

# Chapter 12

## Perspectives of Rhizobacteria with ACC Deaminase Activity in Plant Growth Under Abiotic Stress



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### 12.1 Introduction

Plant growth in its entire lifetime is challenged by many biotic and abiotic stresses. The abiotic stresses may be extremes of temperature, salt, high light, flooding, drought, presence of toxic metals and organic contaminants, radiation, and wounding, and the biotic stresses may include insect predation and attack by various pathogens like viruses, bacteria, and fungi (Abeles et al. 1992). Most of the adverse effects of stress on plant metabolism occurs in the form of osmotic stress or salt toxicity, ROS production, ethylene production, and nutrient imbalance, which overall affect the plant physiology and inhibit seedling growth, vigor, flowering, and fruit setting (Sairam and Tyagi 2004).

Soil which adheres the plant and provides water and nutrients is rich in microbial diversity. The soil microbial diversity includes the bacteria, actinomycetes, fungi, algae, and protozoa. A fertile soil per gram contains  $9 \times 10^7$  bacteria,  $4 \times 10^6$  actinomycetes,  $2 \times 10^5$  fungi,  $3 \times 10^4$  algae,  $5 \times 10^3$  protozoa, and  $3 \times 10^1$  nematodes (Alexander 1991). The rhizospheric bacterial count is 10–1000 times higher than the count in bulk soil as the root exudates contain carbohydrates (sugars and oligosaccharides), organic acids, vitamins, nucleotides, flavonoids, enzymes, hormones, and volatile compounds that diffuse into the rhizosphere and support microbial growth and activity. Plant growth-promoting rhizobacteria (PGPR) are a group of free-living saprophytic bacteria living in plant rhizosphere that aggressively colonize the root system and promote plant growth and act as biocontrol agents against plant diseases (Kloepper and Beauchamp 1992). Plant growth-promoting bacteria promote plant growth and development through many direct and indirect

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mechanisms. The indirect mechanisms include their ability to act as biocontrol agent on phytopathogens, and the direct include fixation of nitrogen, enhanced nutrient uptake through iron sequestration and phosphate solubilization, and production of hormones like indole-3-acetic acid (IAA) and cytokinin (Glick et al. 1999). Plant growth-promoting rhizobacteria elicit the so-called induced systemic tolerance (IST) in plants under different abiotic stresses by altering the plant metabolism. Production of IAA is a common growth-promoting trait observed in up to 80% of the soil bacteria and bacterial endophytes (Patten and Glick 1996). Besides the above benefits, PGPR also benefit the plants by lowering plant ethylene levels through the activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme present in them (Glick et al. 1998). The ethylene level gets elevated during stresses which may be abiotic like temperature, salt, high light, flooding, drought, toxic metals, organic contaminants, and radiation or biotic like attack of viruses, bacteria, and fungi (Abeles et al. 1992). Ethylene was originally regarded as a “stress hormone” due to its accelerated synthesis by plant in response to stress signals (Kende 1993; Johnson and Ecker 1998). Ethylene hormone induces physiological changes in plant growth like overcoming dormancy, differentiation, formation of adventitious roots, abscission of leaf and fruit, induction of flowering and femaleness in dioecious plants, senescence, and fruit ripening (Arshad and Frankenberger 2002; Owino et al. 2006). However, high level of ethylene leads to senescence and abnormal root growth. As ethylene production in plant roots gets accelerated under biotic and abiotic stress factors which have an inhibitory effect on root growth which in turn leads to abnormal plant growth, it becomes vital to regulate the ethylene production in the rhizosphere of plant to achieve normal growth and development. Bacterial strains with ACC deaminase activity are capable of overcoming the ethylene-induced negative responses in plants to a great extent. Bacterial ACC deaminase activity is a widespread character of the rhizospheric bacteria most commonly observed in bacteria residing in stressful conditions (Timmusk et al. 2011). ACC deaminase activity of bacteria endows plants with the capability to withstand the stress better and therefore survive in harsh environmental conditions. Inoculation with PGPR containing ACC deaminase activity has come up as an alternative sustainable approach in improving plant growth and development under stress conditions by reducing stress-induced ethylene production.

