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Bacterial Modulation of Plant Ethylene Levels

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A focus on the mechanisms by which ACC deaminase-containing bacteria facilitate plant growth.Bacteria that produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, when present either on the surface of plant roots (rhizospheric) or within plant tissues (endophytic), play an active role in modulating ethylene levels in plants. This enzyme activity facilitates plant growth especially in the presence of various environmental stresses. Thus, plant growth-promoting bacteria that express ACC deaminase activity protect plants from growth inhibition by flooding and anoxia, drought, high salt, the presence of fungal and bacterial pathogens, nematodes, and the presence of metals and organic contaminants. Bacteria that express ACC deaminase activity also decrease the rate of flower wilting, promote the rooting of cuttings, and facilitate the nodulation of legumes. Here, the mechanisms behind bacterial ACC deaminase facilitation of plant growth and development are discussed, and numerous examples of the use of bacteria with this activity are summarized.

Agricultural development policies and practices in the past sixty years have largely been based on external inputs (pesticides and fertilizers) to control soilborne diseases and increase crop yields. Recently, stimulated by the awareness of potentially serious environmental and human health damage caused by the over use of agricultural chemicals (Alavanja et al., 2004; Leach and Mumford, 2008; Damalas and Eleftherohorinos, 2011), the controversy regarding the use of pesticides and fertilizers has gained prominence. Therefore, worldwide agricultural practice is moving toward a more sustainable and environmentally friendly approach.

In 2002, in the European Union, 5.7 million ha were designated as being cultivated organically, and by 2011, this number had increased to 9.6 million ha (http://ec.europa.eu/agriculture/markets-and-prices/more-reports/pdf/organic-2013_en.pdf). In other words, in 10 years, the area devoted to organic agriculture in the European Union increased by approximately 400,000 ha per year. This growth in organic agriculture notwithstanding, the total amount of organically cultivated land represents only 5.4% of the total agricultural land in Europe. In this context, the use of microbial inoculants instead of traditional chemicals is gaining popularity, and a number of new products have been formulated, marketed, and applied successfully.

The soil surrounding plant roots (the rhizosphere) is one of the main sources of bacteria expressing plant-beneficial activities (i.e. plant growth-promoting bacteria [PGPB]; Bashan and Holguin, 1998). Stimulation of growth and protection of different crops from pathogens and abiotic stressors by PGPB is well documented under both controlled conditions and in the field, and a large number of papers on this topic are available (Reed and Glick, 2005, 2013; Thakore, 2006). The positive effects induced by PGPB on plant growth are based on: (1) the improvement of mineral nutrition (nitrogen fixation, phosphate solubilization, and iron sequestration), (2) the enhancement of plant tolerance to biotic and abiotic stress (largely mediated by 1-aminocyclopropane-1-carboxylate [ACC] deaminase), (3) the modification of root development (via phytohormone synthesis), and (4) the suppression of phytopathogens (by antibiotics, competition, lytic enzymes, systemic resistance, etc.; Fig. 1). The current knowledge of microorganisms living in the rhizosphere, their role, and their biotechnological and environmental applications has been summarized in several reviews (Glick, 2012; Hirsch and Mauchline, 2012; Bakker et al., 2013; Mendes et al., 2013; Reed and Glick, 2013). This review focuses on the role of bacterial ACC deaminase in supporting the growth of plants exposed to environmental stress. In addition, the issues of the distribution and phylogeny of ACC deaminase, and the possible role of ACC as a signaling molecule, are addressed.

RHIZOSPHERIC BACTERIA VERSUS ENDOPHYTES VERSUS RHIZOBIA

Thanks to carbon-rich exudates released from plant roots, bacteria in the rhizosphere establish themselves and proliferate along the roots, giving rise to a biofilm surrounding the roots' surface (Danhorn and Fuqua, 2007). Following rhizosphere colonization, some of these microorganisms can penetrate the root tissue, therefore shifting their habitus from rhizospheric to endophytic. Endophytic bacteria include: (1) facultative endophytes living inside the plants as well as in other habitats, (2) obligate endophytes that can only live inside plant tissues, and (3) opportunistic endophytes that can occasionally enter plants and live

