The Role of ACC Deaminase Producing PGPR in Sustainable Agriculture

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Abstract The plant rhizosphere is a multidimensional and dynamic ecological environment of complicated microbe–plant interactions for harnessing essential macro and micronutrients from a limited nutrient pool. Certain plant growth

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promoting rhizobacteria (PGPR) contain a vital enzyme, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (EC 4.1.99.4), which regulates ethylene production by metabolizing ACC (an intermediate precursor of ethylene biosynthesis in higher plants) into α-ketobutyrate and ammonia. The microbial enzyme 1-aminocyclopropane-1-carboxylate deaminase cleaves ACC irreversibly, this being the immediate precursor of ethylene in plants. ACC deaminase-expressing PGPR protect plants against the growth inhibition that might otherwise result following flooding, extremes of temperature, the presence of organic and inorganic toxicants, phytopathogens, drought or high salt concentrations. Organisms containing ACC deaminase genes have been reported to be useful in promotion of early root development from either seeds or cuttings, increasing the life of horticultural flowers, protecting plants against a wide range of environmental stresses, facilitating the production of volatile organic compounds responsible for aroma formation and phytoremediation of contaminated soils.

1 Introduction

Certain plant growth promoting rhizobacteria (PGPR) contain a vital enzyme, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (EC 4.1.99.4), which regulates ethylene production by metabolizing ACC (an intermediate precursor of ethylene biosynthesis in higher plants) into α-ketobutyrate and ammonia. This pyridoxal phosphate (PLP) enzyme was first isolated in 1978 from *Pseudomonas* sp. strain ACP and from the yeast *Hansenula satrunus* (Honma and Shimomura 1978) since then, it has been detected in fungi and in a number of other bacteria. When ACC deaminase-containing plant growth-promoting bacteria (PGPB) are bound to a plant, they act as a sink for ACC ensuring that plant ethylene levels do not become elevated to the point.

Conceptually, PGPR can have an impact on plant growth and development in two different ways: indirectly or directly. The indirect promotion of plant growth occurs when bacteria decrease or prevent some of the deleterious effects of a phytopathogenic organism by one or more mechanisms. On the other hand, the direct promotion of plant growth by PGPR generally entails providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment (Glick 1995; Glick et al. 1999). Rhizosphere bacteria multiply to high densities on plant root surfaces where root exudates and root cell lysates provide ample nutrients. Sometimes, they exceed 100 times to those densities found in the bulk soil (Campbell and Greaves 1990). Certain strains of these plant-associated bacteria stimulate plant growth in multiple ways: (1) they may fix atmospheric nitrogen, (2) reduce toxic compounds, (3) synthesize phytohormones and Siderophores, or (4) suppress pathogenic organisms (Bloemberg and Lugtenberg 2001). Research on the "biocontrol" activity of rhizobacteria has seen considerable progress in recent years. Disease suppression of soilborne pathogens includes competition for nutrients and production of antimicrobial compounds or lytic

enzymes for fungal cell walls or nematode structures (Persello-Cartieaux 2003). By contrast, systemic resistance can also be induced by rhizosphere-colonizing *Pseudomonas* and *Bacillus* species where the inducing bacteria and the challenging pathogen remained spatially separated excluding direct interactions (Van Loon et al. 1998; Ryu et al. 2004).

Etiolated pea seedlings are very sensitive to ethylene. The most widely renowned example of the effect of ethylene on plant growth is the classical "triple" response in etiolated dicot seedlings in the presence of ethylene. This effect consists of three distinct morphological changes in the shape of seedlings, inhibition of stem elongation, increase in stem diameter and horizontal growth (Akhtar et al. 2005; Khalid et al. 2006). This "triple" response reaction of etiolated seedlings has been a reliable bioassay for ethylene action (Guzman and Ecker 1990). Shaharoona et al. (2007) observed the effect of inoculation with ACC utilizing and ethylene-producing rhizobacteria and compared through highly ethylene specific classical "triple" response bioassay. In this study, the effect of inoculation with rhizobacteria having different ACC-deaminase activities on extenuating the classical "triple" response in etiolated pea seedlings was investigated.

ACC deaminase-containing PGPB up-regulate genes involved with plant growth and protein production while down-regulating plant genes involved with ethylene stress and defence signaling pathways (Hontzeas et al. 2004a). The ACC deaminase-containing PGPB, in part, alleviate the need for the plant to actively defend itself against various environmental stresses (Hontzeas et al. 2004b; Van Loon and Glick 2004). The crystal structure has been determined for the yeast (Minami et al. 1998), and recently for the bacteria (Karthikeyan et al. 2004) ACC deaminase enzymes; the biochemical and thermodynamic properties of the ACC deaminase from *Pseudomonas putida* UW4 have been measured (Hontzeas et al. 2004b).

