



Bacteria with ACC deaminase can promote plant growth and help to feed the world[☆]



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ABSTRACT

To feed all of the world's people, it is necessary to sustainably increase agricultural productivity. One way to do this is through the increased use of plant growth-promoting bacteria; recently, scientists have developed a more profound understanding of the mechanisms employed by these bacteria to facilitate plant growth. Here, it is argued that the ability of plant growth-promoting bacteria that produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase to lower plant ethylene levels, often a result of various stresses, is a key component in the efficacious functioning of these bacteria. The optimal functioning of these bacteria includes the synergistic interaction between ACC deaminase and both plant and bacterial auxin, indole-3-acetic acid (IAA). These bacteria not only directly promote plant growth, they also protect plants against flooding, drought, salt, flower wilting, metals, organic contaminants, and both bacterial and fungal pathogens. While a considerable amount of both basic and applied work remains to be done before ACC deaminase-producing plant growth-promoting bacteria become a mainstay of plant agriculture, the evidence indicates that with the expected shift from chemicals to soil bacteria, the world is on the verge of a major paradigm shift in plant agriculture.

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1. The problem

As a consequence of increases in and environmental damage and worldwide population growth, global food production may soon be insufficient to feed all of the world's people. In this regard, the world's population, which is currently around 7 billion people has been projected to increase to around 10 billion in the next 50 years. To feed all of these individuals it is absolutely essential that agricultural productivity be significantly increased within the next few decades. However, providing sufficient food for an ever-increasing world population is not an easy task; it will require a variety of different strategies and approaches. To produce more food, the world will require (at least initially) more agricultural land; greater use of chemicals including fertilizers, pesticides and herbicides; more farm mechanization; greater use of transgenic crops; and the expanded use of plant growth-promoting microorganisms. Of course, many of the solutions to this problem that will be attempted are not sustainable and will only be effective in the short term. Since we live in a finite world with limited resources, any effective and longer term solutions to providing food for the world (once the population has leveled off) must include sustainable and environmentally friendly biological solutions. To this end,

the purposeful use of PGPB in agriculture is an attractive technology to address this problem. While scientists have dramatically increased our knowledge of the mechanisms employed by PGPB in the past 15–20 years (Glick 2012), additional understanding of the fundamental mechanisms employed by these bacteria will likely hasten the acceptance of these organisms as suitable and effective adjuncts to agricultural practice (Reed and Glick 2004). Therefore, of necessity, any efforts to increase the practical use of PGPB must start with a better understanding of how these bacteria promote plant growth.

2. Plant growth-promoting bacteria

Most soils contain an enormous diversity of microorganisms including bacteria, actinomycetes, fungi, algae and protozoa. It has been suggested that a typical gram of soil contains $\sim 9 \times 10^7$ bacteria, 4×10^6 actinomycetes, 2×10^5 fungi, 3×10^4 algae, 5×10^3 protozoa and 3×10^1 nematodes (Alexander 1991). Of course, the numbers of these organisms in any one soil compared to another may vary greatly.

Soil bacteria in particular have the ability to grow rapidly and to utilize a very wide range of different substances as nutrient sources. While many bacteria are dispersed within the soil, often attached to soil particles, many interact with the roots of plants. It is quite common for the concentration of bacteria that is found around the roots of plants, i.e. in the rhizosphere, to be much greater than the

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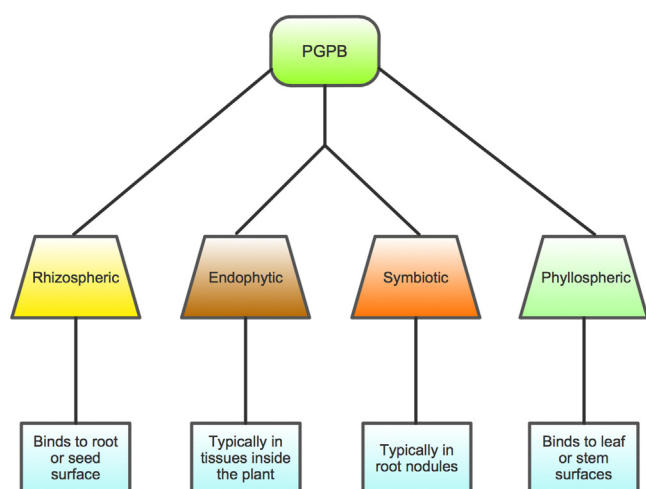


Fig. 1. PGPB includes bacteria that interact with plants in a range of different ways.

bacterial concentration in the rest of the soil (Lynch 1990). The high concentration of bacteria around the roots of plants presumably occurs because of the presence of high levels of nutrients including amino acids, sugars and organic acids that are exuded from the roots of plants, and are then used by rhizosphere bacteria to support their growth and metabolism (Whipps 1990). In this regard, various estimates of the amount of photosynthetically fixed carbon that is exuded by the roots of various plants range from 5% up to around 30%.

The interaction between soil bacteria and plant roots may be classified as being beneficial, harmful or neutral for the plant, and sometimes the effect of a particular bacterium may vary as the soil conditions change (Lynch, 1990). For example, a bacterium that facilitates growth only by providing plants with fixed nitrogen would not provide any benefit to plants when the soil contains large amounts of chemical nitrogen fertilizer. Similarly, bacteria that promote plant growth by decreasing the inhibitory effects of various environmental stresses (abiotic or biotic) are unlikely to have much effect on plant growth when the conditions are optimal (Glick et al. 2007).