## 12.2 Mechanism of Action

Ethylene is an endogenously produced gaseous plant growth hormone by plants. Plants undergoing any stressed situation show an increased production of ethylene, and for this reason it is also known as a stress hormone. On the onset of stress, an initial small peak of ethylene of low magnitude for a few hours is observed, and then a second much larger peak of high magnitude for 1–3 days is observed (Stearns and Glick 2003; Pierik et al. 2006; Van Loon et al. 2006). The second peak initiates protective response in plants, like transcription of pathogenesis-related genes and

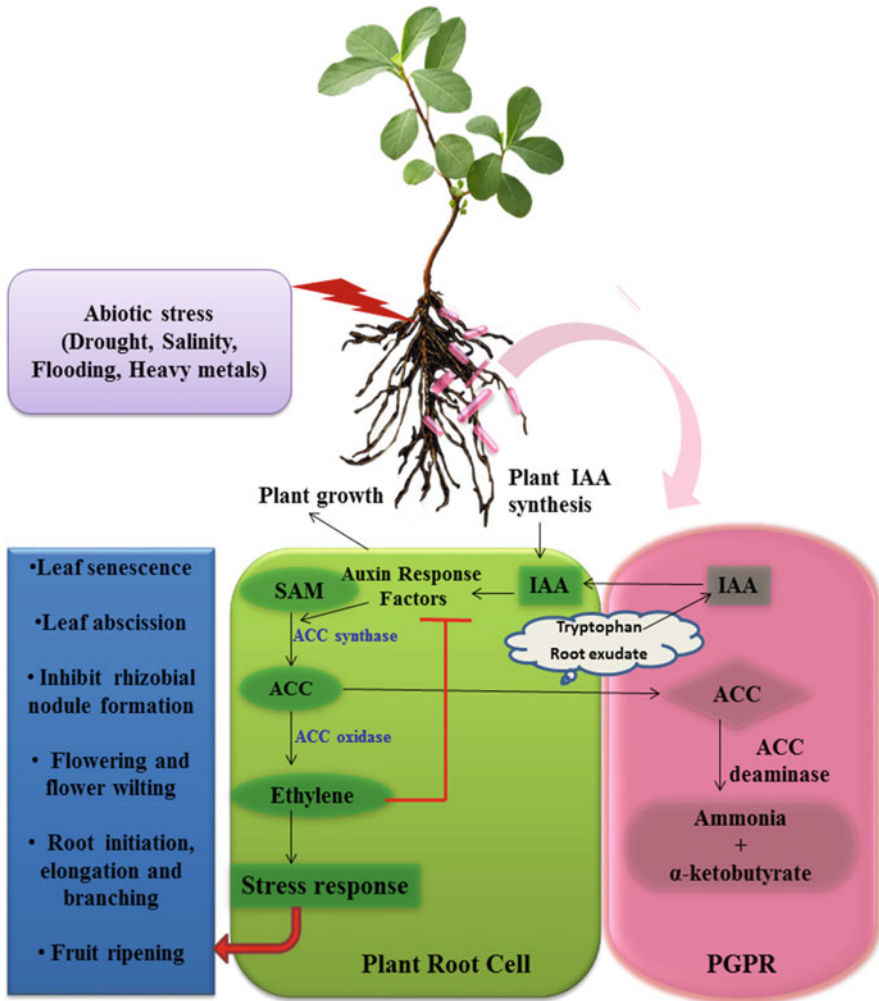
acquired resistance (Ciardi et al. 2000; Van Loon and Glick 2004). The second ethylene peak is so large that processes such as senescence, chlorosis, and abscission are initiated, the overall effect of which is generally inhibitory to plant survival. Plant can be released from the inhibitory levels of ethylene by degrading its precursor like *S*-adenosylmethionine (derived from *L*-methionine) or ACC, which effectively reduces the ethylene levels. Among these, the enzymes ACC deaminase, *S*-adenosylmethionine (SAM) hydrolase, and SAM decarboxylase are much being worked on.

Normally the root exudates are estimated to contain 5–30% of the photosynthetically fixed carbon. The plant root exudates contain tryptophan which is taken by the rhizospheric bacteria and used as a precursor in the synthesis of indole-3-acetic acid (IAA), some of which is taken up by the plant. This bacterial IAA together with endogenously synthesized plant IAA stimulates cell proliferation and cell elongation. It also induces the transcription of ACC synthase which leads to the formation of 1-aminocyclopropane-1-carboxylic acid (ACC) which is the intermediate precursor of ethylene in higher plants (Yang and Hoffman 1984). Small amount of this ACC is exuded from the seeds or roots (Penrose et al. 2001; Grichko and Glick 2001a) which may be taken up by the rhizospheric bacteria. The enzyme present in rhizobacteria ACC deaminase hydrolyzes the ethylene precursor ACC into ammonia and  $\alpha$ -ketobutyrate (Glick et al. 1994, 1998; Mayak et al. 1999; Shaharoon et al. 2006). This activity of the rhizobacteria decreases the amount of ACC, and in turn the ethylene level, in the spermosphere and rhizoplane and thereby eliminates its potential inhibitory effects on plants (Glick et al. 1998). Thus the rhizobacterium acts as a sink for the plant-synthesized ACC (Fig. 12.1). Plants inoculated with PGPR possessing ACC deaminase activity are relatively more tolerant to environmental stress (Naveed et al. 2014). When plants get exposed to stress conditions, the gaseous hormone ethylene endogenously regulates plant homeostasis, resulting in reduced root and shoot growth.