ACC deaminase from *Pseudomonas* sp. ACP, *P. putida, P. fluorescens* (Glick 1995), *Enterobacter cloacae* CAL2 and UW4 (Shah et al. 1998), *Kluyvera ascorbata* SUD165 (Burd et al. 1998), *Hansenula saturnus* (Honma and Shimomura 1978), and *Penicillium citrinum* (Jia et al. 2006) have been reported.

This enzyme facilitates plant growth as a consequence of the fact that it sequesters and cleaves plant produced ACC, thereby lowering the level of ethylene in the plant. In turn, decreased ethylene levels allow the plant to be more resistant to a wide variety of environmental stresses, all of which induce the plant to increase its endogenous level of ethylene; stress ethylene exacerbates the effects of various environmental stresses. The ACC deaminase-containing soil bacteria decrease a significant portion of the physiological damage to plants following environmental stresses including phytopathogen infection, exposure to extremes of temperature, high salt, flooding, drought, exposure to metals and organic contaminants, and insect predation. For many plants a burst of ethylene is required to break seed dormancy but, following germination, a sustained high level of ethylene can be inhibitory to root elongation. PGPB that contain the enzyme ACC deaminase, when bound to a plant root or to the seed coat of a developing seedling, may act as a mechanism for insuring that the ethylene level within the plant's tissues does not become elevated to the point where root (or shoot) growth is impaired. By facilitating the formation of

longer roots and shoots, these bacteria may enhance the survival of some seedlings, especially during the first few days after the seeds are planted.

2 Ethylene Biosynthesis in Higher Plants

Ethylene, which is produced in almost all plants, mediates a range of plant responses and developmental step. Ethylene is involved in seed germination, tissue differentiation, formation of root and shoots primordial, root elongation, lateral bud formation, flowering initiation, anthocyanin synthesis, flower opening and senescence, fruit ripening and degreening, production of aroma, leaf and fruit abscission and response of plant to biotic and abiotic stresses. (Saraf and Tank 2005). Ethylene is a potent plant growth regulator that affects diverse developmental processes, including fruit ripening, senescence, and stress responses (McKeon and Yang 1987; Reid 1987). Chemical inhibitors of ethylene synthesis or action completely block ripening in fruits and senescence in flowers of many plant species.

At a molecular level, ethylene is known to induce expression of a number of genes involved in ripening (Lincoln and Fischer 1988) and pathogen response (Ecker and Davis 1987). In some instances, ethylene is stimulatory while in others it is inhibitory.

When plants are exposed to conditions that threaten their ability to survive, the same mechanism that produces ethylene for normal development instead produces "stress ethylene" which may be defined as an acceleration of ethylene biosynthesis associated with biological and environmental stresses, and pathogen attack (Abeles et al. 1992; Hyodo 1991; VanLoon 1984). Ethylene is synthesized from S-adenosyl L-methionine (AdoMet) by way of the intermediate ACC (McKeon and Yang 1987).

While working on the ethylene biosynthesis pathway, Adams and Yang (1979) found that when ACC was applied to various plant organs, an increase in ethylene production was obtained. From their observations, ACC, as a key intermediate that linked the methionine cycle and ethylene biosynthesis, was deemed to be the direct precursor of ethylene production with its level directly controlling ethylene synthesis in plants (Fig. 1).

Ethylene biosynthesis consists of three steps (1) L-methionine is converted to AdoMet, a reaction catalyzed by methionine S-adenosyl transferase. AdoMet is also utilized in other cellular reactions such as ethylation and polyamine synthesis, (2) The conversion of AdoMet to ACC which is catalyzed by ACC synthase. The ACC synthase step is considered to be the rate-limiting step in the pathway (3) ACC is further metabolized to ethylene, carbon dioxide and cyanide by ACC oxidase.

Since all plants respond differently to stress, it has been difficult to detail the functioning of stress ethylene. Increased ethylene levels in plants exposed to various types of stress including chilling, heat, wounding, pathogen infection, salt, metals and nutritional stress, with increased damage as the result has been documented. Stress ethylene, though its role is unclear, is deleterious to plants in many instances (Saravanakumar and Samiyappan 2007).