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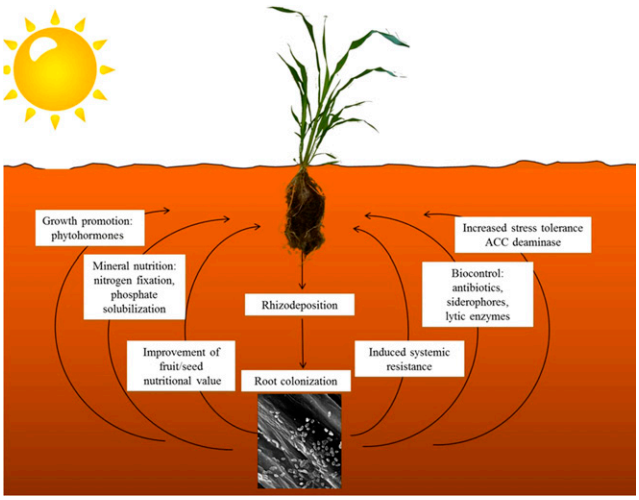

Figure 1. Schematic overview of the main mechanisms used by PGPB. Following the release of root exudates, a variety of soil microorganisms are attracted to the root. Some of them can efficiently colonize the root surface while others (endophytes) can penetrate the root tissue and spread inside the plant. Plant growth promotion by beneficial microorganisms may occur by either direct or indirect mechanisms. Direct promotion of plant growth involves the improvement of mineral nutrition via nitrogen fixation, phosphate solubilization, and iron chelation, as well as the modulation of phytohormones levels (auxins, cytokinins, GAs, and ethylene). In addition to the increase of biomass, PGPB can positively affect the nutritional value of fruits and edible seeds. The indirect mechanisms are based on the improvement of plant health via suppression of soil-borne diseases by antibiotics, lytic enzymes, siderophore production, induced systemic resistance involving jasmonate and ethylene signaling within the plant, and other molecules (the *O*-antigenic side chain of the bacterial outer membrane protein lipopolysaccharide, flagellar fractions, pyoverdine, 2,4-diacetylphloroglucinol, cyclic lipopeptide, surfactants, and salicylic acid) that stimulate the host plant's resistance to pathogens.

inside their tissues. However, the fact that scientists can isolate and culture specific endophytic strains means that they are likely dealing exclusively with facultative endophytes that may be isolated from rhizosphere soil samples as well as from inside the plant.

According to Wilson (1995), endophytes are those microorganisms living inside plant tissues without harming the plant. Internal colonization typically starts in the zone of lateral root emergence or in root wounds and cracks; from there, endophytic bacteria proliferate, spread through xylematic vessels, and reach different plant compartments (Compant et al., 2008). Bacterial endophytes have been detected inside the endorhiza in stems, leaves, and flowers (Compant et al., 2010; Reinhold-Hurek and Hurek, 2011) of a number of plant species.

Inside plant tissues, endophytic bacteria express their physiological activities, synthesize secondary metabolites, and may, both directly and indirectly, facilitate plant development through phytopathogen suppression, mineral nutrition improvement, and enhancement of plant tolerance to stress. Consequently, a number of studies have focused on the application of bacterial endophytes as biofertilizers for phytostimulation and as biological control agents (Kuklinsky-Sobral et al., 2004; Gaiero et al., 2013).

Recently, based on genome sequences of 304 Proteobacteria, Bruto et al. (2014) analyzed the distribution of 23 genes that may contribute to the ability of these bacteria to promote plant growth. These authors suggest that gene transfers, predominantly ancient, resulted in characteristic gene combinations according to taxonomic subgroups of PGPB strains. In other words, genes associated with plant growth, such as the ACC deaminase structural gene (*acdS*), are found in rhizospheric bacteria as a consequence of ancient horizontal gene transfer, and are also present in endophytic bacteria. Thus, understanding the mechanisms utilized by rhizospheric bacteria also provides insight into the mechanisms used by endophytic bacteria.

Rhizobia represent a particular group of endophytic microorganisms able to improve plant mineral nutrition, primarily through nitrogen fixation. They colonize plant roots and establish a mutualistic symbiosis with compatible legume plants. The strict and highly specific relationship between these bacteria and the plant host induces physiological, genetic, and morphological changes in the plant. This includes the formation of root nodules containing bacteria (bacteroids), where nitrogen fixation occurs, under limited oxygen concentration via the action of the enzyme nitrogenase. However, rhizobia, moving from the root toward the shoot (Chi et al., 2005) can, to some extent, colonize internal root tissues of cereal crop plants, such as rice (*Oryza sativa*), maize (*Zea mays*), barley (*Hordeum vulgare*), and wheat (*Triticum aestivum*), increasing plant biomass and grain yield independently of root nodule formation and nitrogen fixation (Biswas et al., 2000; Gutiérrez-Zamora and Martínez-Romero, 2001; Lupway et al., 2004; García-Fraile et al., 2012).

