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REVIEW PAPER

Promotion of plant growth by ACC deaminase-producing soil bacteria

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Abstract Plant growth-promoting bacteria that contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase facilitate plant growth and development by decreasing plant ethylene levels, especially following a variety of environmental stresses. In this review, the physiological basis for this growth-promotion effect is examined in some detail. In addition, models are presented that endeavour to explain (i) the seemingly paradoxical effects of ethylene on a plant's response to stress, (ii) how the expression of this enzyme is transcriptionally regulated in many bacterial strains and (iii) how ACC deaminase-containing plant growth-promoting bacteria alter plant gene expression and positively modulate plant growth.

Keywords ACC deaminase · Ethylene · Plant growth-promoting bacteria · Plant stress · Plant growth

Abbreviations

ACC	1-Aminocyclopropane-1-carboxylate
AOA	Aminooxyacetic acid
AVG	L-α-(aminoethoxyvinyl)-glycine
CRP	Cyclic AMP receptor protein
FNR	Fumarate–nitrate reduction regulatory protein
IAA	Indole-3-acetic acid
Lrp	Leucine-responsive regulatory protein
1-MCP	1-Methylcyclopropene
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polycyclic biphenyls
RAP PCR	RNA arbitrarily primed PCR

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Introduction

The growth of plants in the field may be inhibited by a large number of both biotic and abiotic stresses. These stresses include extremes of temperature, high light, flooding, drought, the presence of toxic metals and environmental organic contaminants, radiation, wounding, insect predation, high salt, and various pathogens including viruses, bacteria and fungi (Abeles et al. 1992). As a consequence of these environmental stresses, plant growth is invariably lower than it would be in their absence. Moreover, during its life, the plant is subject to a number of non-lethal stresses that limit plant growth until either the stress is removed or the plant is able to adjust its metabolism to overcome the stress so that, in the field, plant growth often consists of periods of maximal growth interspersed with periods of growth inhibition (Fig. 1).

In addition to the ability of a plant to modify its physiology and metabolism, including the synthesis

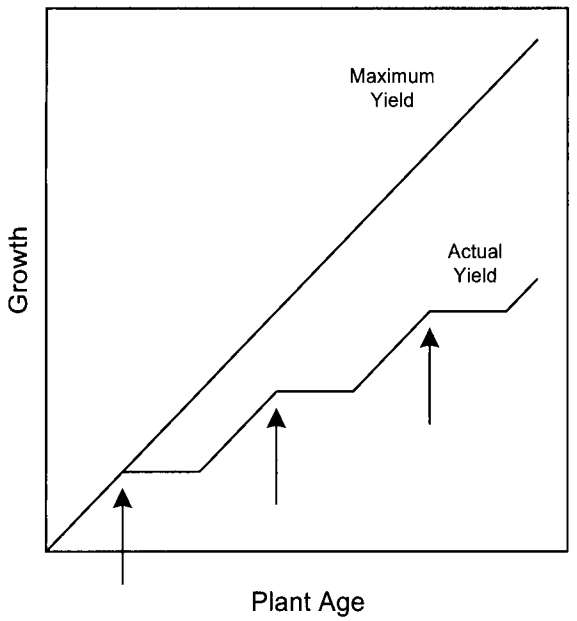

Fig. 1 Plant growth as a function of age. The slope of the maximum yield curve is the maximum growth rate. The arrows indicate the onset of a growth inhibitory, but non-lethal, stress which causes growth to cease or slow down for some period of time so that the actual yield is a direct result of the number and intensity of the stresses that a plant experiences during its lifetime

of a range of defensive proteins, certain soil bacteria can help plants to either avoid or partially overcome a variety of environmental stresses. These plant growth-promoting bacteria facilitate plant growth either by (i) aiding in the acquisition of nutritional resources such as nitrogen, phosphorus or iron; (ii) preventing the proliferation of pathogenic organisms (e.g. by synthesizing antibiotics); or by (iii) directly stimulating plant growth by either providing plant hormones such as auxin or cytokinin, or lowering plant ethylene levels through the action of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Glick 1995; Glick et al. 1999).