Bacteria that facilitate plant growth (Fig. 1) may do so either by binding to the plant's outer surface such as the roots (the rhizosphere) or the leaves (the phyllosphere), or they may inhabit the interior surfaces of the plant forming an endophytic relationship (Hallmann et al. 1997; Sturz et al. 2000; Davison 1988; Kloepper et al. 1989). A bacterial endophyte may be localized in only certain plant tissues such as roots and stems, it may be distributed throughout the plant's tissues, or it may form specific structures such as nodules, depending upon the bacterium and the plant. Endophytic bacteria that form and occupy nodules on specific host plants have previously been called symbiotic bacteria and have been studied extensively (Oldroyd et al. 2011; Wang et al. 2012). These organisms were among the first soil bacteria to be utilized commercially as a biological means of promoting plant growth (Reed and Glick 2004). Plant beneficial soil bacteria, regardless of where they are primarily found on the plant, are now commonly referred to as plant growth-promoting bacteria or PGPB (Bashan and Holguin 1998).

While many different soil bacteria are considered to be plant growth-promoting bacteria, not all strains of a particular bacterial genus and species have the same genetic makeup and metabolic capabilities. Thus for example, some strains of *Pseudomonas fluorescens* may actively promote plant growth while other strains of this species have no measurable effect on plants. This reflects the fact that the so-called core genes that define the fundamental

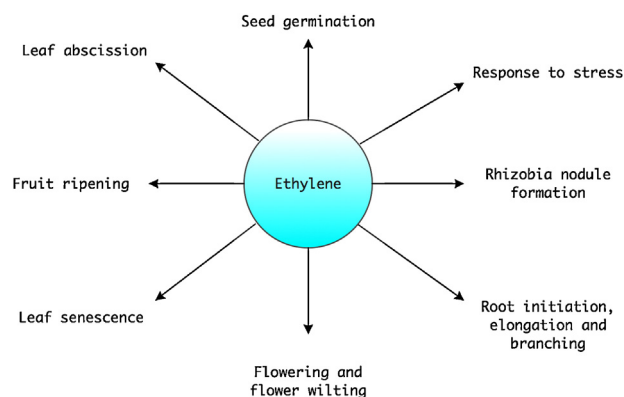


Fig. 2. The phytohormone ethylene affects a large number of different processes in the growth and development of a plant.

metabolic capabilities of a bacterium are not typically involved in plant growth promotion (Huang et al. 2013).

Plant growth-promoting bacteria may facilitate plant growth and development either indirectly or directly. Indirect plant growth promotion occurs when these bacteria decrease or prevent some of the deleterious effects of a plant pathogen (usually a fungus) by any of one of several different mechanisms (Glick 2012). The direct promotion of plant growth by plant growth-promoting bacteria generally entails facilitating the acquisition of nutrient resources from the environment including fixed nitrogen, iron and phosphate, or in specifically modulating plant growth by altering plant hormone levels such as auxin, cytokinin and ethylene (Glick 2012).

3. Ethylene and plants

The hormone ethylene, which is found in all higher plants, is an important modulator of normal plant growth and development in plants as well as a key feature in the response of plants to a wide range of stresses (Abeles et al. 1992). Many aspects of the growth of plant tissues such as roots, stems, leaves, flowers and fruits, as well as all stages of plant development are affected by ethylene (Fig. 2). A variety of other plant processes involve ethylene including rhizobia nodulation of legumes (Goodlass and Smith 1979), rooting of cuttings (Li et al. 2005; Mayak et al. 1999; Montero-Calasanz et al. 2013), as well as a plant's interaction with beneficial mycorrhizal fungi (Beyrle 1995; Gamalero et al. 2008).

Within any particular plant, ethylene synthesis is affected by a number of different factors including temperature, light, gravity, nutrition, the presence and level of other plant hormones, and the presence of various types of biological stress to which the plant may be subjected (Abeles et al. 1992). Regarding a plant's response to stress, an increased level of ethylene is typically formed in response to the presence of metals, organic and inorganic chemicals, temperature extremes, too much or too little water, ultraviolet light, insect damage, nematode damage, phytopathogens (both fungi and bacteria), and mechanical wounding (Abeles et al. 1992). One model that describes the synthesis of "stress ethylene" includes the ethylene being synthesized in two peaks (Glick et al. 2007; Pierik et al. 2006; Van Loon et al. 2006). The first ethylene peak is typically only a small fraction of the magnitude of the second peak. This small peak of ethylene, which consumes the existing pool of 1-aminocyclopropane-1-carboxylate (ACC, the precursor of ethylene) in stressed plant tissues, is believed to be responsible for initiating the transcription of genes that encode plant defensive/protective proteins (Robison et al. 2001a). The first ethylene peak is often technically difficult to measure depending upon the sensitivity of the instrumentation that is utilized. The second, much larger, ethylene peak occurs following synthesis by the plant of additional ACC in

response to a stress (and may occur up to several days after the initial ethylene peak). The second ethylene peak is generally detrimental to plant growth and is often involved in initiating processes such as senescence, chlorosis and leaf abscission. In this case, the high level of plant ethylene that is formed can significantly exacerbate the effects of the stress (that triggered the ethylene response) so that it is predicted that any treatment, chemical or biological, that can significantly lower the magnitude of the second peak of stress ethylene should simultaneously decrease the damage to the plant that occurs as a consequence of the stress.

4. ACC deaminase

4.1. A model of plant growth promotion

Although plant growth-promoting bacteria use a number of different mechanisms to promote the growth of plants (Glick 2012), arguably, the bacterial trait that is key in facilitating plant growth is the possession of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. This enzyme is responsible for the cleavage of the plant ethylene precursor, ACC, into ammonia and α -ketobutyrate (Honma and Shimomura 1978). By decreasing ACC levels in plants, ACC deaminase-producing organisms decrease plant ethylene levels (Glick et al. 1998, 2007), which when present in high concentrations can lead to plant growth inhibition or even death.

