi (AMF) in PGP. For explanation, see text and Table 1A. Colours: green, plant; pink, microbes; blue: processes.

Table 1. Selection of PGP microbes and their relevant PGP

A. Arbuscular Mycorrhizal Fungi	
Trait	**Reference**
Functions as extension of the root system	Parniske, 2013
AMF branching and contact formation with roots stimulated by SLs	Lopez-Raez, 2013
AMF secrete Myc-factors which stimulate root growth and branching	Maillet et al., 2011
Uptake of water, P, Zn, Cu and other nutrients	Clark and Zeto, 2000
Improve of soil structure	Smith and Read, 2008
Protection against (a)biotic stresses	Smith and Read, 2008
Mycorrhiza helper bacteria promote pre-symbiotic survival and fungal growth	Frey-Klett et al., 2007
B. TRICHODERMA	
Trait	**Reference**
Can act as endophyte; increases uptake of water and nutrients; increases solubilization of soil nutrients; increase of nitrogen use efficiency; enhancement of plant vigor; enhanced growth and development of roots and above-ground plant parts; increases root hair formation; causes deeper rooting; improved photosynthetic efficiency	Harman, 2006 ; Lorito et al., 2010; Shoresh et al., 2010; Hermosa et al., 2012
Degrades phenolic compounds secreted by plants.	Ruocco et al., 2009
Produces auxin	Contreras-Cornejo et al., 2009
Accelerates seed germination	Mastouri et al., 2010
Increase of plant resistance, especially under sub-optimal growth conditions	Lorito et al., 2010
Amelioration of abiotic stress; alleviation of physiological stresses, e.g. seed aging	Mastouri et al., 2010 ; Shoresh et al., 2010
The secondary metabolite harzianic acid promotes plant growth	Vinale et al., 2009
C. BACILLUS	
Trait	**Reference**
N_2-fixer	Borriss, 2011
Phosphate solubilizer	Rodríguez et al., 2006; Borriss, 2011
Release of Pi from phytate	Idriss et al., 2002
Potassium solubilizer	Wu et al., 2005
D. PSEUDOMONAS	
Associative N-fixer	Dobbelaere et al., 2003
Phosphate solubilizer	Rodríguez et al., 2006
Siderophore producer	Lemanceau et al., 2009
Auxin producer	Kamilova et al., 2006
Cytokine producer	García de Salmone et al., 2001
ACC deaminase producer	Glick et al., 2007a

[a]Note that not all strains of the mentioned species have the listed traits and that all listed traits are not present in a single strain.

are present in the root exudates of both mono- and dicotyledonous plants. Their synthesis is upregulated by phosphate limitation. SLs from root exudate cause branching of neighbouring AMF spores, thereby increasing their chances to encounter a plant root. SLs also influence auxin transport. In principle, SLs or some of their analogues have the potential to be used for weed control: they are able to induce germination of spores of the weed Striga, which causes massive crop losses of cereals in developing countries. If this induction takes place in the absence of crop plants, the Striga will die (see Schachtschabel and Boland, 2009).

AMF produce diffusible symbiotic signals, recently identified as lipochitooligosaccharides and designated as Myc factors (see **Fig 1**; **Table 1A**). They stimulate root growth and branching. It is expected that (derivatives of) these compounds will be used in future agriculture (Maillet et al., 2011).

Some bacteria help AMF (MHB's; Frey-Klett et al., 2007; Frey-Klett et al., 2011; see also **Fig. 1**). In the case of the *Pseudomonas fluorescens* helper bacterium strain BBc6R8 it was shown that this bacterium promotes the pre-symbiotic survival and growth of the fungus (Deveau et al., 2007).

A.2. *Trichoderma*

Although the soil fungus *Trichoderma* is mainly known as a biocontrol agent (Harman et al., 2004; Lorito, 2010), it has also a large set of direct plant growth-promoting properties (see **Table 1B**). *Trichoderma* is claimed to increase plant resistance under sub-optimal growth conditions, to increase nutrient uptake, to increase nitrogen use efficiency, to enhance solubilization of soil nutrients, to enhance growth, vigor, photosynthetic efficiency, and development of roots and above-ground plant parts, to increase root hair formation and to enhance deeper rooting (Harman, 2006; Shoresh et al., 2010; Lorito et al., 2010; see **Table 1B**). Moreover, it can reduce abiotic and physiological stresses. The latter may be due to ACC deaminase (Viterbo et al., 2010). The secondary metabolite harzianic acid has been identified as a plant growth promoter (Vinale et al., 2009; see **Table 1B**). We conclude that *Trichoderma* has properties similar to those of AMF. However, *Trichoderma* has the advantage that it can be grown in pure culture. Products with *Trichoderma* as the active ingredient have been commercialized.

B. Biofertilisers for specific nutrients.

Plant growth-promoting microbes which fix N_2, solubilise phosphate, and/or produce siderophores are classified as biofertilisers, since they increase the availability of these nutrients to plants (Fuentes-Ramirez and Caballero-Mellado, 2006).