Plant stress and ethylene

When plants are exposed to stressful conditions, they often respond by producing what is known as stress ethylene. Various plants respond differently to stress, however, and also have a range of sensitivities to ethylene. In addition, there is a complex web of interactions between ethylene and other plant hormones that varies somewhat from one plant to another, so that it is difficult to explain the functioning of stress ethylene in one simple model. Nevertheless, plants exposed to various types of stress invariably show increased ethylene levels leading, as a result to increased damage (Hyodo 1991).

In an apparent paradox, stress ethylene has been suggested to both alleviate and exacerbate some of the effects of pathogen infection, depending upon the plant species, its age and the nature of the pathogen (Abeles et al. 1992; Arshad and Frankenberger 2002; Van Loon and Glick 2004). A model that explains these seemingly contradictory effects of stress ethylene on plants has been proposed (Stearns and Glick 2003; Pierik et al. 2006; Van Loon et al. 2006). In one description of this model, there is an initial small peak of ethylene close in time, usually a few hours after, to the onset of the stress and then a second much larger peak some time later, usually one to three days (Fig. 2A). The first peak is only a small fraction of the magnitude of the second peak and is thought to initiate a protective response by the plant, such as transcription of pathogenesis-related genes and acquired resistance (Ciardi et al. 2000; Van Loon and Glick 2004). The first small wave of ethylene production is thought to consume the existing pool of ACC within plant tissues (Robison et al. 2001a). On the other hand, the second ethylene peak is so large that processes such as senescence, chlorosis and abscission are initiated, the overall effect of which is generally inhibitory to plant survival. Thus, following a severe infection by pathogens, a large portion of the damage that occurs to a plant is due to autocatalytic ethylene synthesis and not from direct pathogen action (Van Loon 1984). In this regard, not only can exogenous ethylene increase the severity of a pathogen infection, but as well, inhibitors of ethylene synthesis or ethylene action can significantly decrease the severity of a fungal or bacterial infection. The second peak of ethylene production occurs as a consequence of increased transcription of ACC synthase genes triggered by environmental and developmental cues (Yang and Hoffman 1984).

ACC deaminase

The enzyme ACC deaminase (EC: 4.1.99.4) which catalyzes the cleavage of ACC to ammonia and α-ketobutyrate was first discovered in 1978 (Honma and Shimomura 1978). This enzyme has subsequently