Open-field application of rhizobia as biofertilizers for legume or cereal crop plants of agricultural importance facilitates plant development and high productivity when cultivated under low fertilization regimes. In this regard, rhizobia have been used to promote plant growth in the field for more than 100 years.

ACC DEAMINASE

Biochemistry of ACC Deaminase

The (largely bacterial) enzyme ACC deaminase (3.5.99.7) cleaves ACC, the immediate precursor of ethylene in plants, producing ammonia and α-ketobutyrate (Honma and Shimomura, 1978), reducing the amount of ethylene that the plant can synthesize (Glick et al., 1998). Ethylene is a gaseous hormone displaying a wide range of biological effects in plants at concentrations as low as 0.05 μL L^{-1} (Abeles et al., 1992). Ethylene is involved in seed germination, tissue differentiation, formation of root and shoot primordia,

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root branching and elongation, lateral bud development, flowering, flower senescence, fruit ripening and abscission, anthocyanin production, synthesis of volatile organic compounds responsible for aroma formation in fruits, storage product hydrolysis, leaf senescence, and abscission (Abeles et al., 1992; Glick, 2014). Local increases in the concentration of this hormone also occur during the establishment of symbioses between plants and microorganisms, including rhizobia and mycorrhizal fungi. In these cases, by locally lowering ethylene levels, ACC deaminase-producing bacteria can facilitate symbiosis development (Ma et al., 2003; Gamalero et al., 2008).

In all higher plants, ethylene is produced from *S*-adenosyl-Met via the action of the enzyme ACC synthase, both during normal plant development and when the plant is exposed to various environmental stresses (Abeles et al., 1992). By modulating ethylene levels, ACC deaminase represents one of the key bacterial physiological activities supporting plant growth under stressed conditions, where the ethylene concentration inside the plant might otherwise reach levels inhibitory to plant growth (Glick et al., 1998, 2007; Glick, 2014).

As a consequence of the wide range of potential applications of bacteria that produce ACC deaminase, there has been considerable interest in the biochemistry and functioning of this enzyme. Thus, a number of different ACC deaminases have now been characterized. ACC deaminase is a multimeric enzyme, cytoplasmically localized, that utilizes the coenzyme pyridoxal phosphate as a tightly bound cofactor. Its subunit mass is approximately 35 to 42 kD, while its native size is estimated to be approximately 100 to 112 kD (Sheehy et al., 1991; Jacobson et al., 1994; Hontzeas et al., 2004). Based on its protein fold, ACC deaminase has been classified as belonging to the Trp synthase β superfamily of pyridoxal phosphate-binding proteins (Glick et al., 2007). The affinity of this enzyme for the substrate is not particularly high (K_m = 1.5–6.0 mM). Most organisms with ACC deaminase contain a basal level of enzyme activity. However, ACC deaminase synthesis is induced by ACC, at levels as low as 100 nM (Jacobson et al., 1994), with full induction requiring up to 10 h. The amino acids L-Ala, DL-Ala, and DL-Val can also induce enzyme activity to a small extent, and γ-aminoisobutyric acid can induce activity to almost the same level as ACC (Honma, 1983). Maximal enzyme activity typically occurs at 30°C and pH 8.5. The affinity for the substrate ACC and the competitive inhibitors L-Ala and L-Ser is also highest at pH 8.5 (Hontzeas et al., 2006).

Yoon and Kieber (2013) have recently posited a model in which, in addition to acting as the immediate precursor to ethylene, ACC may also act as a signaling molecule in several plant processes, including root-to-shoot communication. With this scenario, the interaction of plants with ACC deaminase-producing PGPB might be expected to decrease the extent of ACC signaling of specific plant functions such as the regulation of cell wall function. Unlike experiments that utilize chemical inhibitors of ethylene biosynthesis or ethylene perception, ACC deaminase specifically decreases ACC levels. Thus, to test the ability of ACC to act directly as a signaling molecule, one might repeat some of the experiments cited by Yoon and Kieber (2013) in the presence of ACC deaminase. In this regard, while ACC deaminase may not completely breakdown all of the available ACC, the resultant low levels of ACC may be readily quantified (Penrose et al., 2001).