B.1. Nitrogen fixation.

N_2 is abundant in the atmosphere, but is unavailable to plants. Plants receive their nitrogen in the form of ammonium (NH_4^+) and nitrate (NO_3^-). Uptake of NO_3^- occurs together with influx of protons whereas uptake of NH_4^+ occurs together with release of protons. These processes therefore cause alcalinization and acidification of the rhizosphere, respectively, and substantially influence rhizosphere processes.

Conversion of atmospheric N_2 to ammonium is known as the process of biological nitrogen fixation or diazotrophy. The ability to fix nitrogen is widespread among prokaryotes with representatives in both bacteria and archaea (Dekas et al., 2009). This reaction is catalyzed by the nitrogenase enzyme complex which in most bacteria contains molybdenum-iron (Mo-Fe) as the cofactor. Some bacteria have an additional

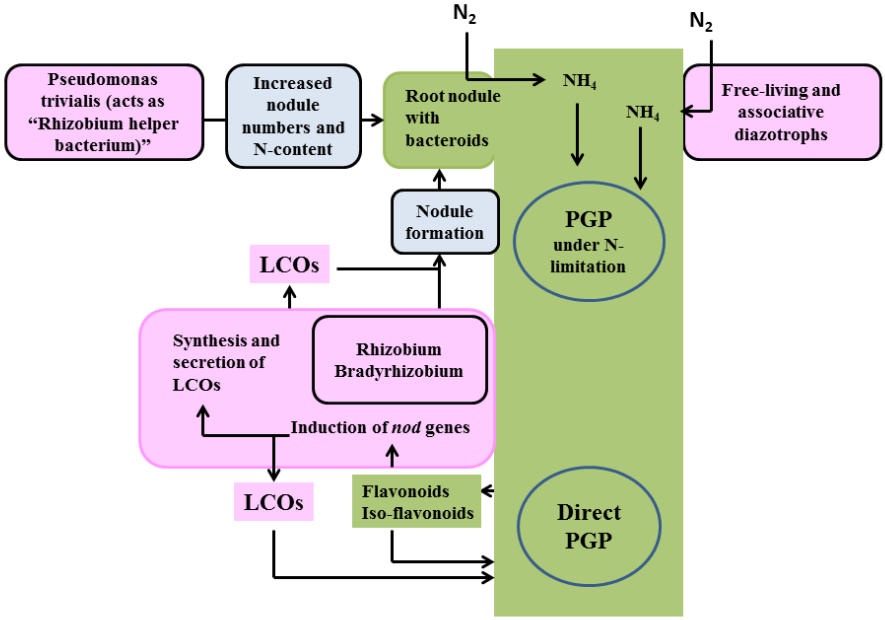

Fig. 2. Microbial contribution to plant N-nutrition. For explanation, see text. Colours: green, plant; pink, microbes; blue: processes.

nitrogenase containing vanadium (Robson et al., 1986) or only iron (Chisnell et al., 1988). However, the alternative nitrogenases have a lower efficiency of nitrogen fixation compared with the conventional ones (Joerger and Bishop, 1988).

Many diazotrophic bacteria are able to establish a symbiotic relationship with plants. The best studied symbiotic diazotrophs belong to the gram-negative rhizobia which induce nodules on leguminous plants (Fabales; see **Fig. 2**). The only exception is the genus *Parasponia* which belongs to Rosales but is nodulated by rhizobia (Markmann and Parniske, 2009).

The rhizobium-legume symbiosis is considered to be the major source of fixed nitrogen. It has been estimated that this symbiosis contributes more than 45 million metric tons of N per year to the terrestrial ecosystems (Vance, 2001). The current taxonomy of rhizobia includes 12 genera with more than 90 species (Weir, 2011) and it is still expanding. The best known rhizobia are those of the α–subclass of Proteobacteria (*Allorhizobium*, *Azorhizobium*, *Rhizobium*, *Mesorhizobium*, *Ensifer* (former *Sinorhizobium*) and *Bradyrhizobium*). In addition, several beta-proteobacteria belonging to *Burkholderia* and *Cupriavidus* have been shown to nodulate plants (Moulin et al., 2001). Rhizobia and other N-fixing bacteria share essential *nod* and *nif* genes encoding nodulation and nitrogen fixation functions, respectively (Zehr and Turnet, 2001). These genes are often carried on symbiotic plasmids which are highly transferable (Brom et al., 2004). Moreover, recipient bacteria are able to obtain a symbiotic function after being transformed with these plasmids (Rogel et al., 2001). Since this can happen under both laboratory and field conditions, it might partly explain the diversity of root-nodulating bacteria.