The increased ethylene levels in plant cause a feedback inhibition of the IAA signal transduction pathway and thereby limit the ACC synthase transcription (Burg and Burg 1966; Glick et al. 2007) by IAA. Bacteria capable of synthesizing IAA and possessing ACC deaminase activity are more beneficial in plant growth promotion as they do not allow the elevated levels of ethylene being formed in the plants. This prevents feedback inhibition of IAA signal transduction by ethylene, and the plant growth continues in response to the auxin.

### 12.3 Biochemistry of ACC Deaminase

ACC deaminase belongs to the tryptophan synthase beta superfamily of pyridoxal phosphate-binding proteins (Glick et al. 2007). ACC deaminase is a multimeric enzyme that is cytoplasmically localized. ACC deaminase subunit mass is approximately 35–42 kD, while its native size is estimated to be approximately 100–112 kD (Sheehy et al. 1991; Jacobson et al. 1994; Hontzeas et al. 2004a). The affinity of this



**Fig 12.1** A schematic model of interactions between plant gene expression and ACC deaminase-producing bacteria during stress

enzyme for the substrate is not particularly high ( $K_m = 1.5\text{--}6.0\text{ mM}$ ). The coenzyme pyridoxal phosphate is a cofactor of ACC deaminase (Honma 1985), and even ACC synthase, the enzyme that catalyzes the formation of ACC, requires pyridoxal phosphate for its enzyme activity. ACC deaminase enzyme is present in bacteria at a very low amount, and at the same time, ACC oxidase has a much higher affinity for ACC compared to ACC deaminase (Glick et al. 1998). The ethylene levels in bacteria depend upon the ratio of the two, i.e., ACC oxidase and ACC deaminase (Glick et al. 1998). However, ACC deaminase synthesis is induced by ACC, at levels as low as 100 nM (Jacobson et al. 1994), with full induction requiring up to 10 h. The

amino acids L-Ala, DL-Ala, and DL-Val can also induce enzyme activity to a small extent, and g-aminoisobutyric acid can induce activity to almost the same level as ACC (Honma 1983). Maximal enzyme activity typically occurs at 30 °C and pH 8.5. The affinity for the substrate ACC and the competitive inhibitors L-Ala and L-Ser is also highest at pH 8.5 (Hontzeas et al. 2006). *acdS* genes that have been traced in some stramenopiles, bacteria, and various fungi (*Ascomycota* and *Basidiomycota*) are believed to have a common ancestor (Nascimento et al. 2014). The genes are commonly transmitted vertically in various microorganisms, and occasional horizontal gene transfer is also observed including inter-kingdom transfer events. ACC deaminase genes (including both the structural gene *acdS* and the regulatory gene *acdR*) have been found in many different rhizobacteria (rhizospheric, endophytic, and rhizobia), including *Azospirillum* spp., *Rhizobium* spp., *Agrobacterium* spp., *Achromobacter* spp., *Burkholderia* spp., *Ralstonia* spp., *Pseudomonas* spp., and *Enterobacter* spp. (Blaha et al. 2006). More importantly, even if some strains of a particular genus and species have an *acdS* gene, not all strains do.

## 12.4 Role of ACC Deaminase Bacteria in Ameliorating Various Stress Responses in Plants

A common invariable observation in plants exposed to stress is an increased ethylene level, which leads to damage. Ethylene production upregulates in response to the presence of metals, organics, salt, temperature extremes, drought, ultraviolet light, damage by insects, nematode, and phytopathogens (Abeles et al. 1992). The second ethylene peak observed in plants exposed to stress is more detrimental to plant growth as this initiates processes such as senescence, chlorosis, and leaf abscission. Any treatment whether it is chemical or biological that lowers the magnitude of the second peak of ethylene reduces the damage caused to the plant as a stress consequence. Microorganisms exhibit wide range (>100-fold) in ACC deaminase activity, and it has been observed that organisms that express high ACC deaminase are beneficial as they are nonspecific toward their host (Glick 2005). This group encompasses most of the rhizospheric and phyllospheric microbes along with the endophytes. Such microbes reduce the ethylene by acting as a sink for ACC produced as a consequence of stress. Addition of an ACC deaminase producing PGPR and its negative mutant strain in canola roots (Hontzeas et al. 2004b) showed down regulation of genes involved in ethylene induced plant stress responses and up-regulation of genes involved in plant growth. The results supported that plant growth-promoting bacteria expressing ACC deaminase are able to overcome the stress response in plants. Different ACC deaminase-producing bacteria have been demonstrated for their efficacy in protecting plants against yield loss induced by various abiotic stresses as listed in Table 12.1.