Distribution and Phylogeny of ACC Deaminase

The bacterium *Pseudomonas* sp. ACP and the yeast *Cyberlindnera saturnus* (previously *Hansenula saturnus*) were the two first microorganisms reported to synthesize ACC deaminase (Honma and Shimomura, 1978; Minami et al., 1998). Subsequently, ACC deaminase activity has been found in numerous bacteria, both gram positive and negative with a variety of different types of metabolism (for review, see Gamalero and Glick, 2012; Glick, 2014). ACC deaminase genes (including both the structural gene *acdS* and the regulatory gene *acdR*) have been found in many different rhizobacteria (rhizospheric, endophytic, and rhizobia), including *Azospirillum* spp., *Rhizobium* spp., *Agrobacterium* spp., *Achromobacter* spp., *Burkholderia* spp., *Ralstonia* spp., *Pseudomonas* spp., and *Enterobacter* spp. (Blaha et al., 2006). More importantly, even if some strains of a particular genus and species have an *acdS* gene, not all strains do.

The frequency of ACC deaminase activity in various soil bacteria has been estimated, especially in rhizobia. Of 13 rhizobial strains tested, five (38%) isolates were able to synthesize ACC deaminase, while seven out of 13 (54%) possessed the *acdS* gene. This discrepancy was related to the fact that two strains, belonging to the genus *Mesorhizobium* are only able to produce the enzyme during the symbiotic phase, when localized inside a root nodule (Ma et al., 2003). It subsequently was shown that ACC deaminase genes in *Mesorhizobium* spp. were, unlike all other known ACC deaminases genes, under the transcriptional control of the nitrogen fixation positive regulatory gene *nifA2* promoter and expressed only within root nodules (Nukui et al., 2006). In this regard, it has been suggested that the expression of ACC deaminase genes within nitrogen-fixing nodules may decrease the rate of nodule senescence, as nitrogen fixation with its high-energy demand could activate stress ethylene synthesis (Murset et al., 2012). Another study, including a much larger number of rhizobial isolates (233; Duan et al., 2009), revealed that 27 strains (12%) expressed ACC deaminase. These 27 strains were characterized for the presence of the *acdS* gene; while 17 of them had genes that were 99% identical to the previously characterized ACC deaminase structural gene (*acdS*) from *Rhizobium leguminosarum* bv *viciae* 128C53K, the other 10 strains were found to be 84%

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identical compared with the *acdS* gene from strain 128C53K (Duan et al., 2009). The observation that rhizobia strains with ACC deaminase activity from a wide geographic area showed very little diversity was somewhat surprising. It was then argued that given the harsh winters and lack of diverse vegetation in southern Saskatchewan (where these strains were isolated), there might be intrinsic limits to the diversity of these microorganisms (Duan et al., 2009).

Bacterial ACC deaminase activity is relatively common in rhizosphere bacteria, especially in soils that are often subjected to stressful conditions (Timmusk et al., 2011). Thus, rhizosphere bacteria that contain ACC deaminase may endow some plants with the ability to better withstand, and therefore survive in, harsh environmental conditions.

When analyzing the sequences of *acdS* genes, Blaha et al. (2006) found a high level of polymorphism. Consequently, they defined three *acdS* groups: groups I and II included sequences originating from the β- and γ-Proteobacteria, while group III was composed of α-Proteobacteria. Looking at their geographical origin and habitat, strains from a given *acdS* group originated from different plant hosts. Moreover, by comparing the sequences of 45 different *acdS* genes, from seven α-Proteobacteria, 35 β-Proteobacteria, and three γ-Proteobacteria, Prigent-Combaret et al. (2008) found a high similarity (62.1%–89.4%) with the *acdS* gene of the model strain *Pseudomonas putida* UW4 and 53.9% to 93.5% with the gene from *Azospirillum lipoferum* 4B.

A complete description of the phylogeny and evolution of the genes encoding *acdS* and its major regulatory gene, *acdR*, has been recently elaborated (Bruto et al., 2014; Nascimento et al., 2014). Information regarding *acdS*/*acdR* sequences must be considered together with the habitat, the origin, and the enzymatic activity of completely sequenced bacterial strains to obtain a comprehensive view. Overall, the data show that ACC deaminase activity is prevalent in some bacteria, fungi, and members of stramenopiles. Stramenopiles are a monophyletic eukaryotic group of organisms bearing an immature flagellum with tripartite hairs comprising more than 100,000 species and including a variety of life forms (single cells, large plasmodia, and complex multicellular thalli). The best known members of the group are the colorless oomycetes (aquatic fungi, including plant pathogens for cultivated crops), diatoms, chrysophyte algae, and giant kelp seaweeds. Stramenopiles able to perform photosynthesis are the predominant eukaryotes in most aquatic environments, where they are major primary producers (Yoon et al., 2009). In parallel, through multiple searches of the National Center for Biotechnology Information database, *acdS* genes have been found in Actinobacteria, members from the Deinococcus-Thermus phylum (*Meiothermus* spp.), α-, β- and, γ-Proteobacteria, various fungi (Ascomycota and Basidiomycota), and some stramenopiles (Nascimento et al., 2014).