In a previously proposed model (Glick et al. 1998), it was suggested that ACC deaminase-producing plant growth-promoting bacteria first bind to the surface of a plant (usually seeds or roots), although these bacteria may also be found on leaves and flowers or within a plant's internal tissues (i.e., as an endophyte). Plants typically exude a large fraction of their photosynthetically fixed carbon (estimated to generally be in the range of 5–30%) through their roots. Roots exudates generally contain large amounts of sugars, organic acids and amino acids, and the ability of these compounds to act as a bacterial food source is the main reason why the numbers of bacteria around the roots of plants (i.e., the rhizosphere) are 10–1000 times higher than in the bulk soil. In response to the presence of tryptophan and other small molecules in the plant root exudates, the associated bacteria synthesize and secrete the phytohormone indole-3-acetic acid (IAA), some of which is taken up by the plant. This IAA, together with endogenous plant-synthesized IAA can affect plants in several different ways. It can stimulate plant cell proliferation and/or plant cell elongation, or it can induce the transcription of the plant enzyme ACC synthase that catalyzes the formation of ACC. In this case, IAA acts to stimulate the synthesis of ethylene in the plant. IAA also acts to loosen plant cell walls, thereby facilitating cell elongation and increasing the extent of root exudation. Along with other small molecule components of root exudates, some of the plant ACC (a non-ribosomal amino acid) is exuded from seeds, roots or leaves (Penrose et al. 2001) and may be taken up by the bacteria associated with these tissues, and subsequently cleaved by ACC deaminase Penrose and Glick (2003).

The net result of the cleavage of exuded ACC by bacterial ACC deaminase is that the bacterium is de facto acting as a sink for ACC. Moreover, as a result of lowering either the endogenous or the IAA-stimulated ACC level, the amount of ethylene that could potentially form in the plant is reduced. Subsequently, as a consequence of lowering plant ethylene levels, ACC deaminase-containing plant growth-promoting bacteria can reduce a portion of the ethylene inhibition of plant growth following a wide range of abiotic and biotic stresses. As a result, plants which grow in association with ACC deaminase-containing plant growth-promoting bacteria generally have longer roots and shoots and are more resistant to growth inhibition by a variety of ethylene-inducing stresses.

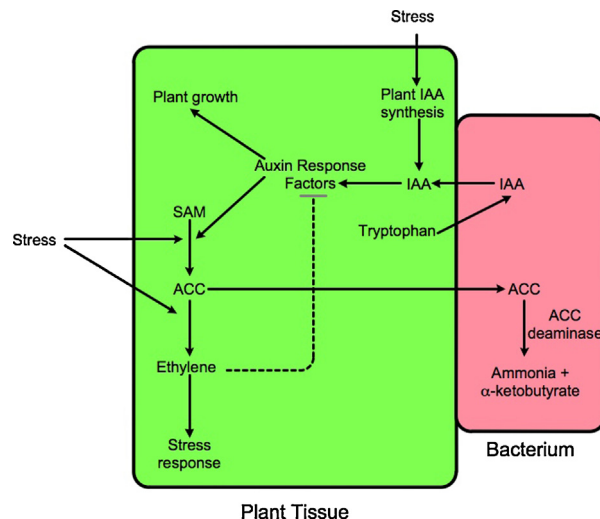


Fig. 3. A schematic model of how plant growth-promoting bacteria that both produce ACC deaminase and synthesize IAA may facilitate plant growth. The only enzyme shown in this scheme is ACC deaminase. SAM is converted to ACC by the enzyme ACC synthase; ACC is converted to ethylene by ACC oxidase. IAA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

The question arises, how does bacterial ACC deaminase selectively lower deleterious ethylene levels (the second ethylene peak) without affecting the small first peak of ethylene that is thought to activate plant defense responses. In this regard, ACC deaminase is generally present in bacteria at a relatively low level until it is induced, and the induction of enzyme activity is a rather slow and complex process (see Section 4.3). Immediately following an abiotic or biotic stress, the pool of ACC in the plant is low as is the level of ACC deaminase in the associated bacterium. Stress induces the induction of ACC oxidase (Fig. 3) in the plant so that there is an increased flux through ACC oxidase resulting in the first (small) peak of ethylene that in turn induces the transcription of protective/defensive genes in the plant. At the same time, bacterial ACC deaminase is induced by the increasing amounts of ACC that ensue from the induction of ACC synthase in the plant so that the magnitude of the second, deleterious, ethylene peak is decreased significantly (typically by 50–90%). Because ACC oxidase has a greater affinity for ACC than does ACC deaminase, when ACC deaminase-producing bacteria are present, plant ethylene levels are dependent upon the ratio of ACC oxidase to ACC deaminase (Glick et al. 1998). That is, to effectively reduce plant ethylene levels, ACC deaminase must function before any significant amount of ACC oxidase is induced.