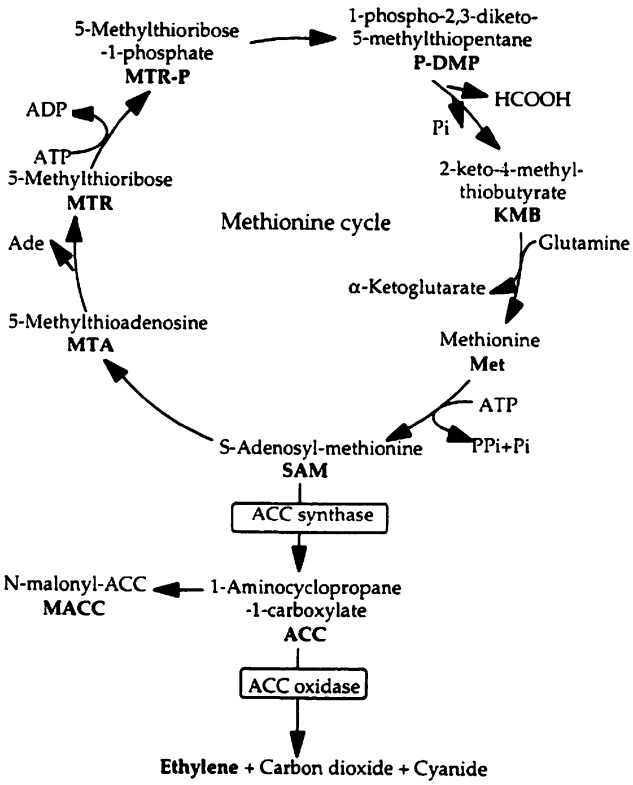

Fig. 1 Pathway of ethylene biosynthesis from the methionine cycle in higher plants. Modified figure adapted from the source reference Li (1999)

3 Characteristics of ACC Deaminase Enzyme

Enzymatic activity of ACC deaminase is assayed by monitoring the production of either ammonia or α-ketobutyrate, the products of ACC hydrolysis. ACC deaminase has been found only in microorganisms, and there are no microorganisms that synthesize ethylene via ACC (Fukuda et al. 1993). ACC Deaminase is a multimeric enzyme (homodimeric or homotrimeric) with a subunit molecular mass of approximately 35-42 kDa. It is a sulfhydral enzyme in which one molecule of the essential cofactor PLP is tightly bound to each subunit. Interestingly, this enzyme is cytoplasmically localized so that the substrate ACC must be exuded by plant tissues and subsequently taken up by an ACC deaminase-containing microorganism before it is cleaved (Glick et al. 1998).

The enzyme–substrate relationship demonstrates Km values of ACC deaminase for ACC 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). Moreover ACC levels in plants are

typically in μM range, therefore in most plant tissues the ACC concentration will be dramatically below the Km of ACC deaminase for this substrate so that based on the Michaelis–Menton rate equation for enzyme catalyzed reaction a small increase in the ACC concentration will result in a parallel increase in the rate of ACC cleavage.

4 Crystal Structure of 1-Aminocyclopropane-1-Carboxylate Deaminase

PLP-dependent enzymes catalyze many important reactions that act upon amino acids, including transamination, decarboxylation, β,γ-replacement/elimination, and racemization. In all of these reactions (except in the case of the glycogen phosphorylase family), the two basic chemical properties of the PLP are conserved; it forms an external aldimine between its aldehyde group and the α-amino group of the substrates and withdraws electrons from the substrate by serving as an electron sink. As a PLP-dependent enzyme, the ACCD's ring opening reaction starts with a transformation reaction from an internal aldimine between the PLP and the enzyme to an external aldimine. These enzymes have been classified based on their three dimensional structure, into four folding types: (1) tryptophan synthase, (2) aspartate aminotransferase, (3) D-amino acid aminotransferase and (4) alanine racemase. In most of the PLP-dependent enzymes, the next step is the nucleophilic abstraction of the α-substituent, either an α-proton or a carboxylate group, to form an α-carbanionic intermediate. This reaction mechanism cannot be applied to ACCD because the substrate (ACC) does not contain α-hydrogen and the carboxyl group is retained in the product. Therefore, the ring-opening reaction of ACC must be initiated without obvious accessibility to an α-carbanionic intermediate, which is, for PLP-dependent enzymes, the common entry for catalysis. One proposed reaction mechanism is the nucleophilic addition to Cγ followed by the cleavage of the Cα–Cγ bond and β-proton abstraction. As PLP, acts as an electron sink, external aldimine is fairly electrophilic, and the nucleophilic addition to Cγ to rupture the cyclopropane ring of ACC is mechanistically feasible (Yao et al. 2000) (Fig. 2).

5 Mechanism of ACC Deaminase Action

A model is proposed to explain how ACC deaminase-containing PGPB can lower plant ethylene levels and in turn stimulate plant growth (Glick et al. 1998), especially under stress conditions. PGPB bind to the surface of either the seed or root of a developing plant in response to tryptophan and other small molecules in the seed or root exudates the PGPB synthesize and secrete the auxin, Indoleacetic acid (IAA), some of which is taken up by the plant. This IAA together with endogenous plant IAA can stimulate plant cell proliferation and elongation, or it can induce the activity