Although ACC deaminase genes are mainly transmitted vertically in various microorganisms, occasional horizontal gene transfer, including interkingdom transfer events, occur. It is possible that *acdS* genes had an ancient origin in a eukaryote and bacterial common ancestor. Then, during vertical transmission, different constraints, such as adaptation to specific niches, induced *acdS* divergence or gene loss. The advantages conferred by ACC deaminase activity have been positively selected by evolution, leading to intragenomic transfers of *acdS* genes from primary chromosomes to plasmids and increased divergence of *acdS* genes. In fact, *acdS* genes in most *Burkholderia* and *Cupriavidus* spp. strains are located on a second smaller chromosome, while in other β-Proteobacteria (e.g. *Ralstonia solanacearum*), it is located on the primary chromosome or on megaplasmids (Nascimento et al., 2014). Here, it should be noted that some strains of *Burkholderia* spp. are exclusively rhizospheric, while others are facultative endophytes. Because plasmids are transmissible between bacteria via conjugation, it's possible that some dispersion of *acdS* genes occurred. This is in agreement with work that previously reported the occurrence of horizontal *acdS*/*acdR* genes transfer in Proteobacteria and in many *Mesorhizobium* species (Hontzeas et al., 2005; Blaha et al., 2006; Nascimento et al., 2012). Moreover, due to intragenomic transfer events, many microorganisms may have lost *acdS* genes. Consistently, it has been reported that, during phenotypic variation events, *A. lipoferum* strain 4B readily loses the plasmid containing an *acdS* gene (Prigent-Combaret et al., 2008).

Model Including IAA Feedback Inhibition of Ethylene Action

In addition to being rich in sugars, root exudates contain high amounts of amino acids. Among them, Trp is released by the roots and may be taken up by bacterial cells in the rhizosphere. Bacteria use Trp to synthesize the phytohormone indole-3-acetic acid (IAA), some of which is then taken up by the plant. Production of IAA is widespread among soil bacteria; it has been estimated that approximately 80% of rhizosphere bacteria and a significant fraction of bacterial endophytes produce IAA (Patten and Glick, 1996).

The bacterial IAA, together with endogenous plant IAA, can regulate several phases of plant development, such as seed and tuber germination, xylem formation, plant cell proliferation and elongation, vegetative growth, emergence of lateral and adventitious roots, plant responses to light and gravity, and florescence and fructification (Tsakelova et al., 2006). IAA can also affect the synthesis of ACC deaminase by activating the transcription of the plant enzyme ACC synthase (that catalyzes the conversion of ACC from *S*-adenosyl-Met). As a consequence of an increased amount of ACC, the ethylene level inside a plant is increased inducing a plant stress response. Bacteria that produce high levels of IAA often inhibit plant growth. However, this does not necessarily occur

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because as plant ethylene levels increase, the transcription of auxin response factors is inhibited (Pierik et al., 2006; Prayitno et al., 2006; Czarny et al., 2007; Glick et al., 2007; Stearns et al., 2012), thereby limiting the extent that IAA can activate ACC synthase transcription. Moreover, some ACC is released by the roots (Bayliss et al., 1997; Penrose and Glick, 2001), taken up by the bacteria, and through the action of ACC deaminase, converted to ammonia and α-ketobutyrate. As a result, the amount of ethylene produced by the plant is reduced. Therefore, root colonization by bacteria that synthesize ACC deaminase prevents a rise in ethylene levels that might otherwise become growth inhibitory (Glick, 1995). In plants inoculated with bacteria that produce both IAA and ACC deaminase, ethylene levels do not become elevated to the same extent as when plants interact with bacteria that synthesize IAA but not ACC deaminase. When bacterial ACC deaminase is induced and expressed, ethylene is synthesized at a relatively low level, and the bacterial IAA can continue to both stimulate plant growth and enhance the transcription of ACC synthase. However, a large portion of the ACC synthesized is released by the root, taken up by the bacterial cells and finally cleaved by ACC deaminase (Fig. 2). Consequently, the cross talk between IAA and ethylene enables ACC deaminase to effectively facilitate the stimulation of plant growth by IAA. Bacteria that synthesize both ACC deaminase and IAA may facilitate plant growth in the presence of several ethylene-producing environmental stresses (Gamalero and Glick, 2010).