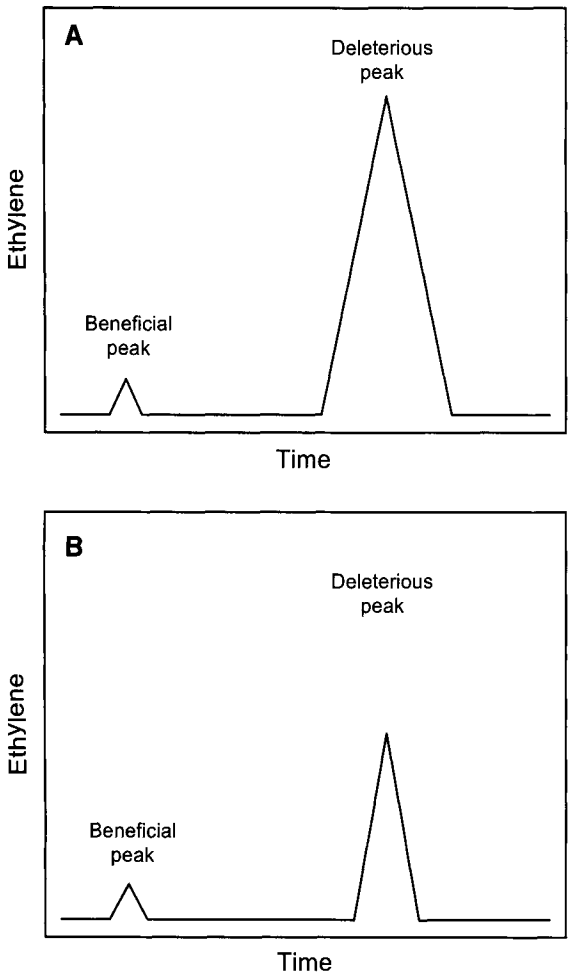

Fig. 2 Plant ethylene production as a function of time following an environmental stress. (**A**) In the absence of any exogenous bacteria. (**B**) In the presence of an ACC deaminase-producing plant growth-promoting bacterium. In both cases, there is an initial small peak of ethylene that is thought to activate transcription of plant defense genes, which is often difficult to detect, followed some time later by a much larger ethylene peak that can cause adverse responses in the plant. The amount of ethylene produced in response to an environmental stress is related to the plant age as well as the nature and severity of the stress

been detected in a wide range of bacterial strains and fungi (Klee et al. 1991; Sheehy et al. 1991; Honma 1993; Jacobson et al. 1994; Glick et al. 1995; Campbell and Thomson 1996; Burd et al. 1998; Minami et al. 1998; Jia et al. 1999; Belimov et al. 2001; Mayak et al. 2004a; Babalola et al. 2003; Ghosh et al. 2003; Ma et al. 2003a, b; Dey et al. 2004; Uchiumi et al. 2004; Belimov et al. 2005; Hontzeas et al. 2005; Blaha et al. 2006; Madhaiyan et al. 2006), and for many of these strains, the ACC deaminase gene has been isolated and characterized. The presence of ACC deaminase is relatively common amongst soil microorganisms. For example, in one study, 27 out of 233 newly isolated *Rhizobium* spp. from various sites in southern and central Saskatchewan contained this activity (Duan et al. 2006). In another study, ACC deaminase activity/genes were found in a wide range of bacterial isolates including *Azospirillum*, *Rhizobium*, *Agrobacterium*, *Achromobacter*, *Burkholderia*, *Ralstonia*, *Pseudomonas* and *Enterobacter* (Blaha et al. 2006). Moreover, 62 out of 88 *Pseudomonas* strains exhibiting biocontrol activity isolated from locations worldwide contained ACC deaminase (Wang et al. 2001).

K_m values of ACC deaminase for ACC have been estimated at pH 8.5, in all instances examined, to be approximately 1.5–17.4 mM indicating that the enzyme does not have a particularly high affinity for ACC (Honma and Shimomura 1978; Klee and Kishore 1992; Jacobson et al. 1994; Hontzeas et al. 2004a). Moreover, ACC levels in plants are typically in the μM range, therefore in most plant tissues the ACC concentration will be dramatically below the K_m of ACC deaminase for this substrate so that—based on the Michaelis-Menten rate equation for enzyme catalyzed reactions—a small increase in the ACC concentration (e.g. a doubling) will result in a parallel increase in the rate of ACC cleavage.

ACC deaminase is a member of a large group of enzymes that require the co-factor pyridoxal 5′-phosphate for enzymatic activity (Walsh et al. 1981). These enzymes have been classified based on their three dimensional structure, into four folding types: (i) tryptophan synthase, (ii) aspartate aminotransferase, (iii) D-amino acid aminotransferase and (iv) alanine racemase (Jansonius 1998). According to this classification scheme, ACC deaminase fits into the tryptophan synthase family. The coenzyme pyridoxal phosphate is a tightly bound cofactor of ACC deaminase in the amount of approximately one mole of pyridoxal phosphate per trimeric subunit (Honma 1985). Interestingly, ACC synthase also requires pyridoxal phosphate for enzyme activity.

ACC deaminase lowering of stress ethylene

A model was previously proposed by which plant growth-promoting bacteria can lower plant ethylene