The symbiosis is initiated by root exudate components, flavonoids or isoflavonoids, which, upon uptake by the bacterium, activate *nod* genes in the bacterium (see **Fig. 2**). The bacterial answer in this molecular dialogue is secretion of products encoded by the *nod*-genes, the NOD factors. NOD factors are lipo-chitin oligosaccharides differing from each other in the length of the chitin fragment, in the unsaturation of their fatty acyl chain and in the presence of several molecular decorations. This makes NOD factors major determinants of the host-specificity of the symbiosis (Spaink et al., 1998; see also **Fig. 2**). Specific perception of NOD factors by plants results in activation of a set of plant genes leading to the formation of root nodules and entry of bacteria (Geurts and Bisseling, 2002). However, certain photosynthetic bradyrhizobia lacking *nod* genes rely on a different, yet to be characterized, strategy for plant signaling (Giraud et al., 2007). *nod* genes have also not been detected in the genome of *Frankia*, Gram-positive

bacteria from the family of Actinobacteria which nodulate non-leguminous plants belonging to the Rosales, Fagales and Cucurbitales. These interesting findings represent a promising source for developing nitrogen-fixing cereals.

Rhizobia can interact with other plant-associated bacteria in the rhizosphere. Such a cooperation can have a beneficial effect on plant growth. For example, Egamberdieva et al. (2010) recently showed that co-inoculation of fodder galega with *Rhizobium* and biocontrol pseudomonads improves shoot and root dry matter of the plant. One of these strains, the cellulase-producing *Pseudomonas trivialis* 3Re27 (Scherwinski et al., 2008), significantly increased nodule numbers and nitrogen content of the co-inoculated plant. The authors coined the term "*Rhizobium* helper bacteria" for this biocontrol strain (see **Fig. 2**).

In addition to symbionts, there are also free-living and associative diazotrophs; these include bacteria from a number of genera: *Acetobacter*, *Azoarcus*, *Azospirillum*, *Azotobact*er, *Bacillus* (**Table 1C**), *Beijerincka*, *Burkholderia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Paenibacillus*, *Pseudomonas* (**Table 1D**), and *Stenotrophomonas* (Dobbelaere et al*.,* 2003; see **Fig. 2**). Using mutants unable to fix nitrogen, Hurek et al. (2002) showed that the beneficial effects of the endophytic diazotrophic bacteria *Azoarcus* sp. on Kaller grass are directly associated with their nitrogen-fixing ability and this is also true for *Acetobacter diazotrophicus* on sugarcane (Sevilla et al., 2001).

Klebsiella pneumoniae and *Azospirillum* are free-living nitrogen-fixing rhizosphere bacteria. In the past, the plant growth-promoting properties of *Azospirillum* were thought to be due to its N_2-fixing property but recent developments show that this property is mainly due to its ability to produce the root architecture influencing hormone auxin. See section C.1 in this Chapter.

B.2. Phosphate solubilization.

After water and nitrogen, phosphorus is the third plant growth-limiting compound. Phosphorus plays a role in numerous plant processes including energy generation, nucleic acid synthesis, photosynthesis, respiration and cellular signaling (Vance et al., 2003).

Plants can absorb phosphorus only as $H_2PO_4^-$ and HPO_4^{2-} ions. Most soils contain amounts of phosphate which are in principle sufficient to support plant growth. However, many of these organic and inorganic forms are not accessible for the plant. Also phosphorus added to the soil as a soluble chemical fertiliser can be rapidly fixed

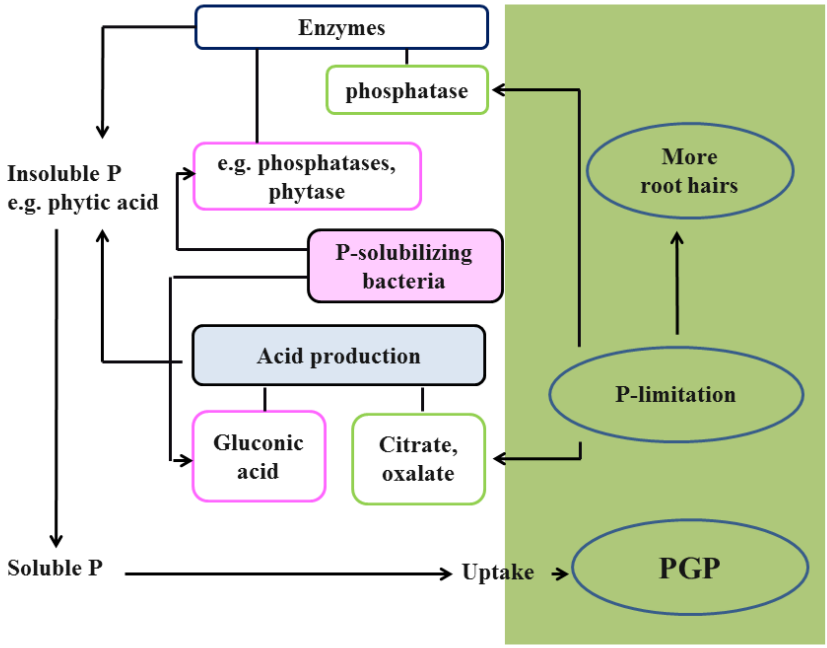

Fig. 3. Microbial contribution to plant P-nutrition. For explanation, see text. Colours: green, plant; pink, microbes; blue: processes.

into insoluble forms and thus made unavailable to plants (Rodriguez and Fraga, 1999; Igual et al., 2001; Smyth, 2011).