Galland et al. (2012) reported that treatment of Arabidopsis (*Arabidopsis thaliana*) seedlings with the rhizospheric plant growth-promoting bacterium *Phyllobacterium brassicacearum* STM196 caused a significant increase in plant root hair elongation. Following this bacterial treatment, these workers were unable to detect any significant increase in ethylene biosynthesis. Moreover, this signaling pathway activation does not depend on local plant auxin biosynthesis. However, this bacterium also produces and secretes IAA so plant IAA biosynthesis is not needed to activate ACC synthase transcription. By using ethylene-insensitive mutants of Arabidopsis, Zamioudis et al. (2013) clarified which plant growth parameter is affected by the ethylene pathway. They concluded that the main impact of a PGPB strain, able to directly affect auxin signaling in plants, is on the length of the primary root; moreover, they demonstrated that other plant parameters such as lateral root and the root hair formation are affected by the strain independently by the ethylene pathways.

AMELIORATING PLANT STRESS VIA ACC DEAMINASE

In the past, stress ethylene has been suggested to both alleviate and exacerbate some of the effects of pathogen infection (Abeles et al., 1992). However, a simple model (originally developed to explain the effects of stress ethylene following biotic stress and later extended to include abiotic stress as well) was proposed to explain these seemingly contradictory results (Glick et al., 2007). That is, a short time following the onset of the stress, a small peak of ethylene is produced. This small peak of ethylene is thought to consume the existing pool of ACC within plant tissues and likely activates the synthesis of defensive genes within the plant (Stearns et al., 2012). Subsequently, following the synthesis of additional ACC within the plant, a

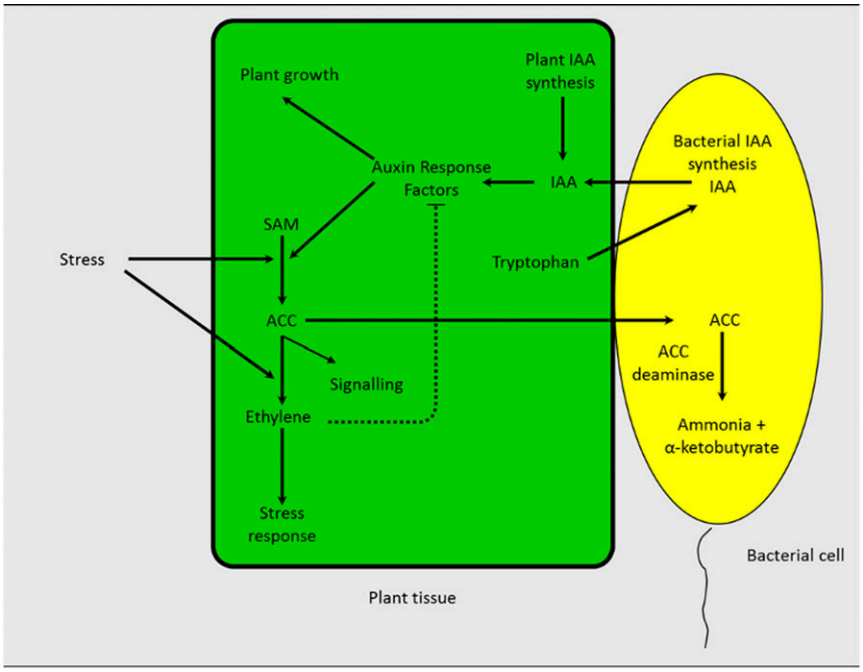

Figure 2. Schematic representation of how PGPB that produce both ACC deaminase and IAA facilitate plant growth. A detailed explanation is given in the text. SAM, *S*-Adenosyl Met.

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second much larger peak of ethylene is typically observed. The second peak of ethylene occurs as a consequence of increased transcription of ACC synthase genes, mostly triggered by environmental cues, and acts as a signal to initiate processes such as senescence, chlorosis, and abscission, all of which are inhibitory to plant growth and survival. Thus, a significant fraction of the damage that occurs to a plant following a biotic or abiotic stress is due to the second (large) peak of ethylene that is synthesized by the plant rather than to the direct effects of the stress itself. Based on this model, it was predicted that bacteria, which produce an amount of ACC deaminase that can reduce the magnitude of the second ethylene peak, should decrease the damage to plants that occurs as a consequence of a wide range of biotic and abiotic stresses.