**Table 12.1** Reports on PGPR showing alleviation of abiotic stress impacts on plants mediated by ACC deaminase activity

S. no.	ACC deaminase-producing bacteria	Abiotic stress	Host plant	References
1	<i>Achromobacter piechaudii</i> ARV8	Drought, salt	<i>Lycopersicon esculentum</i>	Mayak et al. (2004a, b)
2	<i>Achromobacter xylosoxidans</i> (SF2), <i>Bacillus pumilus</i> (SF3 and SF4)	Drought	<i>Helianthus annuus</i>	Castillo et al. (2013)
3	<i>Azospirillum brasilense</i>	Drought	<i>Phaseolus vulgaris</i>	German et al. (2000)
4	<i>Azospirillum brasilense</i> Sp245	Drought	<i>Triticum aestivum</i>	Creus et al. (2004)
5	<i>Azospirillum lipoferum</i> AZ1, <i>A. lipoferum</i> AZ9, <i>A. lipoferum</i> AZ45	Drought	<i>Triticum aestivum</i>	Arzanesh et al. (2011)
6	<i>Bacillus cereus</i> strain AR156, <i>B. subtilis</i> strain SM21, <i>Serratia</i> sp. Strain XY21	Drought	<i>Cucumis sativus</i>	Wang et al. (2012)
7	<i>Bacillus subtilis</i>	Drought	<i>Trigonella foenum-graecum</i>	Barnawal et al. (2013)
8	<i>Bacillus subtilis</i> B26	Drought	<i>Phleum pratense</i>	Bourque et al. (2016)
9	<i>Bacillus licheniformis</i> K11	Drought	<i>Capsicum annum</i>	Lim and Kim (2013)
10	<i>Burkholderia phytofirmans</i> PsJN, <i>Enterobacter</i> sp. FD17	Drought	<i>Zea mays</i>	Naveed et al. (2014)
11	<i>Paenibacillus polymyxa</i>	Drought	<i>Arabidopsis</i>	Timmusk and Wagner (1999)
12	<i>Proteus penneri</i> strain (Pp1), <i>Pseudomonas aeruginosa</i> strain (Pa2), <i>Alcaligenes faecalis</i> strain (AF3)	Drought	<i>Zea mays</i>	Naseem and Bano (2014)
13	<i>Pseudomonas aeruginosa</i> GGRJ21	Drought	<i>Vigna radiata</i>	Sarma and Saikia (2014)
14	<i>P. fluorescens</i> ACC-5	Drought	<i>Pisum sativum</i>	Zahir et al. (2008)
15	<i>Pseudomonas</i> sp.	Drought	<i>Pisum sativum</i>	Arshad et al. (2008)
16	<i>Pseudomonas syringae</i> , <i>Pseudomonas fluorescens</i>	Drought	<i>Zea mays</i>	Zafar-ul-Hye et al. (2014)
17	<i>Variovorax paradoxus</i> 5C-2	Drought	<i>Pisum sativum</i>	Jiang et al. (2012)
18	<i>Arthrobacter protophormiae</i>	Salt	<i>Pisum sativum</i>	Barnawal et al. (2014)
19	<i>Brachybacterium paraconglomeratum</i> SMR20	Salt	<i>Chlorophytum</i>	Barnawal et al. (2016)
20	<i>Enterobacter hormaechei</i>	Salt	<i>Lycopersicon esculentum</i>	Egamberdieva et al. (2014)
21	<i>P. fluorescens</i> YsS6	Salt	<i>Lycopersicon esculentum</i>	Ali et al. (2014)

(continued)