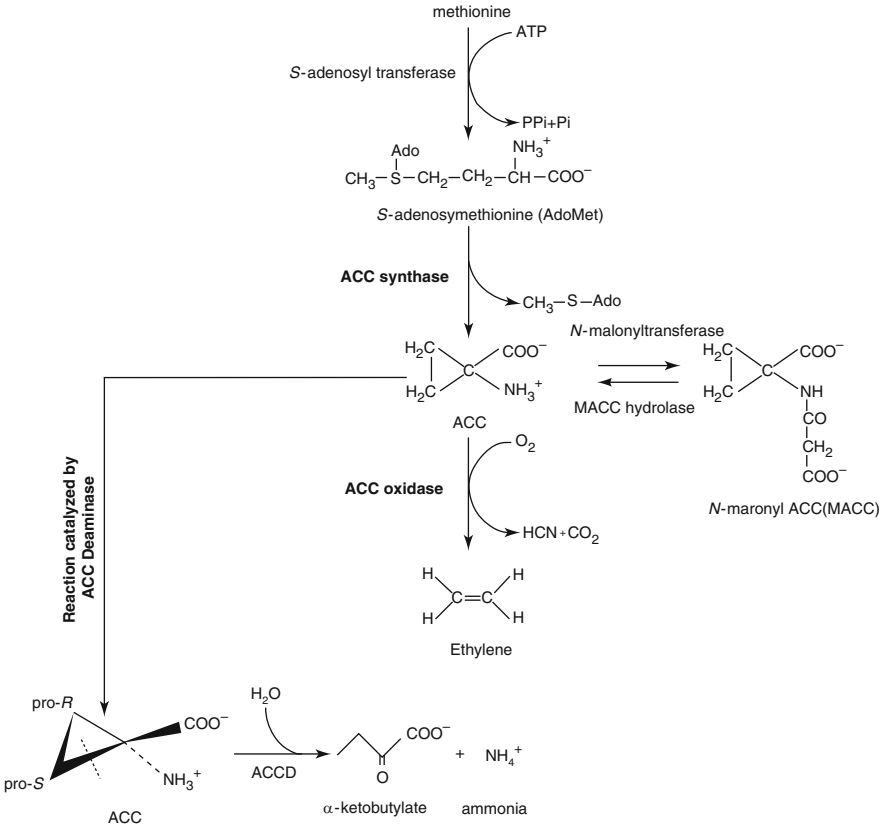

Fig. 2 The enzymatic reaction catalyzed by ACCD. Modified figure adapted from the source reference Ose et al. (2003)

of ACC synthase to produce ACC (Penrose and Glick 2001). Some of the plant's ACC will be exuded along with other small molecules such as sugars, organic acids and amino acids. The exudates may be taken up by the bacteria and utilized as a food source of the rhizosphere bacteria. ACC may be exuded together with the other components of the root or seed exudates. ACC may be cleaved by ACC deaminase to form ammonia and α-ketobutyrate, compounds that are readily further metabolized by the bacteria (Holguin and Glick 2001). The presence of the bacteria induces the plant to synthesize more ACC than it would otherwise need and also, stimulates the exudation of ACC from the plant (some of which may occur as a consequence of plant cell wall loosening caused by bacterial IAA). Thus, PGPB are supplied with a unique source of nitrogen in the form of ACC that enables them to proliferate/survive under conditions in which other soil bacteria may not readily flourish (Hontzeas et al. 2006). As a result of acting as a sink for ACC and lowering its level within the plant, the amount of ethylene that is produced by the plant is also

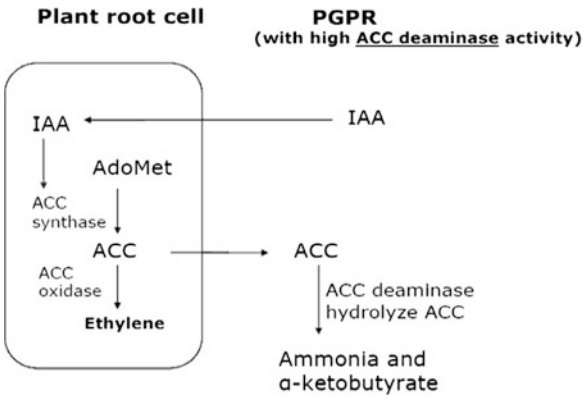

Fig. 3 The ACC deaminase in PGPR degrades the ethylene precursor ACC. The ACC deaminase in PGPR lowers ethylene level in plants by degrading ACC to ammonia and α-Ketobutyrate. Lowering ethylene in plants can alleviate stress and thereby improve plant growth. Some PGPR can also produce plant regulator IAA and further stimulate plant growth. Modified figure adapted from the source reference Glick and Pasternak (2003)

reduced. Thus, the inhibition of plant growth by ethylene (especially during periods of stress) is decreased and these plants generally have longer roots and shoots and greater biomass (Fig. 3).

6 Role of Bacterial ACC Deaminase Under Stress Agricultural Conditions

PGPR containing ACC Deaminase activity eliminates heavy metal toxicity, imparts resistant to drought, other abiotic stresses such as salinity, extremes of temperature and pH in soil apart from antagonism against phytopathogens. Ethylene regulation in plants due to PGPR is now well established (Table 1).