levels and in turn facilitate plant growth (Glick et al. 1998). In this model the plant growth-promoting bacteria bind to the surface of a plant (usually seeds or roots, although ACC deaminase-producing bacteria may also be found on leaves and flowers). In response to tryptophan and other small molecules in the plant exudates, the bacteria synthesize and secrete indole-3-acetic acid (IAA), some of which is taken up by the plant. This IAA together with endogenous plant IAA can stimulate plant cell proliferation, plant cell elongation or induce the transcription of ACC synthase which is the enzyme that catalyzes the formation of ACC. Some of the ACC is exuded from seeds, roots or leaves (Penrose et al. 2001; Grichko and Glick 2001a) along with other small molecules normally present in these exudates and may be taken up by the bacteria and subsequently cleaved by the enzyme, ACC deaminase, to ammonia and α-ketobutyrate. In this model, the bacterium acts as a sink for plant ACC and as a result of lowering either the endogenous or the IAA-stimulated ACC level, the amount of ethylene in the plant is also reduced. As a direct consequence of lowering plant ethylene levels, plant growth-promoting bacteria that possess the enzyme ACC deaminase can reduce the extent of ethylene inhibition of plant growth following a wide range of stresses. Thus, plants grown in association with these bacteria should have longer roots and shoots and be more resistant to growth inhibition by a variety of ethylene-inducing stresses.

The question arises, as to how bacterial ACC deaminase can selectively lower deleterious ethylene levels but not affect the small peak of ethylene that is thought to activate some plant defense responses (Fig. 2A). As discussed later in this review, ACC deaminase is generally present in bacteria at a low level until it is induced, and the induction of enzyme activity is a relatively slow and complex process. Immediately following an environmental stress, the pool of ACC in the plant is low as is the level of ACC deaminase in the associated bacterium. Following the relatively rapid induction of a low level of ACC oxidase in the plant, it is likely that there is increased flux through this enzyme resulting in the first small peak of ethylene which is of sufficient magnitude to induce a protective/defensive response in the plant (Fig. 2B). With time, bacterial ACC deaminase is induced (by the 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 (Fig. 2B). The second ethylene peak may be reduced dramatically, but it is never completely abolished since ACC oxidase has a much higher affinity for ACC than does ACC deaminase (Glick et al. 1998). Thus, when ACC deaminase-producing bacteria are present, ethylene levels are ultimately dependent upon the ratio of ACC oxidase to ACC deaminase (Glick et al. 1998).

Several different chemicals have been used to lower ethylene levels in plants including rhizobitoxine, L-α-(aminoethoxyvinyl)-glycine (AVG) which is a synthetic analog of rhiobitoxine, aminooxyacetic acid (AOA) and the ethylene perception inhibitor 1-methylcyclopropene (1-MCP) and its analogs (Yuhashi et al. 2000; Sisler and Serek 1997). While 1-MCP has been approved for commercial use, it has been utilized primarily to limit post-harvest fruit spoilage and flower wilting. However, unlike ACC deaminase-producing plant growth-promoting bacteria, chemical ethylene inhibitors cannot readily be used in the field as a means of limiting the inhibitory effects of biotic and abiotic stresses.

Phytopathogens

In recent years, the engineering of plants that are resistant to a variety of pathogens including viruses, bacteria and fungi has become popular. Unfortunately, it is impractical to attempt to engineer plants against all of the pathogens (and other stresses) that they might encounter in the environment, as these can vary from one locale to another and from one season to the next. Alternatively, one can either select or engineer biocontrol bacteria that protect plants against a range of different pathogens. A more general strategy, however, might include treating plant seeds or roots with plant growth-promoting or biocontrol bacteria that contain ACC deaminase. Thus, for example, ACC deaminase-producing biocontrol bacteria were more effective at preventing (i) growth inhibition of cucumber plants by the plant root pathogen *Pythium ultimum* and (ii) rotting of potatoes by *Erwinia carotovora* than were biocontrol bacteria that lacked this enzyme (Wang et al. 2000). In addition, transgenic tomato plants expressing a bacterial ACC deaminase gene under the transcriptional control of a root-specific promoter (which

mimics the effect of adding ACC deaminase-producing plant growth-promoting bacteria to the plant roots) are significantly protected against damage from Verticillium wilt compared to non-transformed tomato plants (Robison et al. 2001b).

High salt and drought

Soil salinity is an enormous problem for agriculture under irrigation. In the hot and dry regions of the world the soils are frequently saline with low agricultural potential. In these areas most crops are grown under irrigation, and to exacerbate the problem, inadequate irrigation management leads to secondary salinization that affects 20% of irrigated land worldwide (Mayak et al. 2004b).