Flooding and Anoxia

Plant roots typically respond to flooding by synthesizing a high level of ACC, and as a consequence of a lack of oxygen, the ACC is translocated to shoots, where it becomes a substrate for ACC oxidase and is converted to ethylene (Bradford and Yang, 1980; Else and Jackson, 1998). Ethylene synthesis in flooded plants induces the expression of various symptoms such as epinasty, leaf chlorosis, and necrosis (Li et al., 2013). Bacteria able to limit the increase of ethylene through the action of ACC deaminase can be useful in supporting plant growth in such adverse conditions (Grichko and Glick, 2001; Barnawal et al., 2012; Li et al., 2013)

The protein profile of cucumber (*Cucumis sativus*) roots, inoculated or not with *P. putida* UW4 and able to synthesize ACC deaminase, in normoxic (no oxygen limitation) and hypoxic conditions has been characterized (Li et al., 2013). In normoxic conditions, no significant change in protein expression occurred in cucumber seedling roots treated with *P. putida* UW4. However, expression of several root proteins changed following the plant's inoculation with *P. putida* UW4 under hypoxic stress, including those involved in carbohydrate and nitrogen metabolism, defense stress, antioxidant activity, and binding to host plants (Li et al., 2013).

Drought

The first report of ACC deaminase-producing bacteria facilitating the growth of plants under drought stress was by Mayak et al. (2004a), who used *Achromobacter piechaudii* ARV8, from the rhizosphere of *Lycium shawii* from the Arava region of the Negev desert, to inoculate tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) plants exposed to drought stress. Plants inoculated with the bacterial strain had 4 times the biomass compared with noninoculated controls, concomitant with a significant reduction of the ethylene level.

Similar experiments (in the laboratory and in the field) with several plants (pea [*Pisum sativum*], maize, wheat, mung bean [*Vigna radiata*], and *Trigonella foenum-graecum*) and different ACC deaminase-producing bacteria have since demonstrated the efficacy of using bacteria able to synthesize ACC deaminase in protecting plants against yield loss induced by drought stress (Arshad et al., 2008; Belimov et al., 2009; Shakir et al., 2012; Barnawal et al., 2013; Sarma and Saikia, 2014; Zafarul-Hye et al., 2014).

Salt

Worldwide, the total area of salt-affected soil is about one billion ha, mainly in the arid-semiarid regions of Asia, Australia, and South America. In addition, salinity affects about 1 million ha in the European Union and is a major cause of desertification. In Spain, for example, 3% of the 3.5 million ha of irrigated land is severely affected, while another 15% of this land is considered to be under serious risk (Soil Atlas of Europe, European Soil Bureau Network European Commission 2005, http://eusoils.jrc.ec.europa.eu/projects/soil_atlas/pages/117.html).

Salt stress inhibits plant growth, inducing osmotic stress, Na^+ and Cl^- toxicity, ethylene production, plasmolysis, nutrient imbalance, production of reactive oxygen species, and interference with photosynthesis. Inhibition of seed germination, seedling growth and vigor, flowering, and fruit set occur as a consequence of these physiological changes (Sairam and Tyagi, 2004).

The initial responses of most plants to drought and salinity are very similar; both are attributed to water stress. When plants are exposed to high salt, a decrease in the growth rate followed by a slow recovery to a new reduced growth rate is the plant's first response to the decrease in water potential caused by salt, rather than to any salt-specific toxicity. Subsequently, metabolic toxicity in plants caused by sodium ions is attributed to these ions competing with potassium ions for binding sites essential for cellular functioning (Gamalero et al., 2009a).

Mayak et al. (2004b) first reported on the ability of *A. piechaudii* ARV8 to promote tomato plant growth in the presence of up to 172 mM NaCl salt. This work has served as a model for other researchers employing similar bacterial strains to facilitate the growth of plants in the presence of inhibitory salt levels (Gamalero et al., 2010; Nadeem et al., 2010; Ahmad et al., 2011; Siddikee et al., 2011; Chookietwattana and Maneewan, 2012; Karthikeyan et al., 2012; Bal et al., 2013; Ramadoss et al., 2013; Akhgar et al., 2014; Ali et al., 2014; Barnawal et al., 2014; Chang et al., 2014).