Plants react to P-limitation by acidification of the rhizosphere, by increased growth of roots towards unexploited soil zones, by increasing the number of root hairs, and by secreting phosphatases. Acidification is the result of secretion of organic anions together with protons. Organic anions, with citrate and oxalate being more effective than others, can directly facilitate the mobilisation of phosphate (Richardson, 2009; see **Fig. 3**).

Phosphorus is widely applied as a chemical fertilizer, and the excessive and unmanaged application of phosphorus can have negative impacts on the environment, including the eutrophication and hypoxia of lakes and marine estuaries (Smyth, 2011).

Some bacteria, referred to as phosphate-solubilising bacteria (Igual et al., 2001; Kim et al., 1998) are able to solubilise bound phosphorous from organic or inorganic molecules, thereby making it available for the plant (Lipton et al., 1987; see **Fig. 3**). Phosphate-solubilizing bacteria are ubiquitous and *Bacillus* **(Table 1C**), *Enterobacter*, *Erwinia* and *Pseudomonas* spp. **(Table 1D)** are among the most potent species.

Production of organic acids such as gluconic acid is a major factor in the release of phosphorous from mineral phosphate (Rodríguez et al., 2006). Also the release of a range of enzymes results in the generation of phosphate forms which can be taken up by the plant (see **Fig. 3**). These include non-specific phosphatases that dephosphorylate phosphor-ester and/or phosphoanhydride bonds in organic matter, phytases that release phosphorus from phytic acid (Idriss et al., 2002), and phosphonatases and C-P lyases that dissociate C-P bonds in organophophonates (Rodriguez et al., 2006). Vyas and Gulatti (2009) showed that phosphate-solubilising *Pseudomonas* spp. are able to increase both the growth and phosphorus content of maize. Sundara et al. (2002) showed that a phosphate-solubilising *Bacillus megaterium* increases both the amount of plant-available phosphorus as well as the yield of sugarcane. De Freitas et al. (1997) showed that phosphate-solubilising *Bacillus* spp. increase the yield of canola. Using molecular techniques, it was possible to identify a possible new mechanism involved on P solubilization: assessing a genomic library of *Pseudomonas fluorescens* B16, pyrroloquinoline quinone (PQQ) biosynthetic genes were identified responsible for plant growth promotion in this strain (Choi et al., 2008).

AMF were initially thought to provide the plant with phosphorous only. Since it is now known that AFM has a more general function, AFM has been described under section A.1.

B.3. Fe and siderophores.

Iron is an essential element for all organisms. Iron is an abundant element on the earth crust but it is hardly soluble and therefore not suitable for uptake by living organisms. The concentration of Fe^{3+}, the form of iron ions available for living organisms, is only 10^{-18} M.

Plants produce and excrete chelators and/or phytosiderophores which bind Fe^{3+} and transport it to the root surface where it is either reduced to Fe^{2+}, that is subsequently taken up by the plant, or it is absorbed as a Fe^{3+}-phytosiderophore complex by the plant (Lemanceau et al., 2009; see **Fig. 4**).

Bacteria growing under low Fe^{3+} concentrations also produce a variety of siderophores which bind this ion with high affinity (see **Fig. 4**). A number of plant species can absorb bacterial Fe^{3+}-siderophores complexes, but it is unclear whether the uptake of these complexes has any significance to plant iron nutrition and/or direct plant growth promotion (Zhang et al., 2008).

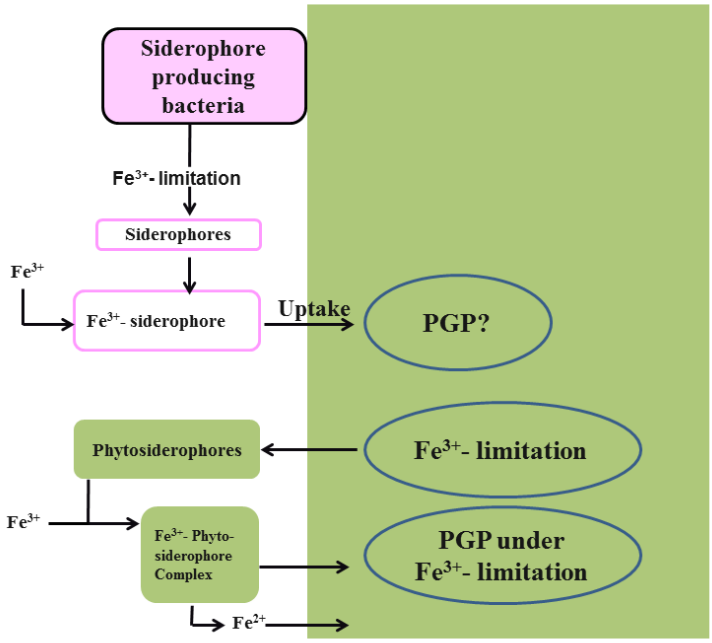

Fig. 4. Possible microbial contributions to plant Fe-nutrition. For explanation see text. Colours: green, plant; pink, microbes; blue: processes.