In recent years, considerable attention has been directed toward genetically engineering plants to be more salt tolerant, with moderate success (e.g. Apse et al. 1999). An alternative approach to overcoming some of the problems associated with growing plants in saline soils involves employing an ACC deaminase-producing bacterium, *Achromobacter piechaudii* ARV8, isolated from the rhizosphere of a *Lycium shawii* plant growing in a dry riverbed in the Arava region of Israel (Mayak et al. 2004b). This strain dramatically lowered the level of ethylene and prevented inhibition of plant growth in tomato plants grown in the presence of high concentrations of salt (Mayak et al. 2004b). The same bacterial strain lowered the ethylene level and significantly decreased the growth inhibition of peppers and tomatoes from drought stress (Mayak et al. 2004a).

Flooding

Periods of flooding can occur several times a growing season and may last for periods of from 1 or 2 days to several weeks. During these periods, the root environment rapidly becomes anaerobic causing an induction in the expression of ACC synthase, resulting in the accumulation of ACC in root tissues (Else and Jackson 1998). With other stresses, a significant portion of the newly synthesized ACC might be converted to ethylene in the roots; however, this is not possible when roots are flooded since the enzyme ACC oxidase, which catalyzes this reaction, requires oxygen for the conversion to proceed. Instead, the accumulated ACC is transported to the shoots where there is an aerobic environment and ethylene can be produced. Unfortunately, this causes epinasty, leaf chlorosis, necrosis and reduced fruit yield. On the other hand, when flooded plants are first treated with ACC deaminase-producing plant growth promoting bacteria, or plants are genetically engineered to express this enzyme in a root specific manner, much less ACC accumulates in the roots. Consequently, the damage to the plant that would otherwise occur from the newly synthesized ethylene is significantly decreased (Grichko and Glick 2001a, b).

Metal and organic contamination

In the presence of high levels of metals most plants synthesize growth inhibitory amounts of stress ethylene and also become severely iron depleted. This is readily remedied in the laboratory by adding ACC deaminase- and siderophore-producing plant growth-promoting bacteria which can help plants to overcome many of the effects of high levels of metal (Burd et al. 1998, 2000; Reed and Glick 2005). Similarly, transgenic plants that express a bacterial ACC deaminase gene under the control of a root-specific promoter are more resistant to the toxic effects of metals than are non-transformed plants (Grichko et al. 2000; Nie et al. 2002; Stearns et al. 2005; Li et al. 2006).

Field experiments aimed to facilitate plant growth in metal-contaminated soils so that the plant can take up and concentrate the metal (i.e., metal phytoremediation/phytoaccumulation) are considerably more complex than laboratory experiments. In the field, both ACC deaminase-producing plant growth-promoting bacteria and transgenic plants that express a bacterial ACC deaminase gene under the control of a root-specific promoter grow better than non-transformed and untreated plants. Although many metal contaminants are present at high levels in the field, in this environment they are generally not especially bioavailable so that only a small fraction of the metals are taken up by the plants (Farwell et al. 2006).

Considerable success has been achieved in the phytoremediation of organic environmental contaminants such as oil spills, polycyclic aromatic hydrocarbons (PAHs) and polycyclic biphenyls (PCBs) such that this technology is ready for commercialization. Many varieties of plants and trees can take up

and degrade some organic compounds; however, larger molecules are less water soluble and more difficult to degrade, often requiring degradative bacteria as well as plant roots for their breakdown. While the degradative bacterial population in the bulk soil is insufficient to efficiently break down complex organic molecules, the bacterial population in the rhizosphere is typically 100–1,000 times greater than in bulk soil so that most of the degradation of organic environmental contaminants occurs in the rhizosphere. Despite the fact that many plants, together with rhizosphere degradative bacteria, can readily degrade many organic environmental pollutants, most of these compounds are somewhat inhibitory to plant growth. Not surprisingly, a significant part of this growth inhibition is a consequence of the production of stress ethylene by the plant. Thus, treatment of plant seeds or roots with ACC deaminase-producing plant growth-promoting bacteria relieves much of this growth inhibition, allowing the plant to grow to near normal size, and degradation of the contaminants to proceed at a much faster rate than would otherwise be possible (Huang et al. 2004; Reed and Glick 2005; Huang et al. 2005; Greenberg et al. 2006).