Fungal and Bacterial Pathogens

Ethylene levels inside plants increase following pathogen infection, and this induces the appearance of

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specific symptoms (van Loon et al., 2006). In this context, seedling inoculation with bacteria expressing ACC deaminase may reduce pathogen-induced ethylene, e.g. for soil-borne disease caused by pathogenic bacteria such as *Pseudomonas syringae* pv *tomato* (Indiragandhi et al., 2008), *Agrobacterium tumefaciens* (Toklikishvili et al., 2010; Hao et al., 2011), *Erwinia* spp. (Wang et al., 2000), and fungi, including *Pythium ultimum* (Wang et al., 2000), *Pythium aphanidermatum* (El-Tarabily, 2013), and *Pyricularia oryzae* (Amutharaj et al., 2012).

Nematodes

Recently, bacterial ACC deaminase has been identified as a key trait in suppression of the pathogenic nematode *Bursaphelenchus xylophilus* causing pine wilt disease (Nascimento et al., 2013). Thus, seedling inoculation with bacteria able to synthesize ACC deaminase may lead to plant resistance to nematode-induced diseases.

Metals and Organic Contaminants

Phytoremediation is the use of plants, able to tolerate/accumulate/degrade organic or inorganic chemicals and/or producing high biomass, to clean up polluted soils (Pilon-Smits, 2005). However, plants tolerant to xenobiotics do not develop high biomass, often limiting the practical application of this technology (Khan et al., 2000). PGPB can often facilitate phytoremediation (Glick, 2010) by promoting plant growth, improving their health, enhancing root development, or increasing plant tolerance to the stress imposed by environmental toxicants (Burd et al., 1998; Huang et al., 2004; Reed and Glick, 2005; Gamalero et al., 2009b; Gurska et al., 2009; Glick, 2012).

Flower Wilting

To extend the shelf life of cut flowers, treatments with, potentially environmentally harmful, chemical ethylene inhibitors are routinely performed (Reid and Wu, 1991). The application of bacteria that produce ACC deaminase to lower the amount of ethylene in cut flowers represents a safer alternative. To prolong the lifetime of cut flowers, bacterial cells must be taken up by the cut flowers. In this context, the use of ACC deaminase-expressing endophytes, which are adapted to live inside plant tissues, may assure the efficacy of this treatment. Consistent with this hypothesis, Ali et al. (2012) demonstrated that two endophytic bacterial strains, *Pseudomonas fluorescens* YsS6 and *Pseudomonas migulae* 8R6, both of which internally colonize the stems of the cut flowers, lower the flower ethylene levels and delay flower senescence by 2 to 3 d.

Rooting of Cuttings

The impact of inoculating plant cuttings with bacteria that are able to produce ACC deaminase was described by Mayak et al. (1999), who treated mung bean cuttings with *P. putida* GR12-2 or with its mutant lacking ACC deaminase activity. While the number of adventitious roots was similar in the two treatments, the length of the newly generated roots was significantly greater in mung bean cuttings inoculated with the wild-type strain. Similarly, carnation cuttings treated with a strain of *Azospirillum brasilense* engineered to synthesize ACC deaminase produced significantly more and longer roots than untreated cuttings (Li et al., 2005). Montero-Calasanz (2013) measured the rooting efficiency of olive (*Olea europaea*) cuttings following inoculation with five bacterial strains with different physiological traits: *Pantoea* spp. AG9, the only strain able to express ACC deaminase, was the most efficient strain in enhancing the rooting of these cuttings.

SUMMARY AND CONCLUSION

Plants that are grown in the field are subject to more or less continuous exposure to one stress after another, all of which can potentially inhibit plant growth and development. These stresses may be caused by biotic factors such as viruses, nematodes, insects, bacteria, or fungi or by abiotic factors such as extremes of temperature, high light, flooding, drought, the presence of toxic metals, and organic contaminants. While various plants may respond somewhat differently to stresses, nearly all plants respond to stress by producing ethylene. Lowering the amount of ethylene that is synthesized in response to stress through the application of ACC deaminase-producing bacteria can significantly decrease the extent of plant growth inhibition that accrues from the stress. From a practical perspective, as a consequence of the fundamental knowledge of plant growth-promoting bacterial modes of action that has been gained over the past 10 to 20 years, specifically emphasizing an understanding of the key role of ACC deaminase, this technology is currently accessible for use in agriculture, horticulture, and environmental cleanup technologies in both the developed and the developing world.

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