6.1 Pathogenicity Stress

Pathogenic microorganisms are a major and serious threat to food production and ecosystem stability worldwide. PGPR mediated biocontrol in terms of competition for an ecological niche or a substrate and producing allelo-chemicals and inducing systemic resistance (ISR) in host plants to a broad spectrum of pathogens (Compant et al. 2005).

ACC deaminase bacteria, apart from directly antagonizing pathogens, support the plant resistance against pathogen attack. Beneficial rhizobacteria do not obviously damage their host/cause localized necrosis, therefore, the eliciting factors

Table 1 List of ACC deaminase producing bacteria

Strain	ACC deaminase activity (nM αKB $mg^{-1}h^{-1}$)	Reference(s) or Sources
Achromobacter xylosoxidans A551	400 ± 4	Belimov et al. (2001, 2005)
A. xylosoxidans Bm1	90 ± 4	Belimov et al. (2001, 2005)
Achromobacter sp. strain CM1	130 ± 3	Belimov et al. (2001, 2005)
Acidovorax facilis 4p-6	3,080 ± 120	Belimov et al. (2001, 2005)
Azospirillium brasilense Cd1843	–	Holguin and Glick (2003)
Enterobacter aerogenes CAL3	16 ± 12	Shah et al. (1998)
Pseudomonas putida UW4	3,030 ± 60	Hontzeas et al. (2006)
P. syringae GR12-2	3,470 ± 30	Belimov et al. (2001, 2005)
P. brassicacearum Am3	5,660 ± 12	Belimov et al. (2001, 2005)
P. putida BM3	3,780 ± 32	Belimov et al. (2001, 2005)
P. marginalis DP3	4,054 ± 27	Belimov et al. (2001, 2005)
Rhizobium leguminosarum128C53K	5 ± 1	Belimov et al. (2001, 2005)
R. hedysari ATCC 43676	20 ± 0.1	Ma et al. (2003)
R. leguminosarum 99A1	8 ± 3	Ma et al. (2003)
Rhodococcus sp. strain Fp2	7,320 ± 400	Belimov et al. (2001, 2005)
Rhodococcus sp. strain 4N-4	12,970 ± 440	Belimov et al. (2001, 2005)
Serratia quinivirans SUD165	12 ± 15	Belimov et al. (2001, 2005)
Variovorax paradoxus 3P-3	3,700 ± 90	Belimov et al. (2001, 2005)
V. paradoxus 5C-2	4,322 ± 100	Belimov et al. (2001, 2005)
V. paradoxus 2C-1	3,588 ± 26	Belimov et al. (2001, 2005)
P. putida ATCC17399	–	Shah et al. (1998)
Schizosaccharomyces pombe	–	Wood et al. (2002)
Hansenula saturnus	–	Honma and Shimomura (1978), Minami et al. (1998)
Penicillium citrinum	–	Jia et al. (2006)
Yersinia pestis	–	Parkhill et al. (2001)
Caulobacter crescentus	–	Nierman et al. (2001)
Bacillus anthracis	–	Read et al. (2002)
Mesorhizobium loti	–	Sullivan et al. (2002)
Burkholderia fungorum	–	NCBI microbial genome annotation project

produced by ISR-triggering rhizobacteria must be different from elicitors of pathogens. Expression of ISR is similar to systemic acquired resistance (SAR) upon challenge inoculation with pathogen wherein disease severity is reduced; the number of diseased plants also diminishes. This reduction is associated with decreased growth of the pathogen and reduced colonization of induced tissues which reflects upon the ability of plant to resist the pathogen (Dobbelaere et al. 2003). Salicylic acid is an important signaling molecule in both locally and systemically induced resistance responses; however, research on rhizobacteria mediated ISR signaling has demonstrated that jasmonic acid and ethylene play the key roles. Thus, expression of ISR is phenotypically quite similar to SAR, and relies not only on a different type of biological induction but occurs also through different defense-related

activities (Domenech et al. 2006). It is emphasized that ISR-inducing PGPR is a useful tool to reduce diseases caused by pathogens that are sensitive to jasmonic acid and ethylene-dependent defenses. Rasche et al. 2006 reported that ACC deaminase bacteria were capable of antagonizing at least one of the two potato pathogens *Ralstonia solanacearum* and *Rhizoctonia solani*.

6.2 Remediation of High/Heavy Metal Concentration

High metal concentrations in soil have also been shown to cause increased ethylene production and inhibition of root development, to reduce CO_2 fixation and limit sugar translocation. ACC deaminase and siderophore producing PGPB can help plants to overcome many of the effects of high levels of metal (Burd et al. 1998, 2000). Phytoremediation of metals poses a significant challenge because most metal contaminants are tightly bound by soil particles and are not readily bioavailable to plants. Moreover, although many plants can tolerate the presence of excess metals in the soil, most will experience a decrease in plant growth and viability due to either the synthesis of stress ethylene and/or iron depletion. PGPR can alleviate some of the effects of metal toxicity in plants via several different mechanisms. For example bacterial siderophore bind iron with extremely high affinity and plants are able to take up and utilize the iron from these complexes. Thus PGPR are able to protect plants against the inhibitory effects of high concentration of metals by providing the plants with sufficient iron. Belimov et al. (2005) reported 11 cadmium-tolerant strain of PGPR isolated from the rhizosphere of *Brassica juncea* grown in cadmium-containing soils. *Variovorax paradoxus, Rhodococcus* sp. and *flavobacterium sp*. all stimulated root elongation in untreated and Cd-treated soils.