**Table 12.1** (continued)

S. no.	ACC deaminase-producing bacteria	Abiotic stress	Host plant	References
22	<i>Pseudomonas putida</i>	Salt	<i>Vigna radiata</i>	Mayak et al. (1999)
23	<i>Pseudomonas</i> sp. ST3	Salt	<i>Vigna unguiculata</i>	Trung et al. (2016)
24	<i>P. putida</i> UW4	Salt	<i>Lycopersicon esculentum</i>	Yan et al. (2014)
25	<i>Pseudomonas putida</i> UW4	Salt	<i>Brassica napus</i>	Cheng et al. (2007)
26	<i>Pseudomonas fluorescens</i>	Salt	<i>Arachis hypogea</i>	Saravanakumar and Samiyappan (2007)
27	<i>Bacillus licheniformis</i> HSW-16	Salt	<i>Triticum aestivum</i>	Singh and Jha (2016)
28	<i>Pseudomonas putida</i> UW4	Flood	<i>Lycopersicon esculentum</i>	Grichko and Glick (2001a)
29	<i>P. fluorescens</i> REN <sub>1</sub>	Flood	<i>Oryza sativa</i>	Etesami et al. (2014)
30	<i>Alcaligenes</i> sp., <i>Bacillus pumilus</i>	Heavy metals	<i>Brassica napus</i>	Belimov et al. (2001)
31	<i>E. cloacae</i> CAL2	Arsenate	<i>Brassica napus</i>	Nie et al. (2002)
32	<i>Enterobacter intermedius</i> MH8b	Zn toxicity	<i>Sinapis alba</i>	Płociniczak et al. (2013)
33	<i>Kluyvera ascorbata</i> SUD165	Nickel	<i>Brassica napus</i>	Burd et al. (1998)
34	<i>P. putida</i> UW4, <i>P. putida</i> HS-2	Nickel	<i>Brassica napus</i>	Farwell et al. (2007)
35	<i>K. ascorbata</i> SUD165/26	Lead	<i>Lycopersicon esculentum</i>	Burd et al. (2000)
36	<i>Sinorhizobium</i> sp. Pb002	Lead	<i>Brassica juncea</i>	Di Gregorio et al. (2006)
37	<i>Burkholderia</i> sp. J62	Lead	<i>Lycopersicon esculentum</i>	Jiang et al. (2008)
38	<i>Pseudomonas koreensis</i> AGB-1	Cd, AS, Cu, Pb and Zn toxicity	<i>Miscanthus sinensis</i>	Babu et al. (2015)
39	<i>Variovorax paradoxus</i>	Cadmium	<i>Brassica juncea</i>	Belimov et al. (2005)
40	<i>Burkholderia phytofirmans</i> PsJN	Low temperature	<i>Vitis vinifera</i>	Ait Bakra et al. (2006)
41	<i>P. putida</i>	Low temperature	<i>Lycopersicon esculentum</i>	Cheng et al. (2007)
42	<i>Enterobacter aerogenes</i> NBRIK24	Fly-ash soil	<i>Brassica juncea</i>	Kumar et al. (2008)

### 12.4.1 Salt Stress

Salinity, which was a natural feature of ecosystems in arid and semiarid regions, has now become a major constrain due to the anthropogenic activities, primarily due to irrigation of agricultural fields (Abrol et al. 1988). Of the total global cultivable area, 20% is under salinity stress, and this is continuously increasing as a direct consequence of irrigation (Flowers 2004). Around 800 million hectares of land is estimated to be affected by salinity throughout the world (FAO 2008). Salinity stress creates an oxidative burst in cells resulting in an increased accumulation of reactive oxygen species (ROS) which affects the plasma membrane, cell metabolism, and homeostasis. Salt stress imbalances the ethylene production and causes its overproduction which accelerates leaf and petal abscission and organ senescence, leading to premature death (Cheng et al. 2007; Mayak et al. 2004a, b; Zahir et al. 2009).

Reducing the ethylene level, one can alleviate some of the effects of stresses on plants (Glick 2004). Plant losses approximately 40% of photosynthates, through root exudates (Lynch and Whipps 1991), and it has been estimated that during stress much of the released carbon is in the form of ACC, which is a precursor of ethylene, and is exuded from plant roots (Bayliss et al. 1997). Thus, PGPR, with ACC deaminase activity, can be used to convert ACC to ammonia and  $\alpha$ -ketobutyrate that are used up by the plant as a nitrogen source simultaneously reducing the negative effects of salinity stress (Cheng et al. 2007; Mayak et al. 2004a, b; Zahir et al. 2009). However, the efficiency of PGPR depends on environmental factors such as the climate, weather conditions, soil characteristics, and interaction with other indigenous microbial flora in the soil (Giongo et al. 2008; Sinha and Raghuwanshi 2015). Salt-tolerant ACC deaminase-producing bacteria can survive well in a saline environment, and their beneficial properties help plants to overcome stress effects (Mayak et al. 2004a, b). Halotolerant bacteria are a group of microorganisms able to grow in media containing a wide range of NaCl up to 1–33% or in the absence of NaCl (Larsen 1986). A significant decrease in the level of ethylene was observed in tomato plants exposed to high salt concentration on inoculation with *Achromobacter piechaudii* ARV8, an isolate obtained from the rhizosphere of *Lycium shawii* plant wildy growing in the Arava region of Israel (Mayak et al. 2004b). The inoculated tomato seedlings showed an increased fresh and dry weight, but this however did not reduce the content of sodium in the plant. Plants inoculated with *Achromobacter piechaudii* ARV8 had four times higher biomass compared to controls, as there was a significant reduction of the ethylene level (Mayak et al. 2004b). Similar effect of the bacterial strain, i.e., lowering the ethylene level, was observed in peppers and tomatoes growing under drought stress (Mayak et al. 2004a). Studies done on maize plant growing in saline–sodic soil when treated with fertilizer along with ACC deaminase-producing *Pseudomonas* strains showed 198% augmented plant dry weight (Zafar-ul-Hye et al. 2014). Studies done on wild-type bacterial endophytes showed protection against salt stress in plants by limiting the buildup of salt and thereby improving plant survival. Inoculating ACC