A naive view of the interaction of IAA-producing bacteria with plants might posit that since IAA activates the transcription of ACC synthase, these bacteria should all ultimately result in the production of relatively high concentrations of ACC and subsequently inhibitory levels of ethylene. Thus, in the absence of some other mechanism, IAA-producing bacteria might all be expected to ultimately be inhibitory to plant growth. However, this is in fact not the case because as plant ethylene levels increase, the ethylene that is produced feedback inhibits IAA signal transduction thereby limiting the extent that IAA can activate ACC synthase transcription (Burg and Burg 1966; Czarny et al. 2007; Glick et al. 2007; Morgan and Gausman 1966; Pierik et al. 2006; Prayitno et al. 2006; Stearns et al. 2012). With plant growth-promoting bacteria that both secrete IAA and synthesize ACC deaminase, plant ethylene levels do not become elevated to the same extent as when plants interact with bacteria that secrete IAA but do not synthesize ACC

deaminase. In the presence of ACC deaminase, there is much less ethylene and subsequent ethylene feedback inhibition of IAA signal transduction so that the bacterial IAA can continue to both promote plant growth and increase ACC synthase transcription. However in this case, a large portion of the additional ACC that is synthesized is cleaved by the bacterial ACC deaminase. The net result of this cross-talk between IAA and ACC deaminase is that by lowering plant ethylene levels, ACC deaminase facilitates the stimulation of plant growth by IAA (Fig. 3).

4.2. Enzymology and biochemical properties

The enzyme ACC deaminase was first purified to homogeneity from *Pseudomonas* sp. strain ACP (Honma and Shimomura 1978), was subsequently partially purified from *Pseudomonas chloroaphis* 6G5 (Klee et al. 1991) and *Pseudomonas putida* GR12-2 (Jacobson et al. 1994) and then purified to homogeneity from *P. putida* UW4 (Hontzeas et al. 2004). Most of the early work in elaborating the biochemical properties of ACC deaminase is the result of the pioneering studies carried out by Honma and his co-workers (Honma and Shimomura 1978; Honma 1983, 1985, 1993; Honma et al. 1993a,b; Minami et al. 1998; Jia et al. 1999; Ose et al. 2003; Sheehy et al. 1991; Walsh et al. 1981). In addition, a few other biochemical studies of ACC deaminase have been reported (Hontzeas et al. 2004; Jacobson et al. 1994).

The various biochemical studies of ACC deaminase indicate that (1) despite the fact the substrate ACC is found largely within plant tissues, the enzyme is not secreted by bacteria but is typically found within the cytoplasm; (2) in all instances examined, the *K_m* value of the enzyme for ACC indicated that the enzyme did not have a particularly high affinity for ACC, ranging from around 1.5 to approximately 15 mM; and (3) the enzyme temperature and pH optima are approximately 25–30 °C and 8.0–8.5, respectively. The affinity of the enzyme for the competitive inhibitors L-alanine and L-serine is also highest at pH 8.5.

Where it has been examined, ACC deaminase activity is present at a low, basal, level but it can be induced in both *P. sp.* strain ACP and *P. putida* GR12-2 by ACC, at levels as low as 100 nM (Honma and Shimomura 1978; Jacobson et al. 1994). Induction of ACC deaminase activity typically includes growing bacterial strains on rich media and then switching the bacteria to minimal medium containing ACC as the sole nitrogen source.

The level of ACC deaminase activity that is observed when strain *P. putida* GR12-2 is grown on minimal medium plus ammonium sulfate represents a basal level of activity of $\leq 5\%$ of the total activity that is measured in extracts grown on minimal medium containing ACC (as a nitrogen source) instead of ammonium sulfate. A few other amino acids including L-alanine, DL-alanine and DL-valine can also induce ACC deaminase enzyme activity, albeit to a limited extent, while α -aminoisobutyric acid can induce activity to nearly the same level that is found with ACC.

ACC deaminase is a member of a rather large group of enzymes that utilize pyridoxal 5'-phosphate (vitamin B6) as an essential co-factor for enzymatic activity (Christen and Metzler 1985). Moreover, Jansonius (1998) who classified pyridoxal 5'-phosphate enzymes based on their three dimensional structure into different folding types considered ACC deaminase to be a member of the tryptophan synthase family. The pyridoxal 5'-phosphate is tightly bound to ACC deaminase in the amount of approximately one mol per trimeric subunit.

4.3. Regulation

Many of the ACC deaminase structural genes (*acdS*) that have been characterized also have a leucine responsive regulatory protein (LRP) gene (*acdR*) located around 50 to a few hundred base

pairs upstream of the start of the *acdS* gene and transcribed in a direction opposite to *acdS* (Blaha et al. 2006; Duan et al. 2009; Grichko and Glick 2000; Li and Glick 2001; Ma et al. 2003a; Prigent-Combaret et al. 2008).

A detailed model, described below, of transcriptional regulation of *acdS* has been developed (Glick et al. 2007) and may be summarized as follows. In the absence of ACC, the *acdR* gene is transcribed until an excess amount of LRP builds up and then binds to an LRP box, part of the DNA sequence immediately upstream of the *acdR* gene and overlapping with the promoter for this gene, preventing further transcription of *acdR*. In the presence of ACC, LRP forms an active octamer that binds to a complex of ACC and another protein, AcdB (Cheng et al. 2008). This tripartite complex activates transcription of *acdS* by binding to its promoter region. Upon synthesis of ACC deaminase, ACC is cleaved to form ammonia and α -ketobutyrate (a precursor of branched chain amino acids such as leucine), and when a cell accumulates a sufficiently high level of leucine, this amino acid binds to the LRP octamer causing it to dissociate into inactive dimers shutting down further transcription of *acdS*. This mode of regulation ensures that ACC deaminase is synthesized only when it is needed. In addition to *acdR*, transcription from some *acdS* promoter regions may be modulated under aerobic conditions by CRP, cyclic AMP receptor protein, and under anaerobic conditions by FNR, fumarate–nitrate reduction regulatory protein (Duan et al. 2013; Grichko and Glick 2000; Li and Glick 2001).