B.4. Mixures of biofertilizers

Wu et al. (2005) performed a thorough greenhouse study to evaluate the effect of a mixture of four biofertilizers, namely an AMF (*Glomus mossae* or *Glomus intraradices*), an N-fixer (*Azobacter chroococcum*), a P-solubiliser (*Bacillus megaterium*) and a K-solubilizer (*Bacillus mucilaginous*) on growth of *Zea* mays and soil properties. Controls were no fertilizer, chemical fertilizer, organic fertilizer, and two types of biofertilizers. The mixture of the four microbes significantly increased the growth of Z*. mays* and resulted in the highest biomass and seedling height. It also increased assimilation of N, P and K. Moreover, soil properties such as organic matter and total N in soil were improved. The presence of the bacteria in the inoculum resulted in an at least 5-fold higher root infection rate by AMF.

C. Microbial plant growth regulators

Plants produce phytohormones or plant growth regulators, i.e. compounds which at concentrations lower than 1 µM can regulate plant growth and development. There are six classes of plant hormones, namely auxins, brassinosteroids, cytokinins,

gibberellins, abscisic acid, ethylene, and the recently discovered strigolactones. Phytohormones regulate processes such as cell division, cell expansion, differentiation, shoot branching and cell death. Phytohormone pathways and cross-talk between them plays a key role in process coordination and cellular responses (Moller and Chua, 1999; Santner et al., 2009).

Many rhizosphere bacteria can produce plant growth regulators *in vitro*, such as auxins, cytokinins, gibberellins, abscisic acid, and ethylene (Zahir et al., 2003). Bacteria which produce abscisic acid, and ethylene are known as stress controllers. As far as presently known, brassinosteroids and strigolactones are not produced by bacteria or fungi.

Phytohormone production by microbes can modulate the endogenous plant hormone levels and consequently can have an enormous influence on plant growth and development (Gray, 2004; van Loon, 2007). For details on hormones produced by plants and rhizosphere bacteria, the reader is referred to excellent reviews by García de Salome et al. (2006), and Spaepen et al. (2009).

C.1. Auxins.

Nonconjugated indole-3-acetic acid (IAA) is the most abundant member of the auxin family. The concentration of auxin and the ratio of auxin to other hormones are critical for the physiological response of the plant (Lambrecht et al., 2000).

It has been estimated that up to 80% of the rhizosphere bacteria can synthesize IAA (Khalid et al., 2004; Patten and Glick, 1996). Bacteria which produce IAA can add to, or influence, the levels of endogenous plant auxin (Patten and Glick, 1996). It is assumed that plant growth promotion by exogenously added auxin acts by increasing root growth, length and surface area, thereby allowing the plant to access more nutrients and water from the soil (Vessey, 2003; see **Fig. 5**).

Rhizosphere bacteria can use several different pathways for IAA biosynthesis. Most of them use tryptophan, secreted by the plant as a component of root exudate, as a precursor (Costacurta and Vanderleyden, 1995; Spaepen et al., 2007; Spaepen et al., 2009; see **Fig. 5**). Indeed, Kamilova et al. (2006) observed that *P. fluorescens* biocontrol strain WCS365, which produces IAA in the presence of tryptophan, is able to stimulate root growth of radish, a plant which secretes high amounts of tryptophane in its exudate, but not of tomato, sweet pepper or cucumber plants which secrete at least 10-fold less tryptophan.

Azospirillum brasilense is an N_2-fixer which promotes plant growth by increasing its

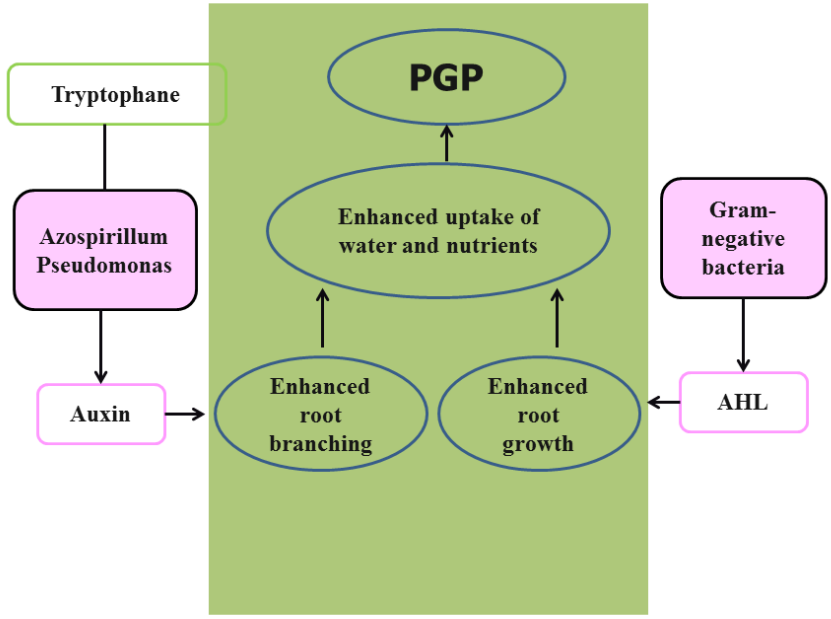

Fig. 5. Stimulation of root branching and growth by auxin. For explanation, see text. Colours: green, plant; pink, microbes; blue: processes.