deaminase bacteria do not alter the sodium level in plants, but the uptake of phosphorous and potassium gets slightly increased, which supports plant growth under salt stress. Similar reports by Saravanakumar and Samiyappan (2007) revealed *Pseudomonas fluorescens* strain TDK1 possessing ACC deaminase activity not only enhanced the resistance toward salinity in groundnut plants but also increased yield. Compared to mutant-inoculated or non-inoculated plants, the plants inoculated with ACC deaminase-producing strain show augmented level of chlorophyll content. High chlorophyll content has been linked with stress tolerance in many plants (Vurukonda et al. 2016). Encouraging results were also obtained by the tripartite interaction of *Arthrobacter protophormiae*, *Rhizobium leguminosarum*, and *Glomus mosseae* which increased plant weight by 53%, reduced proline content and lipid peroxidation, and increased pigment content under 200 mM salt condition (Barnawal et al. 2013). Inoculating plants with wild-type ACC deaminase-producing strain tend to prevent salt buildup in plant tissues; however, few contradictory results have also been observed where more salt was deposited per gram of dry biomass in the plants inoculated with the ACC deaminase-producing strains. Study done to evaluate the growth of canola in the presence of wild-type ACC deaminase-containing plant growth-promoting rhizospheric bacterium *P. putida* UW4 showed the accumulation of much higher concentrations of sodium in the shoots compared to the plants treated with ACC deaminase mutants (Cheng et al. 2007).

Pea crop has been reported to suffer approximately 50% yield loss at 100 mM NaCl (Subbarao and Johansen 1994). Ethylene formed in response to salt stress inhibits the development of rhizobial infection threads in *Pisum sativum* cv. Sparkle (Lee and LaRue 1992). Plants undergoing symbiotic association with microbes, like *Rhizobium*, and mycorrhizal fungi also show a slight increase in ethylene levels during the establishment period. As the nitrogen fixation is a high energy-demanding process, there are fair chances of ethylene production by the plant, which may lead to nodule senescence (Murset et al. 2012). During this period the ACC deaminase-producing bacteria residing in the rhizosphere help in establishment of symbiosis by locally lowering the ethylene levels.

Thus effects of soil salinity on crop productivity can be alleviated by bacterial inoculations having ACC deaminase activity. Microbe-assisted plant stress management has emerged as an important strategy, and their role in improving growth and productivity has been well established (Venkateswarlu et al. 2008; Yang et al. 2009).

### 12.4.2 Waterlogging Stress

Soil flooding or waterlogging causes major changes in the normal functioning of plant roots (Jackson and Drew 1984) as the gas diffusion rates get reduced in flooded soil (Jackson 1985), and at the same time, respiration by microorganisms and plant roots leads to a rapid buildup anaerobic conditions in the soil. Anaerobic conditions of the soil lead to toxicity primarily due to  $\text{Fe}^{2+}$ ,  $\text{Mn}^{+}$  and sulfide and due to accumulated gases like carbon dioxide, methane, ethane, and ammonia (Ernst