4.4. Nodulation

Rhizobial strains that express ACC deaminase are up to 40% more efficient at forming nitrogen-fixing nodules than strains that lack this activity (Ma et al. 2003a, 2004). However, strains of rhizobia that express ACC deaminase have only a low level of enzyme activity compared with free-living plant growth-promoting bacteria, i.e. typically around 2–10%. Thus, free-living bacteria bind relatively non-specifically to plant tissues (mainly roots) and have a high level of ACC deaminase activity that can protect plants from different abiotic and biotic stresses by lowering ethylene levels throughout the plant. On the other hand, (symbiotic) rhizobia that generally bind tightly only to the roots of specific plants, have a low level of enzyme activity which facilitates nodulation by locally lowering ethylene levels. It is not known whether the large differences in enzyme activity that are observed when comparing free-living bacteria with rhizobia is a consequence of differences in the amount of enzyme synthesized by one type of bacteria versus the other or of differences in the specific catalytic activity of the enzymes from the different types of bacteria.

In addition to the more common mode of *acdS* transcriptional regulation described above, *acdS* genes from various strains of *Mesorhizobium loti* have been found to be under the transcriptional control of the *nifA* promoter that is normally responsible for activating the transcription of *nif*, nitrogen fixation genes (Kaneko et al. 2000; Nascimento et al. 2012a; Nukui et al. 2006; Sullivan et al. 2002; Uchiumi et al. 2004). The consequence of this somewhat unusual mode of regulation is that, unlike ACC deaminases from other rhizobia, the *M. loti* ACC deaminase does not facilitate nodulation but, rather, is expressed within nodules. The result of this unusual regulation is, in *M. loti*, ACC deaminase may act to decrease the rate of nodule senescence. This is particularly important because of the fact that nitrogen fixation, a process that utilizes a very high level of energy in the form of ATP, could (perhaps inadvertently) activate stress ethylene synthesis resulting in premature nodule senescence. On the other hand, a longer-lived nodule might as a consequence fix an increased amount of nitrogen.

4.5. Genes

Computer searches of databases of sequenced bacterial and fungal genomes typically annotate a relatively large number of genes as *acdS*. However, the precise identity of these putative genes should be approached with some caution. For example, only a small fraction of putative *acdS* genes have been shown to encode active enzyme. In addition, some genes that were tentatively identified as encoding ACC deaminase have subsequently been shown to encode D-cysteine desulphydrase activity (Riemenschneider et al. 2005). Moreover, it has been shown that only two amino acid residues from within the active site of ACC deaminase and D-cysteine desulphydrase may control which of the two reactions is carried out (Todorovic and Glick 2008). Thus, using directed mutagenesis it was possible to convert a gene encoding D-cysteine desulphydrase into a gene encoding ACC deaminase, and vice versa (Todorovic and Glick 2008). Notwithstanding the above mentioned constraints, any microorganism that can grow on minimal medium using ACC as the sole nitrogen source (Glick et al. 1995) most likely contains an actively expressed ACC deaminase gene.

Based upon a phylogenetic analysis of a limited number of *acdS* genes and their comparison to the phylogeny of 16S rRNA genes from the same bacteria, Hontzeas et al. (2005) proposed that some ACC deaminase genes have evolved through horizontal gene transfer (HGT). Based on the same criteria, Blaha et al. (2006) suggested that ACC deaminase genes in Proteobacteria were extensively subjected to horizontal transfers. The phylogeny in Proteobacteria of *acdR* has also been investigated (Prigent-Combaret et al. 2008). The latter study suggested that *acdR*, like *acdS*, may have evolved through horizontal gene transfer (Prigent-Combaret et al. 2008). This conclusion notwithstanding, these authors suggest that the evolution of *acdS* and *acdR* genes is not necessarily coupled. More recently, Nascimento et al. (2012a) suggested that in many *Mesorhizobium* spp. *acdS* genes appear to be horizontally transferred between strains by the exchange of the symbiosis island. This suggestion is based on observing the presence of the *acdS* gene in the symbiosis islands of *M. loti* R7A, *M. sp.* MAFF303099, *Mesorhizobium ciceri* bv. *biserrulae* WSM1271, *Mesorhizobium australicum* WSM2073T and *Mesorhizobium opportunistum* WSM2075T, close to the nitrogen fixation gene cluster. In agreement with this suggestion, it has been observed that “genes required for pathogenic or symbiotic interactions with eukaryotic hosts are often part of the accessory gene pool of the microbe, acquired by horizontal transfer. They may be clustered on plasmids or on the chromosome as genomic islands.” (Finan 2002).

While most phylogenetic studies of *acdS* and *acdR* genes have been focused on Proteobacteria, other studies have demonstrated the presence of ACC deaminase activity in a wide range of bacteria including (but likely not limited to) Actinobacteria (Hontzeas et al. 2005; Siddiquee et al. 2010), Firmicutes (Ghosh et al. 2003; Siddiquee et al. 2010; Timmusk et al. 2011), and Bacteroidetes (Maimaiti et al. 2007; Marques et al. 2010). Thus, the currently extant view of *acdS* and *acdR* gene phylogeny and evolution is somewhat incomplete.

4.6. Environmental distribution

ACC deaminase-containing bacteria are relatively common in soil, having been found in a wide range of environments all over the world. In fact, it has been suggested that, “The ability of *P. putida* GR12-2 to hydrolyze ACC may, in the soil, provide it with a competitive advantage over other rhizosphere microorganisms because it can use ACC as a nitrogen source.” (Jacobson et al. 1994). This argument continues, suggesting that ACC may act as a unique/novel source of nitrogen for some soil bacteria. While, a detailed understanding of the competitiveness of ACC deaminase-containing bacteria is likely considerably more complex than this

early work would suggest, there are several studies that are consistent with the possession of ACC deaminase activity facilitating bacterial competitiveness or persistence in the environment.