Rhizobial infection

Ethylene is an inhibitor of rhizobial nodulation of legumes, and since the infection of plant roots by *Rhizobia* causes plants to locally produce ethylene, rhizobial infection may be viewed as a self-limiting process (Guinel and Geil 2002; Ma et al. 2003a). On the other hand, many strains of *Rhizobia* produce either rhizobitoxine, an inhibitor of the enzyme ACC synthase, or ACC deaminase which allows these bacterial strains to lower the ethylene levels and increase nodulation (and subsequent biomass formation) by 25–40% (Nukui et al. 2000; Ma et al. 2003b, 2004). Moreover, surveying *Rhizobia* strains for ACC deaminase activity indicates that a large number of commercial strains but only a small number of field strains have this activity. This suggests that the direct screening of field isolates for ACC deaminase activity may be one means of rapidly selecting *Rhizobia* strains with superior commercial inoculant potential.

Rhizobia strains that express ACC deaminase exhibit only a low level of enzyme activity compared with free-living plant growth-promoting bacteria. This has led us to speculate that there are two types of ACC deaminase-producing bacteria. On the one hand, there are free-living bacteria that bind relatively non-specifically to plant tissues (mainly roots) and have a high level of ACC deaminase activity which protects plants from a range of different stresses by lowering ethylene levels throughout the plant. On the other hand, *Rhizobia* bind tightly only to the roots of specific plants and have a low level of enzyme activity which facilitates nodulation by locally (but not globally) lowering ethylene levels. At this point, it is not known whether the 10- to 30-fold differences in enzyme activity observed when comparing free-living bacteria with *Rhizobia* is a consequence of differences in the amount of enzyme synthesized or in the intrinsic catalytic activity of the enzymes from different types of bacteria.

ACC deaminase regulation

Full induction of ACC deaminase gene expression requires the addition of ACC to the growing cells and takes much longer than the generation time of the bacterium (Jacobson et al. 1994). This suggests that ACC deaminase induction, and hence its mode of regulation in this bacterium, is relatively complex. In fact, analysis of DNA sequence data for the region upstream of the ACC deaminase structural gene (*acdS*) from *Pseudomonas putida* UW4 indicates that this DNA segment contains a CRP (cyclic AMP receptor protein) binding site, an FNR (fumarate–nitrate reduction regulatory protein) binding site (a known anaerobic transcriptional regulator), an Lrp (leucine-responsive regulatory protein) binding site, an open reading frame encoding an Lrp protein and three putative promoter sequences, one controlling the ACC deaminase regulatory gene (*acdR*; encoding Lrp) and two controlling *acdS* (Grichko and Glick 2000; Li and Glick 2001). All of these features were shown to be involved in the transcriptional regulation of *acdS*. More recently, in this same bacterium, a protein (AcdB) that interacts directly with ACC, the Lrp protein and the region of DNA upstream of *acdS* was identified and characterized (Z. Cheng, B.P. Duncker, B. McConkey and B.R. Glick submitted for publication). Although all of the details of how these various proteins and regions of DNA interact have not been completely elaborated, based on a

combination of the published and submitted data, a model of the transcriptional regulation of *acdS* has been developed (Fig. 3). In addition to *P. putida* UW4, genes encoding Lrp proteins have been found immediately upstream from a number of bacterial ACC deaminase structural genes, and in every instance *acdS* and *acdR* were oriented in opposite directions (N. Hontzeas, J. Duan and B.R. Glick unpublished results). In many instances, however, neither the CRP nor the FNR binding site were found to be present. These data suggest that *acdS* and *acdR* are usually inherited together and that this mode of transcriptional regulation is a central feature of the functioning of many bacterial ACC deaminases.