6.3 Drought Stress

Drought is one of the major environmental stresses that limit the growth of plants and the production of crops. The inhibitory effects of ethylene induced by drought stress might have been eliminated through the ACC deaminase activity of the PGPR. Inoculation of plants with PGPR containing ACC deaminase partially or completely eliminated the "drought stress imposed effects" on root and shoot growth, fresh and dry weights, and number of leaves per plant of peas. This might be due to suppression of the stress-induced accelerated synthesis of ethylene by the ACC deaminase activity of these PGPR in the inoculated roots. Sharp increases in ACC levels and, consequently, ethylene synthesis in plants under drought stress conditions has been frequently reported. (Apelbaum and Yang 1981). The rhizobacteria having ACC deaminase activity are effective in promoting plant growth and water use efficiency under drought conditions, by lowering the ethylene or ACC accumulation whose higher levels have inhibitory effects on root and shoot

growth. It is highly likely that rhizobacteria containing ACC deaminase might have decreased the drought-stress induced ethylene in inoculated plants, which resulted in better growth of plants even at low moisture levels. Therefore, inoculation with rhizobacteria containing ACC deaminase could be helpful in eliminating the inhibitory effects of drought stress on the growth of plants. Dodd et al. (2005) investigated the physiological responses of pea (*pisum sativum* L.) to inoculation with ACC deaminase bacteria *V. paradoxus* 5C-2 under moisture stress and watering condition. The bacterial effects were more pronounced and more consistent under controlled soil drying (moisture stress conditions).

6.4 Organic Contaminants Stress

Many organic contaminants are recalcitrant and highly persistent in the environment, making them particularly difficult to remediate. Many of these compounds are hydrophobic and are bound tightly to soil particles. A few studies have revealed an accelerated production of ethylene in soil and plants treated with organic contaminants (Coupland and Jackson 1991). Reed and Glick (2005) have studied the growth of canola (*Brassica napus*) seeds treated with PGPR in copper-contaminated and creosote-contaminated soil. In creosote-contaminated soils, the native bacterium was the least effective, and the transformed encapsulated ACC deaminase bacterium was the most effective in growth promotion.

6.5 Waterlogging Stress

Waterlogging enhances the biosynthesis of ethylene in roots and stem of plants. In flooding, ACC, which is synthesized in roots, is transported to plant shoots where it is converted to ethylene by ACC oxidase (Bradford and Yang 1980). The molecular basis for the increase in ethylene production observed in shoots of flooded tomato plants is due to an increase in the activity of both ACC synthase in the submerged roots and ACC oxidase in the shoots (Chao et al. 1997). The accelerated production of ethylene in the shoots of flooded tomato plants is responsible for the phenotype to demonstrate abnormal growth under flooding conditions (Jackson 1997).

6.6 Temperature Stress

The heat stress in terms of so-called global warming is a serious threat to world agriculture (Mendelsohn and Rosenberg 1994). A fluctuation in temperature leads to hormonal imbalances in plants and thus their growth is significantly affected

(Cheikh and Jones 1994). It has been reported that PGPR containing ACC deaminase activity performs better when subjected to diurnal temperature regime. *Bacillus globiosporus* was inoculated to analyze the effect of diurnal temperature regime (i.e., 25°C days and 5°C night) on root and shoot length, fresh and dry weight were significantly increased in comparison to *B. subtilis* and magnesium sulphate controls (Ghosh et al. 2003).

6.7 Flower Senescence

Ethylene is a key signal in the initiation of willing in most plants. Typically flowers produce minute amount of ethylene until an endogenous rise of the phytohormone, which is responsible for flower senescence to occur (Mol et al. 1995). However, the senescence symptoms that are covered by ethylene differ from plant to plant. The use of ACC deaminase containing PGPR to lower ACC levels in cut flowers might be an environmentally friendly alternative to the available use of silver thiosulphate. An important characteristic of PGPR containing ACC deaminase activity has been shown to be the enhancement of shelf life of flowers incubated in suspension form (Nayani et al. 1998). On a commercial scale, shelf life of flowers could be increased to manifold by treating them with suspensions of PGPR containing ACC deaminase activity, which portends great prospects for the application of this biotechnological approach to commercial floriculture.