An early effort to search for ACC deaminase amongst different rhizobial strains indicated that five out of the thirteen strains tested displayed enzyme activity while seven out of the thirteen strains appeared positive for the *acdS* gene (Ma et al. 2003b). For one of the two strains positive for the *acdS* gene but negative for enzyme activity, we now know that this *Mesorhizobium* strain only expresses this activity when the bacterium is present within a root nodule.

When a collection of 233 newly isolated rhizobial strains from soil samples collected from 30 different sites across Southern Saskatchewan (in an area ~350 km by ~320 km), Canada was assayed for ACC deaminase activity, 27 strains (nearly 12%) displayed the enzyme activity (Duan et al. 2009). Similarly, upon examination of *acdS* genes from *Mesorhizobium* strains, including a collection of chickpea-nodulating mesorhizobia isolated from various sites all over Portugal, ACC deaminase genes were detected in 10 of 12 *Mesorhizobium* type strains and 18 of 18 chickpea *Mesorhizobium* isolates (Nascimento et al. 2012a).

In a recent experiment, Timmusk et al. (2011) assessed the prevalence of ACC deaminase among bacterial strains isolated from the rhizosphere of wild barley (*Hordeum spontaneum*) growing in Northern Israel. Based on an assessment of soil samples from a region termed ‘Evolution Canyon’, these workers determined that approximately 50% of the bacteria isolated from the South Facing Slope contained ACC deaminase while only approximately 4% of the bacteria from the North Facing Slope contained this enzyme. The South Facing Slope was sparsely vegetated as a result of the harsh conditions on this side of the canyon (including excessive sunlight and frequent drought) while the North Facing Slope featured much more luxuriant plant growth and the apparent absence of drought. These results are consistent with bacterial ACC deaminase being selected for by the plants growing on the more stressful conditions on the South Facing Slope, protecting plants and facilitating their survival; without plant growth and concomitant root exudation the bacteria would not proliferate. On the other hand, given the much better growth conditions on the North Facing Slope, there is apparently much less selective pressure for bacteria to retain ACC deaminase.

5. Overcoming environmental stress

Bacteria that express ACC deaminase can lower the impact on plants of a range of different stresses (Fig. 4). For example, both endogenous and exogenous ACC deaminase genes increase the symbiotic performance of many rhizobial strains (Ma et al. 2003a, 2004; Nascimento et al. 2012b,c). Importantly, using ACC deaminase-producing bacteria in association with plants subjected to a wide range of different kinds of biotic and abiotic stresses, in all instances tested, results in enhanced plant tolerance to the stresses.

5.1. Flooding

Flooding is a common biotic stress that can deleteriously affect the growth of many different plants. As a consequence of flooding, plant roots typically become hypoxic or oxygen limited. This leads to a plant stress response including the synthesis of increased amounts of the enzyme ACC synthase as well as other stress proteins (Li et al. 2012). The stressed plant subsequently synthesizes additional ACC in its roots. However, since the newly synthesized ACC cannot be converted to ethylene in the roots, as ethylene synthesis requires oxygen, the ACC is transported to the shoots where there is an aerobic environment and the ACC can be converted to ethylene (Bradford and Yang 1980; Else and Jackson 1988). The

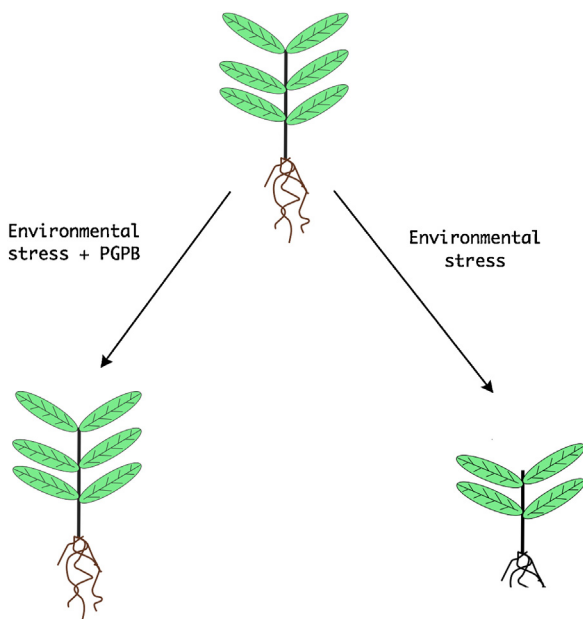


Fig. 4. A schematic view of the inhibition of plant growth as a consequence of stress induced ethylene synthesis and the ability of ACC deaminase-containing PGPB to decrease the stress ethylene level and thereby limit the growth inhibition.

production of ethylene by flooded plants results in epinasty (wilting), leaf chlorosis, necrosis and reduced biomass yield. However, it is possible to protect plants from a significant portion of the damage caused by flooding by treating them with ACC deaminase-producing plant growth-promoting bacteria (Barnawal et al. 2012; Grichko and Glick 2001; Li et al. 2013).

5.2. Drought

In their pioneering work, Mayak et al. (2004a) argued that plant growth-promoting bacteria endemic to sites where rainfall is limited are more likely to be able to protect plants against growth inhibition from drought than are similar bacteria from sites where water is more abundant. Following this reasoning, Mayak et al. (2004a) isolated the ACC deaminase-containing bacterium *Achromobacter piechaudii* ARV8 from a rhizosphere soil sample from a *Lycium shawii* plant growing in a dry riverbed in the Arava region of the southern Negev desert in Israel. When three-week-old tomato plant seedlings were treated with *A. piechaudii* ARV8, not watered for one week and then re-watered every other day for a week, they had four times the biomass and one quarter the level of ethylene of plants not treated with this bacterium (Mayak et al. 2004a). Based on the results of these experiments, several other researchers have subsequently demonstrated the efficacy of protecting a range of different plants against loss of biomass from drought stress using ACC deaminase-containing plant growth-promoting bacteria (Arshad et al. 2008; Belimov et al. 2009; Shakir et al. 2012; Zahir et al. 2008).