In addition to the more common mode of regulation by AcdR (= Lrp), the *acdS* gene from *Mesorhizobium loti* MAFF303099 was found to be under the control of a *nifA* promoter (Uchiumi et al. 2004) and to be expressed within legume nodules (Nukui et al. 2006). This observation leads to the speculation that the expression of *acdS* within nitrogen-fixing nodules might act to decrease the rate at which the nodule senesces—as a consequence of its high energy demand, nitrogen fixation could activate stress ethylene synthesis—and the resultant longer nodule lifetime might effectively increase the amount of fixed nitrogen.

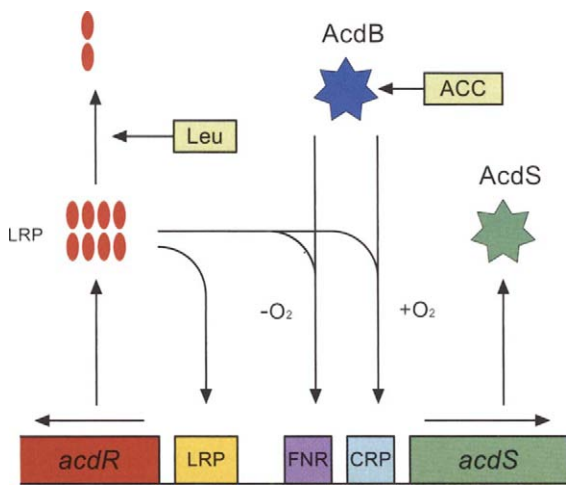

Fig. 3 Model of the transcriptional regulation of ACC deaminase expression in *Pseudomonas putida* UW4. The *acdR* gene encodes an Lrp protein which is thought to function as an octamer (Leonard et al. 2001). This protein can either bind to a DNA sequence known as an LRP box (which overlaps the promoter for *acdR*, not shown), preventing further transcription of this gene, or it can bind to a complex of ACC and the AcdB protein, encoding glycerophosphoryl diester phosphodiesterase (Z. Cheng, B.P. Duncker, B. McConkey and B.R. Glick submitted for publication), and together Lrp and AcdB can bind to either an FNR or CRP box on the DNA (both of which overlap separate promoter sequences, not shown). Binding to FNR is favoured under anerobic conditions while binding to CRP is favoured under aerobic conditions. The binding of these factors facilitates transcription of *acdS* by RNA polymerase. The newly synthesized ACC deaminase (= AcdS) cleaves ACC to form ammonia and α-ketobutyrate with the latter compound being a precursor of branched chain amino acids including leucine. Finally, in the presence of high levels of leucine in the cell, the Lrp octamer is dissociated into an inactive dimeric form thereby shutting down further transcription of *acdS*

Effect of ACC deaminase on plant gene expression

In one study, differential display PCR was used to elaborate some of the changes in plant gene expression caused by the addition of the plant growth-promoting bacterium *Paenibacillus polymyxa* to the roots of *Arabidopsis thaliana* plants (Timmusk and Wagner 1999). These workers identified a small number of genes whose expression was altered significantly and concluded that the plant responded to the presence of the bacterium as if the bacterium was a mild biotic stress.

In a subsequent study, RNA arbitrarily primed (RAP) PCR was used to identify several genes in canola roots whose expression was affected differentially by the addition of an ACC deaminase-producing plant growth-promoting bacterium and an ACC deaminase negative mutant of that strain (Hontzeas et al. 2004b). Interestingly, the ACC deaminase-producing bacterium down-regulated genes involved in ethylene-induced plant stress responses in the plant and up-regulated genes involved in plant growth. These data are consistent with the notion, when plant growth-promoting bacteria express ACC deaminase they are no longer perceived by the plant as a mild biotic stress.