1990). This also affects some of the vital processes like ion uptake in root (Jackson and Drew 1984). These stressful conditions trigger the synthesis of enzyme ACC synthase as well as other stress proteins in the plant which elevate the level of ACC in its roots (Li et al. 2012). This newly synthesized ACC cannot be converted to ethylene in the roots, as ethylene synthesis requires oxygen, so the ACC is transported to the shoots where under aerobic environment the ACC gets converted into ethylene (Bradford and Yang 1980; Else and Jackson 1988) causing epinasty (wilting), leaf chlorosis, necrosis, and stunted growth. Accumulation of ethylene in plants may also lead to adaptive response like shoot elongation (Voeselek and Blom 1989) and formation of aerenchyma (Armstrong et al. 1994), and a few studies also report the formation of adventitious roots (Drew 1992). Waterlogging induces several physiological alterations like reduced photosynthetic rate, stomatal closure, plant growth inhibition, and low yield. A sustainable solution to this problem comes from the ACC deaminase-producing plant growth-promoting bacteria (Barnawal et al. 2012; Grichko and Glick 2001a; Li et al. 2013) which mitigate the stress by lowering the ethylene level in plants and making them more capable to withstand flood (Saleem et al. 2007). Studies have shown that flooded plants inhabiting ACC deaminase-producing microbes are able to overcome the flood response partially. Even plants are genetically engineered to express this enzyme in root-specific manner resulting in less accumulation of ethylene in the roots and thereby minimizing the adverse effects of flooding (Grichko and Glick 2001a, b).

### ***12.4.3 Metal and Organic Pollutants***

Industrial revolution has accelerated the toxic metal accumulation rate in the biosphere and has come up as a serious current environmental problem. Metal in soil beyond a limit becomes toxic to plant growth as they interfere with normal growth and development. Soils dumped with heavy metals also cause a severe stress induction in plants that leads to the synthesis of stress ethylene up to an inhibitory level. Plants interact with these heavy metals present in the environment through phytostabilization, phytoextraction, and phytovolatilization (Pilon-Smits 2005). The easy and preferred way to get rid of the metals is phytoextraction. Cleaning heavy metal pollutants through plants, i.e., phytoremediation (Salt et al. 1995), is an eco-friendly and cost-effective approach compared to the traditional soil remediation approaches of metal removal through chemical and physical extraction. However, the limitation lies that not all plants are capable to naturally tolerate and accumulate heavy metals. Many plants effective in phytoremediation are small sized and slow in growth, which limit their practical use (Khan et al. 2000). An effective plant to remediate the soil must be tolerant to one or more pollutants, highly competitive, and fast growing and produce a high biomass. Healthy and robust plants are preferred as they have better ability to phytoremediate metal contaminants. The commonly used plants in heavy metal accumulation belong to the Brassicaceae family (Kumar et al. 1995). The literature is well documented with the role of metal-resistant ACC

deaminase-producing bacteria in improving plant growth by decreasing the stress effects due to ethylene. The first report on role of ACC deaminase-containing bacterium in phytoremediation of nickel-contaminated soil indicated that toxicity of nickel toward canola plants was reduced in the presence of the bacteria (Burd et al. 1998). Bacteria increased the uptake of Cd in *Brassica napus* (Sheng and Xia 2006) and Ni in *Alyssum murale* (Abou-Shanab et al. 2006). Rhizobial microfloras are known to affect heavy metals mobility and availability to the plant through release of chelating agents, acidification, and redox changes (Abou-Shanab et al. 2003; Smith and Read 1997). The root-associated ACC deaminase-producing bacteria not only reduce the ethylene levels but also provide multifaceted benefits to the plant (Glick 1995; Glick et al. 1999). Bacteria produce indole-3-acetic acid, siderophores, and solubilize phosphate, which stimulate plant growth (Glick 1995; Chabot et al. 1996a, b; Rajkumar et al. 2006). Heavy metal-contaminated soil often become iron depleted, and this effect of heavy metals can be overcome by inoculating ACC deaminase and siderophore-producing bacteria (Burd et al. 1998, 2000; Reed and Glick 2005). Thus, besides reducing the ethylene level in plants, microbes also enhance the mobility and availability of minerals to the plants (Abou-Shanab et al. 2003; Idris et al. 2004) which improve plant growth.

Treatment of plants with ACC deaminase-producing plant growth-promoting bacteria not only relieves the plant with the growth inhibition effects of ethylene but also allows the plant to grow normal and restore the nutrient cycling (Huang et al. 2004, 2005; Reed and Glick 2005; Greenberg et al. 2006). Therefore, bacterial strains utilized in plant growth promotion under metal stress should be screened for their abilities to resist the targeted toxic metal, synthesize IAA to promote root growth (Patten and Glick 2002), secrete siderophore (Burd et al. 2000) that helps plants to acquire iron from the metal-contaminated soil, and possess ACC deaminase activity which can prevent the building up of inhibitory levels of ethylene in the plants (Glick et al. 1998).