5.3. Salt

Salinity is an enormous and growing problem for worldwide agriculture. Not only is salt inhibitory to the growth of a large number of different plants (Cuartero and Fernandez-Munoz 1999), the amount of salt-affected land represents at least 20% of the world's cultivated area (Flowers 2004) and this number is continuously increasing as a direct consequence of irrigation. The physiological consequences of soil salinity are inhibition of plant growth and development including (but not limited to) inhibition of seed

germination, seedling growth and vigor, flowering and fruit set (Sairam and Tyagi 2004).

Since saline-induced stress in plants is at least partially the result of the plant's production of stress ethylene, it was reasoned that lowering ethylene levels using ACC deaminase-containing plant growth-promoting bacteria might afford some protection against this stress (Mayak et al. 2004b). In this regard, Mayak et al. (2004b) decided to test the plant growth-promoting bacterium *A. piechaudii* ARV8, previously successfully employed to facilitate plant growth under drought conditions, for its ability to promote plant growth in the presence of salt. It is worth noting here that many of the early effects of salt stress have been attributed to the water/drought stress that the salt causes in plants. Not only was this approach successful, it served as a model for numerous laboratories from around the world to employ ACC deaminase-containing plant growth-promoting bacteria to promote the growth of plants, both in the lab and in the field, in the presence of otherwise inhibitory levels of salt (Amhad et al. 2011; Bal et al. 2013; Cheng et al. 2007, 2012; Chookietwattana and Maneewan 2012; Gamalero et al. 2010; Jalili et al. 2009; Karthikeyan et al. 2012; Nadeem et al. 2007, 2010; Ramadoss et al. 2013; Sadrnia et al. 2011; Saravanakumar and Samiyappan 2006; Siddikee et al. 2010, 2011; Yue et al. 2007).

5.4. Flower wilting

It is well known that ethylene is a key signal in initiation of the senescence of flowers of most plants. However, not all flowers are equally sensitive to ethylene (Woltering and van Doorn 1988). Nevertheless, many cut flowers (e.g. carnations and lilies) are routinely treated with the chemical ethylene inhibitor, silver thiosulfate, prior to their sale (Reid and Wu 1991). However, high silver thiosulfate concentration is potentially phytotoxic and environmentally hazardous (Abeles et al. 1992). On the other hand, the use of ACC deaminase-containing plant growth promoting rhizobacteria (that naturally limit ethylene production) to treat cut flowers might be an environmentally friendly alternative to the use of silver thiosulfate (Ali et al. 2012; Nayani et al. 1998).

In the first attempts to decrease flower senescence using ACC deaminase-containing plant growth promoting rhizobacteria, the addition of these bacteria to the stems of cut flowers (carnations) did not have any effect on the rate of flower senescence (Nayani et al. 1998). To see any effect of the added bacteria, it was necessary to carefully dissect petals from carnation flowers before treating the petals with the bacteria, a treatment that successfully delayed petal senescence by approximately 5–6 days. To show that the observed effect was not limited to carnation, the senescence of Maltese Cross (*Lychnis*) flowers treated ACC deaminase-containing plant growth promoting rhizobacteria was compared with the senescence of untreated flower petals. In this instance, flower petal senescence was delayed by 2–3 days. Notwithstanding this proof of principle, with ACC deaminase-containing plant growth promoting rhizobacteria mimicking the action of chemical inhibitors of ethylene, to be used practically it is essential that the bacteria be taken up by the cut flowers. In fact, when endophytic ACC deaminase-containing plant growth promoting rhizobacteria (that normally colonize the interior tissues of the plant) instead of root binding ACC deaminase-containing plant growth promoting rhizobacteria were used, the predicted behavior was observed (Ali et al. 2012). That is, the endophytic bacteria were taken up through the stems of the cut flowers (in this case mini-carnations) and decreased flower ethylene levels so that senescence was delayed by 2–3 days (Ali et al. 2012). Based on these endophyte experiments, it is reasonable to expect that the use of ACC deaminase-containing plant growth promoting rhizobacteria might eventually completely replace the use of chemical ethylene inhibitors in commercial efforts to prolong the lifetime of cut flowers.

5.5. Metals

The use of plants to clean up heavy metal polluted soils, i.e., phytoremediation (Salt et al. 1995), is an environmental-friendly and potentially cost-effective alternative to traditional soil remediation approaches such as soil removal or chemical and physical extraction. Although phytoremediation is a clean technology, only a small number of plant species can naturally tolerate/accumulate heavy metals and, many of the plants that are most effective at removing metals from the soil are characterized by their small size and slow growth, thereby limiting their practical use in this technology (Khan et al. 2000). To be considered effective for soil remediation, plants must be tolerant to one or more pollutants, highly competitive, fast growing and produce a high biomass.

Plants interact with heavy metals from the environment in various ways including phytostabilization, stabilization of the metal in soil by reducing its bioavailability; phytoextraction, extraction, transport and accumulation of metals in plant tissues; and phytovolatilization, transformation of metals into volatile forms (Pilon-Smits 2005). By far, the preferred way of dealing with metals in the environment is phytoextraction, a process that may be facilitated by plant growth-promoting bacteria living in association with plant roots (Glick 2010). These bacteria can improve plant growth and health, enhance root development, or increase plant tolerance to metal stress. In turn, larger and healthier plants are better able to phytoremediate metal contaminants. Although the use of plant growth-promoting bacteria as adjuncts as part of a metal phytoremediation strategy can often significantly increase the growth of plants in the presence of high levels of metals, the bacteria typically do little or nothing to increase metal bioavailability.