Recently, canola shoot mRNA was isolated and hybridized to microarrays in which each chip contained more than 20,000 different 60-mer oligonucleotide DNA sequences representing approximately 80% of the *Arabidopsis* genome. In these experiments, non-transformed canola was compared to transgenic canola expressing a bacterial ACC deaminase gene under the control of a root-specific promoter. Analysis of the results indicated that several auxin response factor genes were more highly

transcribed in the transgenic plants (J. Czarny, S. Shah and B.R. Glick unpublished results). While these preliminary experiments need to be repeated, and gene expression needs to be examined in canola plants treated with ACC deaminase-producing bacteria, it is intriguing to speculate as to how these data fit into our understanding of the mode of action of ACC deaminase. One model (Fig. 4) that explains the data is that by lowering the ethylene concentration in plant roots, ACC deaminase relieves the ethylene repression of auxin response factor synthesis, and indirectly increases plant growth (see also Dharmasiri and Estelle 2004). This interaction between ethylene and IAA acts as a feedback loop which decreases the impact of IAA and also may decrease the amount of IAA-stimulated ethylene synthesis that might otherwise occur. In this way, ethylene may limit the amount of its own synthesis. In concert with the model proposed here, it has been reported that ethylene can inhibit the transport of IAA in various plants (Burg and Burg 1966; Morgan and Gausman 1966; Suttle 1988; Prayitno et al. 2006).

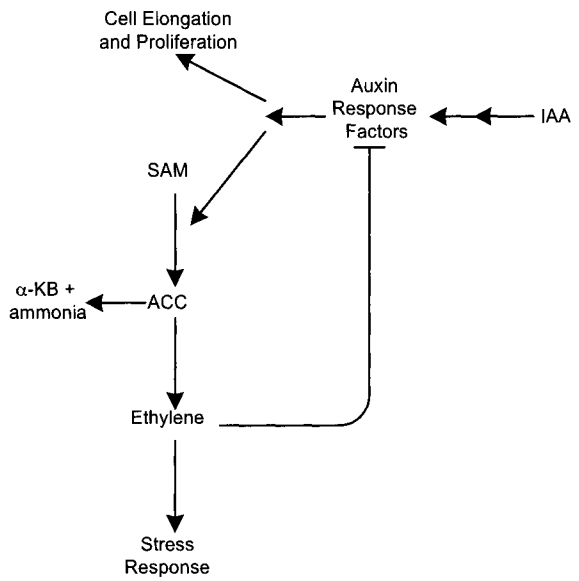

Fig. 4 Model for how the ACC deaminase lowering of ethylene levels leads to an increase in IAA-mediated canola gene expression. While this model was developed from microarray data comparing transgenic plants expressing ACC deaminase under the control of a root-specific promoter, based on physiological data, it is expected that non-transformed plants treated with an ACC deaminase-producing plant growth-promoting bacterium will behave similarly. Here, in the absence of ACC deaminase, root-produced ethylene inhibits transcription of auxin response factors thereby limiting auxin-stimulated plant growth as well as auxin promotion of ACC synthase transcription. In the presence of ACC deaminase, ethylene levels are decreased and the blockage of auxin response factor transcription is relieved thereby facilitating plant growth

Conclusions and future prospects

All of the available data are consistent with the previously proposed model of plant growth facilitation by ACC deaminase-producing plant growth-promoting bacteria (Glick et al. 1998). Moreover, plants respond similarly to ACC deaminase regardless of whether the enzyme is expressed in the roots of transgenic plants or as part of a root-associated bacterium. If anything, the root-associated bacterium provides a greater benefit to the plant, most likely reflecting the fact that in addition to lowering ethylene levels, the bacteria may also provide a variety of other benefits to the plant (Glick 1995; Glick et al. 1999).

Given the very large difference in the cost of engineering, selecting and developing transgenic plants that are protected against a variety of pathogens and other stresses, compared to selecting and testing appropriate plant growth-promoting bacteria, it is more propitious to direct our efforts toward the development of new plant growth-promoting bacteria. One of the major drawbacks in the large scale employment of plant growth-promoting bacteria is that these organisms may not always survive harsh environmental conditions including high concentrations of environmental contaminants, salts, extremes of pH and temperature, and the presence of other organisms that either out-compete or consume these bacteria. A possible solution to this problem may lie in the use of endophytic plant growth-promoting bacteria (Sturz and Nowak 2000).

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