root surface through shortening the root length and enhancing root hair formation. It has been thought for a long time that its plant growth-promoting ability was based on N_2 fixation. However, the present notion is that auxin production is the major factor responsible for its root changes and therefore for its plant growth-promoting properties (Pliego et al., 2011; see **Fig. 5**). This notion is based on the following observations. (i) Dobbelaere et al. (1999) showed that the effect of the wild type strain on the root can be mimicked by the addition of pure auxin. (ii) A mutant strain strongly reduced in IAA production did not induce the root changes and, (iii), a strain constitutive for IAA production showed the same effect on the root changes as the wild type strain but already at lower bacterial cell concentrations (Spaepen et al., 2008). Interestingly, when the amount of root exudate becomes limiting for bacterial growth, *Azospirillum brasilense* increases its IAA production, thereby triggering lateral root and root hair formation which results in more exudation and, therefore in further bacterial growth. In this way, a regulatory loop is created which connects plant root proliferation with bacterial growth stimulation (Spaepen et al., 2009).

C.2. Cytokinins

Zeatin is the major representative of a group of molecules called cytokinins. Cytokinins have the capacity to induce division of plant cells in the presence of auxin. Starting from callus tissue, the ratio between the amounts of auxin and cytokinin determines whether callus differentiates in root or shoot: high auxin promotes root differentiation whereas high cytokinin promotes shoot morphogenesis. Equimolar concentrations induce cell proliferation.

Cytokinin production is linked to callus growth of tobacco. A test based on this principle can be used as a screening method for cytokinin-producing bacteria. Many rhizosphere bacteria can produce cytokinins in pure culture, e.g. *Agrobacterium, Arthrobacter, Bacillus, Burkholderia, Erwinia, Pantoea agglomerans*, *Pseudomonas, Rhodospirillum rubrum, Serratia* and *Xanthomonas* (reviewed in García de Salome et al., 2001). The spectrum of cytokinins produced by rhizobacteria is similar to that produced by the plant (Barea et al., 1976; García de Salome et al., 2001; Frankenberger and Arshad, 1995) of which isopentenyladenine, trans-zeatin, cis-zeatin and their ribosides as the most commonly found.

García de Salome et al. (2001) provided evidence for a role of cytokinin of rhizosphere bacteria in plant growth promotion. They used mutants of *P. fluorescens* strain G20-18 which produce reduced amounts of cytokinin and normal amounts of auxin. In contrast to the wild type strain, the mutants appeared to be unable to promote growth of wheat and radish plants (García de Salome et al., 2006).

Concerning the mechanism of action of cytokinins, one speculates that cytokinin produced by rhizosphere bacteria becomes part of the plant cytokinin pool, and thus influences plant growth and development.

The ability to produce auxins and cytokinins is a virulence factor for the pathogen *Agrobacterium tumefaciens* which produces crown galls. This bacterium can transfer the genes for production of auxins and cytokinins to the plant and incorporate these genes in the plant's DNA (see Spaink et al., 1998). Another bacterium from this genus, *A. rhizogenes*, modifies cytokinin metabolism, resulting in the appearance of masses of roots - instead of callus- from the infection site (Hamill, 1993).

C.3. Gibberellins (GAs)

These hormones consist of a group of terpenoids with 20 carbon atoms, but active GAs only have 19 carbon atoms. This group of compounds consists of over 130 different molecules (Dodd et al., 2010). GAs are mainly involved in cell division and cell

elongation within the subapical meristem, thereby playing a key role in internode elongation. Other processes affected by these hormones are seed germination, pollen tube growth and flowering in rosette plants. Like auxins and cytokinins, GAs mainly act in combination with other hormones.

Bacteria which produce gibberellins, such as *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Azospirillum brasilense*, *A. lipoferum, Azotobacter*, *Bacillus*, *Bradyrhizobium japonicum, Clostridium*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, *Rhizobium* and *Xanthomonas*, secrete it in the rhizosphere (Frankenberger and Arshad, 1995; Gutiérrez Manero et al., 2001; Rademacher, 1994; Tsavkelova et al., 2006). Hardly anything is known about gibberellin synthesis in rhizosphere bacteria.