The first report of the use of ACC deaminase-containing plant growth-promoting bacterium in metal phytoremediation indicated that a nickel resistant bacterium could decrease the toxicity of nickel to canola plants (Burd et al. 1998). Since that time there have been a large number of reports of facilitation of metal phytoextraction through the addition of plant growth-promoting bacteria (summarized in Glick 2012). These studies encompass a wide range of different plants, metals, soils and bacteria. The bacteria that have been used are typically first selected for resistance to the target toxic metal(s) and then either selected or tested for the presence of ACC deaminase and the ability to synthesize IAA and siderophores. The data from these studies is consistent with the involvement of IAA, siderophores and ACC deaminase where (i) IAA promotes plant growth per se (Patten and Glick 2002), (ii) ACC deaminase prevents stress ethylene from becoming overly inhibitory of plant growth (Glick et al. 1998) and (iii) siderophores help the plants to acquire sufficient iron in the presence of overwhelming amounts of other (potentially competing) metals (Burd et al. 2000).

In addition, some other bacterial traits may facilitate metal phytoremediation. For example, bacteria that facilitate phytoremediation sometimes have an active phosphate solubilization system that some workers believe plays a role in assisting metal uptake. In one study where a bacterial strain aided phytoremediation, the bacterium produced biosurfactants, possibly helping to make the metals more bioavailable (Sheng et al. 2008). Notwithstanding the increased amount of phytoextraction of metals that is generally facilitated by plant growth-promoting bacteria, at the present time, the limited bioavailability of many metals in the environment (Wenzel 2009) may prevent this strategy from being used practically.

5.6. Organics

The presence of organic contaminants in the soil acts as an abiotic stress for most plants, resulting in the plant synthesizing increased levels of ethylene (Abeles et al. 1992). Therefore,

the growth of plants exposed to organic contaminants in the soil should be facilitated by the presence of ACC deaminase-containing plant growth-promoting bacteria. In fact, this strategy of bacterially-assisted phytoremediation appears to be particularly effective for removal and/or degradation of organic contaminants from impacted soils, both in the lab (Huang et al. 2004; Reed and Glick 2005) and in the field (Gurska et al. 2009).

A detailed examination of the data from experiments directed toward phytoremediation of organic contaminants indicates that a number of conditions appear to facilitate this process. These include bacteria that can: (i) promote plant growth (typically through the provision of IAA); (ii) degrade soil contaminants (often by harboring specific biodegradative pathways); (iii) lower plant ethylene levels (based on the activity of ACC deaminase); and (iv) colonize the tissues inside of plants (by being bacterial endophytes).

5.7. Pathogens

In response to infection with various pathogens, typically fungi, bacteria or nematodes, plants often produce stress ethylene (Abeles et al. 1992). Following pathogen infection, many of the symptoms that can be observed in an infected plant arise as a direct consequence of the stress imposed by the pathogen (Van Loon 1984). That is, a significant portion of the damage to plants infected with various pathogens occurs as a result of the response of the plant to the increased levels of stress ethylene. In this regard, it has been shown that exogenous ethylene can increase the severity of pathogen infections while chemical inhibitors of ethylene synthesis decrease the severity of those infections. In addition, pretreatment of plants with ACC deaminase-containing plant growth-promoting bacteria can provide significant protection to plants against some of the ethylene caused damage from pathogen infection. This has been shown to be the case for bacteria (Hao et al. 2007, 2011; Toklikishvili et al. 2010; Wang et al. 2000), fungi (Amutharaj et al. 2012; Fürnkranz et al. 2009; Husen et al. 2011; Nascimento et al. 2012c; Rani et al. 2012; Wang et al. 2000) and nematodes (Nascimento et al. 2013).

In addition to protecting plants from stress damage using ACC deaminase-containing plant growth-promoting bacteria, there are several reports wherein transgenic plants that express ACC deaminase were created to the same end. These plants were subsequently shown to be more resistant to growth inhibition by fungal pathogens (Lund et al. 1998; Robison et al. 2001a,b), salt (Sergeeva et al. 2006), arsenate (Nie et al. 2002), and nickel and other metals (Grichko et al. 2000; Stearns et al. 2005).

6. Future prospects

We live in a finite world that can support only a limited number of people. The resources that are required to feed, clothe and house everyone will eventually run out unless we begin now to address the many problems that in the near future may dictate our choices for us. Unless new approaches and technologies are soon developed, it will not be possible to feed the world's growing population in approximately the next 50 years. To obviate this risk, a range of new innovative technologies needs to be developed and implemented. Among, these technologies are the widespread use of plant growth-promoting bacteria, first in addition to, and ultimately instead of the agricultural chemicals that are currently employed.

In the past 30–40 years, researchers have begun to develop a much more in depth and detailed understanding of precisely how plant growth-promoting bacteria facilitate the growth of plants so that the use of these organisms is a technology whose time has come. While a lot more work, both basic and applied, remains to be done, plant growth-promoting bacteria are already being

used successfully, albeit on a small scale, in several countries. If scientists, and the agencies that fund them, direct their efforts toward the perceived problems and bottlenecks of this technology, there is every reason to expect that these difficulties can readily be addressed so that agricultural practice worldwide can finally begin to make more efficacious and serious use of this sustainable approach to agriculture. We are approaching the juncture of a major paradigm shift in agriculture. We need to embrace this change and step into the future.

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