6.8 Salinity Stress

Salinity is one of the most severe environmental stresses on plants (White and Broadley 2001; Tester and Davenport 2003; Munns and Tester 2008). Salt primarily limits plant growth in three ways: (1) osmotic effects that lower the ability of plants to take up water from the soil, (2) ion-specific damage of excess Na^+ and Cl^-, and (3) nutrient deficiencies because elevated levels of Na^+ compete with the uptake of other nutrients by interfering with ion transporters (Tester and Davenport 2003). Symptoms of damage to plants include: growth inhibition, leaf discoloration, anatomical and morphological changes such as changes in cell wall structure (Tester and Davenport 2003). Highly saline soil (ECe $>$ 16 dS/m) can severely interfere with seed germination and growth of plants. As water and nutrients move from areas of low salt concentration to areas of high salt concentration, soil salinity prevents plant roots from taking up water and other nutrients, resulting in osmotic and nutrient imbalances that impair proper plant growth. A sudden increase in soil salinity will cause plant cells to shrink due to water loss and immediate changes in expansion rates resulted from the osmotic effects of salt around the roots (Cramer and Bowman 1991; Munns 2002; Neumann 1993). After several hours, plant cells can restore their original shape; however, a decrease in cell elongation rates is

observed in both leaves and roots (Hsiao and Xu 2000; Munns 2002). Continued exposure for a few days results in a decrease in plant growth (i.e., slower cell division and impaired cell elongation). In this case, leaves are often more sensitive to salinity than roots (Hsiao and Xu 2000; Munns 2002). Changes in plant cell dimension are observed more for an area than depth, therefore, leaves appear to be smaller and thicker (Munns and Tester 2008). The effects of salinity become more apparent after a few weeks of exposure (Munns and Tester 2008). Yellowing or death of older leaves may be visible in salt-sensitive plants, where salt levels are high, due to increase uptake or inability to store salt in vacuoles (Karley et al. 2000; Munns and Tester 2008; Tester and Davenport 2003). Only the salt-tolerant plants are able to grow for several months under moderate salinity; but showed early flowering or decreased production of florets (Munns 2002).

Salinity stress boosts endogenous ethylene production in plants, which in most cases serves as a stress hormone (Blumwald 2000). It is very likely that reducing salinity-induced ethylene by any mechanism could decrease the negative impact of salinity on to plant growth. Recent studies have revealed that plants inoculated with PGPR containing ACC deaminase were able to thrive better through the salinity stress while demonstrating a normal growth pattern. Tank and Saraf (2010) have reported that increase in the salinity is directly proportional to the ACC deaminase activity which increases survival rate in saline soils. As the uptake and hydrolysis of ACC by the PGPR decreases the ACC level in plants, the biosynthesis of the "stress ethylene" is impeded, facilitating plant growth under stress conditions (Glick et al. 1998). It has been shown that PGPR promotes plant growth under saline conditions. The presence of PGPR with ACC deaminase may lower the levels of ethylene in developing or stressed plants, enhance the survival of some seedlings and facilitate the formation of longer roots.

6.9 Ethylene–IAA Cross-talk

It is well known that IAA can activate the transcription of ACC synthase (Kende 1993; Kim et al. 1992) but it is less well known that ethylene may inhibit IAA transport and signal transduction (Pratiyon et al. 2006). This feedback loop of ethylene inhibition of IAA synthesis and/or functioning limits the amount of ACC synthase, ACC and ultimately, ethylene following every stressful event in the life of the plant. When an ACC deaminase containing PGPB lowers the ethylene concentration in plant roots, these relieve the ethylene repression of auxin response factor synthesis, and indirectly increase plant growth. Thus ACC deaminase containing PGPR facilitate plant growth by decreasing ethylene inhibition and permitting IAA stimulation without the negative effects of increasing ACC synthase and plant ethylene levels.

6.10 Air Pollutants Stress

It is very likely that PGPR can be utilized as a gene source for genetic modification of plants expressing the enzyme ACC deaminase against plant damage by air pollutants. Air pollution, in addition to damaging plants, inhibits many enzyme systems and metabolic processes of plants (McCune 1975). Increased ethylene evolution by plants exposed to various environmental stresses i.e., air contaminants has been well documented (Wang et al. 2002) and this hormone is now considered a major regulator of plant defense reactions, including cell death, in response to pathogen attack and air contaminant stresses, i.e., O_3 exposure. Many researchers reported that the inhibition of ethylene biosynthesis resulted in a significant reduction of O_3-induced leaf lesion formation (Moeder et al. 2002). In this direction, the role of ACC deaminase in alleviation of air contaminants stresses has not been studied.