Kang et al. (2009) showed that culture suspensions of GA-producing *Acinetobacter calcoaceticus* were able to increase the growth of cucumber, Chinese cabbage and crown daisy. The mechanism of plant growth stimulation by gibberellins is still rather obscure. It is thought that bacteria may increase GA levels *in planta* by either producing GAs, deconjugating GAs from root exudates or hydroxylating inactive GA to active forms (Bottini et al., 2004). Fulchieri et al. (1993) speculate that gibberellins increase root hair density in root zones involved in nutrient and water uptake.

C.4. Abcisic Acid (ABA)

ABA is a 15-carbon compound which, like ethylene, is involved in plant responses to biotic and abiotic stresses. It inhibits seed germination and flowering. It is involved in protection against drought, salt stress and toxic metals. It also induces stomatal closure (Smyth, 2011).

ABA can be produced in culture media by several bacteria such as *Azospirillum brasilense* (Cohen et al., 2008; Perrig et al., 2007) and *Bradyrhizobium japonicum* (Boiero et al., 2007). ABA levels *in planta* have been increased in *Arabidopsis thaliana* by *Azospirillum brasilense* Sp25 (Cohen et al., 2008).

The effect of inoculation with ABA-producing bacteria on plant growth is experimentally poorly underpinned. Since ABA inhibits the synthesis of cytokinins (Miernyk, 1979) it was speculated that ABA increases plant growth by interfering with the cytokinin pool (Spaepen et al., 2009). It could also alleviate plant stress by increasing the root/shoot ratio (Boiero et al., 2007).

C.5. Ethylene (ET) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase.

Ethylene is a gaseous hormone best known for its ability to induce fruit ripening and flower senescence. ET affects numerous plant developmental processes including root growth, root hair formation, flowering, fruit ripening and abscission, and leaf and petal senescence and abscission (Dugardeyn et al., 2008). ET usually inhibits both primary root elongation and lateral root formation but it can promote root hair formation (Dodd et al., 2010). It generally inhibits stem elongation in most dicots favouring lateral cell expansion and leading to swelling of hypocotyls. ET also breaks seed and bud dormancy. ET production is typically up-regulated in plants in response to pathogen attack, heat and cold stress, waterlogging, drought, excess heavy metals, high soil salinity and soil compaction (Dodd et al., 2010; Glick, 2005).

ET is synthesised under biotic stress conditions following infection by pathogens, as well as by abiotic stress conditions such as drought. It is therefore also known as the stress hormone. In the plant, ethylene is produced from S-adenosylmethionine (SAM) which is enzymatically converted to ACC and 5'-deoxy-5'methylthioadenosine (MTA) by ACC synthase (Giovanelli et al., 1980; see **Fig. 6**).

The enzyme ACC deaminase is present in many rhizosphere bacteria, such as *Achromobacter*, *Pseudomonas*, and *Variovorax* and in the fungus *Trichoderma*. Such microbes can take up ACC secreted by the plant root and convert it into α-ketobutyrate and ammonia (Glick et al., 2007a) **(Fig. 6)**. This results in the decrease of ACC levels, and therefore also of ethylene levels, in the plant and in decreased plant stress. Inoculation of plants with ACC deaminase producing bacteria can protect plants against stress caused by flooding, salination, drought, waterlogging, heavy metals, toxic organic compounds and pathogens (Berg, 2009; Glick, 2005; Glick et al., 2007a; Glick et al., 2007b; Belimov et al., 2005). ACC deaminase activity has been found in fungi such as *Trichoderma* (Viterbo et al., 2010) and in free-living soil bacteria, endophytes, and rhizobia from a wide range of genera, and there have been many correlations between ACC deaminase activity in a range of bacteria and their ability to promote plant growth under various conditions, for example in wheat (Zahir et al., 2009), maize (Shaharoona et al., 2006), and tomato (Grichko and Glick, 2001; Mayak et al., 2004a; Mayak et al., 2004b).

In addition to a direct role of ethylene on plant growth, this hormone can also act as a virulence factor and a signalling molecule in plant protection against pathogen attack. Ethylene production was reported to act as a virulence factor for bacterial