Organic pollutants in the soil, if present above a permissible limit, inhibit plant growth and drag the plant toward senescence by accelerated ethylene production (Abeles et al. 1992). Phytoremediation has come up as a technology to clean soil contaminated with organic oil spills, polycyclic aromatic hydrocarbons (PAHs), and polycyclic biphenyls (PCB), and is being practiced at commercial scale. PGPR possessing ACC deaminase activity has multifold benefits in phytoremediation of organic-, metal-, and salt-contaminated soils. Reduction in stress ethylene partially alleviates the damage caused by the target contaminant (Mayak et al. 2004a, b). 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. *Helianthus annuus* L. seedlings inoculated with *Achromobacter xylosoxidans* (SF2) and *Bacillus pumilus* (SF3 and SF4) bacterial strains increased the production of auxins, salicylic acid, abscisic acid, jasmonic acid, as well as the plant dry matter. High salicylic acid concentration in stressed seedlings played key role in abiotic stress tolerance (Castillo et al. 2013). Plant growth-promoting bacteria

improve plant competitiveness and responses in a stressed ecosystem (Egamberdiyeva and Hoflich 2004).

#### 12.4.4 Drought

Drought stress can adversely affect plant growth and yield and is one of the most fatal reasons for economic losses in agriculture and forestry. It affects plant water relations at the cellular and whole plant levels, altering the plant physiology and leading to specific and nonspecific phenotype (Pereyra et al. 2009; Arzanesh et al. 2011). In order to combat drought, plants adopt altered gaseous exchange and water relation strategies (Sinha and Raghuwanshi 2016). Bacteria adhering to plant roots containing ACC deaminase enzyme hydrolyze ACC and use it as the source of carbon and nitrogen (Glick 2014), and the process continues until a dynamic equilibrium between the roots and rhizosphere bacteria is maintained and the modulated root system starts normal functioning under low water condition. It has been well documented that inoculation of plants with certain PGPR at seedling stage improves biomass production through their effects on root system, which enhance plant growth and yield (Prasad et al. 2017).

Different ACC deaminase-producing bacteria have been demonstrated for their efficacy in protecting plants against yield loss induced by drought stress (Table 12.1). Studies done by Mayak et al. (2004a, b) on ACC deaminase PGPR *Achromobacter piechaudii* ARV8 showed that during water stress although the bacterium did not influence the water content of plants, it improved the recovery of plants when watered. Exposure of *Bacillus subtilis*-inoculated plants to 8 weeks drought stress led to significant increase in shoot and root biomass by 26.6 and 63.8%, and the photosynthesis and stomatal conductance too got enhanced by 55.2% and 214.9%, respectively (Bourque et al. 2016). *Azospirillum brasilense* sp. 245 uninoculated seeds of *Triticum aestivum* when sown under drought conditions had a yield loss of 26.5% and got reduced to 14.1% on inoculation with *Azospirillum brasilense* sp. 245. Grain Mg and K diminished in nonirrigated, non-inoculated plots. Grains harvested from *Azospirillum*-inoculated plants had significantly higher Mg, K, and Ca than non-inoculated plants (Creus et al. 2004). Cucumber plants treated with a consortium of three plant growth-promoting rhizobacterial strains (*Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21) induced tolerance to drought stress. The treatment decreased the leaf monodehydroascorbate (MDA) content and increased the leaf proline content and the root recovery intension by 3.45-fold and 50%, respectively. It also maintained the leaf chlorophyll content in cucumber plants under drought stress (Wang et al. 2012). *Azospirillum lipoferum* strain B3 having phosphate-solubilizing and ACC deaminase activities, when inoculated in wheat under drought, produced the highest amounts of N and auxin and increased wheat yield up to 109% (Arzanesh et al. 2011). Many studies have proven the positive effects of ACC deaminase bacterial activity on plant biomass, leaf area, and transpiration ratio of plants under drought (Saleem et al. 2007). Inoculation with

ACC deaminase bacterial activity has restored nodulation in pea plants under drought which is comparable with the well-irrigated plants (Arshad et al. 2008).

## 12.5 Conclusion and Future Prospects

It is well proven that bacteria with ACC deaminase activity are potent in improving plant growth and productivity under varied abiotic stress. Knowing the potentially serious environmental health damage caused by the excessive use of chemicals and pesticides in the agricultural sector, we need a major paradigm shift in agricultural practices. As the cost of engineering and developing transgenic plants that are able to defend well the variety of pathogens and other abiotic stresses, it is rather economical to isolate and screen an efficient plant growth-promoting bacterium able to combat the adverse conditions. The major challenge in the large-scale application of these bacteria is their survival under varied geographical and harsh environmental conditions, but a potential solution to the problem can be the exploitation of a potent endophytic plant growth-promoting bacteria. Unrevealing the fundamental mechanism of action of these bacteria will facilitate the wider application of this technology and overcome the bottleneck.

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