6.11 Rhizobial Infection

Considerable evidence suggests that the ethylene that is produced following infection of legumes with *rhizobia* is inhibitory to the process of nodulation. The latest evidence has demonstrated that PGPR containing ACC deaminase activity promotes nodulation in legumes through inhibition of ethylene biosynthesis and consequently, they enhance symbiosis and nitrogen fixation in plants (Okazaki et al. 2004). Uchiumi et al. (2004) reported that an up regulated gene in bacteroids, mlr5932, and encoding ACC deaminase activity was involved in enhanced nodulation in *Lotus japonicus*. Pandey et al. (2005) isolated an endophytic ACC deaminase bacterium capable of modulating nodulation in *Mimosa pudica*. Coinoculation with *Bradyrhizobium* plus ACC deaminase rhizobacteria increased nodulation in mung bean compared to inoculation with *Bradyrhizobium* spp. alone (Shaharoona et al. 2006).

7 Microbe–Microbe Interactions Benefiting Sustainable Agro-Ecosystem Development

Direct interactions occurring between members of different microbial types often result in the promotion of key processes benefiting plant growth and health. It is obvious that all interactions taking place in the rhizosphere are, at least indirectly, plant-mediated (Azcon-Aguilar and Barea 1992). However, this section will deal with direct microbe–microbe interactions themselves, with the plant as a

"supporting actor" in the rhizosphere. Three types of interactions have a major role to play in bacteria–plant health development because of their relevance to the development of sustainable agro-ecosystems. These are (1) the cooperation between ACC deaminase producing PGPR and *Rhizobium* for improving N-fixation, (2) microbial antagonism for the biocontrol of plant pathogens, and (3) interactions between rhizosphere microbes and AM fungi to establish a functional mycorrhizosphere (Barea et al. 2005).

8 ACC Deaminase Gene-Containing Transgenic Plants

Transgenic plants express a bacterial ACC deaminase under the control of either the *35S* (constitutive) or *rolD* (root-specific) promoter as a treatment with ACC deaminase containing bacteria, although ethylene levels have been reported to be decreased by more than 95% in some ripen transgenic tomato fruit. Transgenic plants that express ACC deaminase are also significantly protected against the potentially deleterious effects of a variety of stresses including drought, flooding (Grichko and Glick 2001), high salt (Sergeeva et al. 2006), phytopathogens (Robison et al. 2001), arsenic (Nie et al. 2002), and several different metals (Grichko et al 2001). In all instances, transgenic plants, in which ACC deaminase was under the control of the *rolD* promoter, performed significantly better than the nontransformed plants (regardless of whether the plant was tomato, canola or tobacco) and the transgenic lines in which the ACC deaminase gene was under the control of the *rolD* promoter, yielded significantly more root and shoot biomass than either the nontransformed plants or transgenic plants in which the ACC deaminase gene was under the control of the *35S* or *prb-1b* (stress-specific) promoter. Transgenic plants in which ACC deaminase is under the control of the *rolD* promoter appear to mimic the behavior of nontransgenic plants treated with ACC deaminase-containing PGPB. However, the performance of plants treated with ACC deaminase-containing PGPB is almost always superior to the performance of transgenic plants expressing ACC deaminase under the control of the *rolD* promoter. This likely reflects the fact that the bacteria do more than merely lower plant ethylene levels. They also provide the plants with other "benefits" such as plant hormones and siderophores.

9 Conclusions and Future Trends

There is considerable experimental evidence that certain microorganisms are able to colonize the root–soil environments where they carry out a variety of interactive activities known to benefit plant growth and health, and also soil quality. Given the current reluctance of many consumers worldwide to embrace the use as foods of genetically modified plants, it may be advantageous to use PGPB as a means to

promote growth by lowering plant ethylene levels or reduce disease through induction of resistance, rather than genetically modifying the plant itself to the same end.

Rhizobacteria having ACC deaminase activity are effective in promoting plant growth and water use efficiency under drought conditions, by lowering the ethylene or ACC accumulation whose higher levels have inhibitory effects on root and shoot growth. From the previous demonstrations, it is established that the microorganisms that possess ACC deaminase activity have the selective advantage over other bacteria during biotic and abiotic stress conditions. Besides the activity of ACC deaminase in alleviating ethylene-mediated abiotic and biotic stresses, the ecology of bacterium and physiology of the plant may also interact with plant system to increase resistance to stress. However, the defined mechanisms involved in the use of plant growth-promoting rhizobacteria which decrease the damage to plants that occurs under stress conditions is a potentially important adjuvant to agricultural practice in locales where stress is a major constraint.

From the agricultural and ecological viewpoints, the aims will be to increase food quality, and to improve sustainable plant productivity, while maintaining environmental quality. However, to achieve this, basic and strategic studies must be undertaken to improve our understanding of microbial interactions in the rhizosphere. Only then can the corresponding agro-biotechnology be applied successfully. Hence, future investigation in the field of microbial cooperation in the rhizosphere will include: (1) advances in visualization technology; (2) analysis of the molecular basis of root colonization; (3) signaling in the rhizosphere; (4) functional genomics; (5) mechanisms involved in beneficial cooperative microbial activities; (6) engineering of microorganisms for beneficial purposes; and (7) biotechnological developments for integrated management.

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