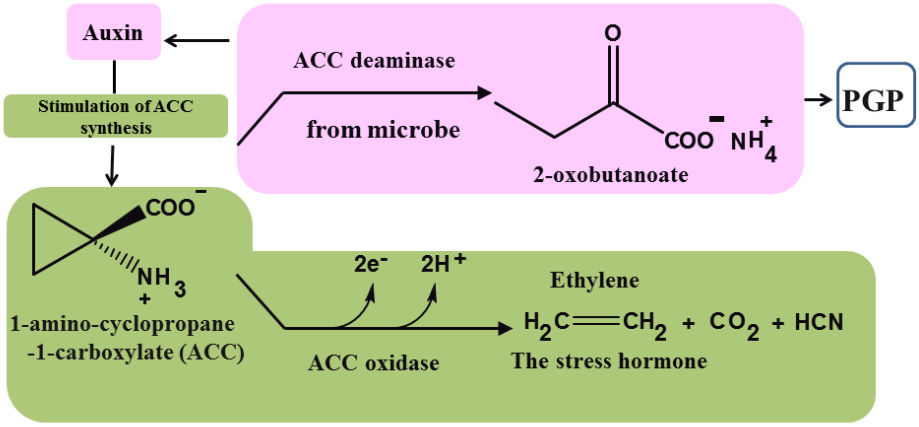

Fig. 6. Role for microbial ACC deaminase in plant stress control. For explanation, see text. The figure is mainly based on papers by the group of B. Glick. According to his hypothesis (Glick et al., 1998), bacterial auxin activates plant ACC synthase. The produced ACC can be used by some microbes as an N-source, thereby decreasing ethylene levels. In order to explain how the ACC produced by the plant is converted by ACC deaminase from the bacterial cytoplasm, Glick et al. (1998) assumed that a significant portion of ACC is exuded from plant roots and seeds and then taken up by the microbe. We would like to suggest the following alternative explanation, namely that the microbe uses the TTSS (type three secretion system) for this purpose since it has been proposed earlier that a beneficial bacterium uses the needle of the TTSS to suck nutrients from the plant root (De Weert et al., 2007). Another possibility is that the bacterium uses its TTSS to deliver the enzyme into the plant. In the case of *Trichoderma*, one can imagine that its endophytic localization facilitates contact between enzyme and substrate.
Colours: green, plant; pink, microbes; blue: processes.

pathogens e.g. *P. syringae* (Weingart and Völksch, 1997; Weingart et al., 2001). Furthermore, ethylene acts as a signalling compound in induced systemic resistance caused by some rhizobacteria (Van Loon et al., 2007).

C.6. Volatiles

Bacteria can produce a wide range of volatiles. While the biological function of most of these volatiles is not fully understood, it is assumed they are involved in a number of processes including cell-cell signaling, inter-species signaling, a possible carbon release valve and that these compounds can promote plant growth and act as microbial inhibiting agents (Wheatley, 2002; Vesperman et al., 2007; Kai et al., 2009).

Bacterial volatiles produced by *Bacillus* spp. have been shown to promote plant growth in *A. thaliana*. The highest level of growth promotion was observed with 2, 3 butanediol and its precursor acetoin (Ryu et al., 2003).

Farag et al. (2006) identified 38 volatile compounds from rhizobacteria. Blom et al. (2011) screened 42 strains grown in four different growth media on the growth response of *A. thaliana*. Under at least one of these conditions each strain showed significant volatile-mediated plant growth modulation. Only one strain, a *Burkholderia pyrrocinia,* showed significant plant growth-promotion on all four media. The volatiles indole, 1-hexanol and pentadecane showed plant growth promotion but the results suggested that this occurred only under stress conditions.

C.7. A-HSLs

N-acyl homoserine lactones (A-HSLs) are signal molecules secreted by many bacteria. When their extracellular concentration reaches a certain value, the quorum, they play a role in many processes such as secretion of antibiotics and exo-enzymes (Vivanco, 2013). In terms of growth promotion, it was shown recently that 10µm C6-AHL and C8-AHL increase root growth in *A. thaliana* (see **Fig. 5**). This is accompanied by an increase in the auxin/cytokinin ratio and in increased expression of over 700 genes in the roots and of a lower number in the stem (von Rad et al., 2008).

C.8. *nod* gene inducers and LCO's (Nod-factors)

The *nod* genes of (*Brady)Rhizobium* are induced by flavonoids or isoflavonoids. LCO's signal molecules are the products of *nod* genes. They initiate root hair curling and subsequent steps in the nodulation of leguminous plants by (*Brady)rhizobium* bacteria (see section B.1).

Interestingly, both the inducers as well as the products of the *nod* genes promote plant growth and this effect is not restricted to leguminous plants (see **Fig. 2**). See http://www.bioag.novozymes.com. For example, one product is based on isoflavonoids and is claimed to activate mycorrhizae before the plant does so, resulting in enhancing nutrient uptake, which in turn leads to lateral root development, and stress tolerance. Formulations for soybean, peanut, alfalfa and pea/lentil, combining the respective LCO and rhizobia, have also been commercialized. When LCOs were applied on seeds of the non-legumes corn, cotton, and wheat, increased plant growth as well as yield increase in the field was observed. In furrow application as well as foliar sprays have similar effects. Possible explanations given are enhanced germination, early seedling growth, increased photosynthesis, enhanced nutrient uptake and enhanced LCO-stimulated mycorrhizal root colonization (Smith et al., 2011).

Conclusions

Nitrogen and phosphorous are the major chemical fertilizers applied to enhance crop yield. This raises a number of concerns such as water contamination leading to euthrophication and health risks for humans. Moreover it results in soil degradation and loss of biodiversity. Presently, the cost for nitrogen fertilizer is steeply increasing as a consequence of the increasing energy prices. The amount of available phosphorous is limited. For these reasons, the interest in sustainable fertilization, using microbes, is strongly increasing. In this chapter we have discussed many microbes which can be applied for a more environmentally friendly agriculture.

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