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REVIEW ARTICLE

Use of plant growth-promoting rhizobacteria to control stress responses of plant roots

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Abstracts Ethylene is a key gaseous hormone that controls various physiological processes in plants including growth, senescence, fruit ripening, and responses to abiotic and biotic stresses. In spite of some of these positive effects, the gas usually inhibits plant growth. While chemical fertilizers help plants grow better by providing soil-limited nutrients such as nitrogen and phosphate, overusage often results in growth inhibition by soil contamination and subsequent stress responses in plants. Therefore, controlling ethylene production in plants becomes one of the attractive challenges to increase crop yields. Some soil bacteria among plant growth-promoting rhizobacteria (PGPRs) can stimulate plant growth even under stressful conditions by reducing ethylene levels in plants, hence the term "stress controllers" for these bacteria. Thus, manipulation of relevant genes or gene products might not only help clear polluted soil of contaminants but contribute to elevating the crop productivity. In this article, the beneficial soil bacteria and the mechanisms of reduced ethylene production in plants by stress controllers are discussed.

Keywords ACC deaminase · Ethylene · Plant growth-promoting rhizobacteria · Phytoremediation · Stress controllers

Introduction

Ever since the cultivation of crop plants, agricultural science and technology have continuously developed to a great extent. The developments have been more notable recently, and one of the major contributions to such development is chemical fertilizers. During the last two decades, the amount of chemical fertilizers used has been increasing by about 3% per year. Currently, about 100 million tons of nitrogen fertilizers and 90 million tons of phosphate fertilizers are used globally per year, and have contributed to increasing crop productivity. However, when overused, the soil-remaining fertilizers not taken up by the plants often cause serious environmental problems. They can be over-enriched in lakes and rivers. This leads to microbial overgrowth in water, resulting in oxygen depletion and subsequently the death of aquatic animals. In addition, the overused fertilizers can threaten human health. For example, NO_3^-, a main form of the nitrogen fertilizers, can be converted to N-nitroso compounds which act as carcinogens. Therefore, it is very important to properly remove the remaining fertilizers from the treated area.

Phytoremediation is the return of the contaminated soil to its original state by using plants to take up the pollutants.

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In nature, plants are continuously interacting with a large number of microorganisms. While pathogenic microbes inhibit plant growth, symbiotic microorganisms help plants grow better by providing substances that are in principle not synthesized or metabolized by plants themselves. Indeed, farmers have already known through their experience that some soil bacteria are useful to increase crop yields in the fields, implying that these bacteria during evolution have become adapted to soil conditions. The size of plants, especially the roots, is critical for absorbing the underground substances and is known to be positively correlated with phytoremediation activity of plants (Gleba et al. 1999). In many cases, however, soil contaminants are known to inhibit plant growth likely by activating plant stress responses, causing the dilemma of using plants as soil pollutant removers. Therefore, in the phytoremediation field, many researchers are trying to generate transgenic plants whose tolerance to soil contaminants is increased and whose growing activity under high concentrations of pollutants is retained. In this context, it might be valuable to use some beneficial soil bacteria as a stimulant of plant growth to take up more soil pollutants by maintaining plant size.

The rhizosphere is defined as the small area of soil where plant roots interact with microorganisms, and is surrounded by bulk soil. In the rhizosphere, the growth of plants and microorganisms is mutually affected by their secreted molecules. The exuded molecules by plant roots are called rhizodeposits, and include carbohydrates, amino acids, fatty acids, nucleotides, organic acids, phenolics, plant growth regulators, putrescine, sterols, and vitamins (Uren 2007). While some of these act as repellants to microbes, others act as attractants to accommodate the microbial growth. The composition of these exudates depends on the physiological status and species of plants and microorganisms (Kamilova et al. 2008; Meharg and Killham 1995). Intriguingly, plants secrete significant amounts (20–40%) of carbon assimilates to the rhizosphere via roots, although how and why is not yet understood. A proportion of these plant-derived small organic molecules is further metabolized as carbon and nitrogen sources by closely located microorganisms, and some microbe-oriented molecules are subsequently re-taken up by plants for their growth.

Plant growth promoting rhizobacteria

Bacteria that can stimulate plant growth by interacting with plants are called plant growth-promoting bacteria (PGPB). Best studied among PGPB are plant growth-promoting rhizobacteria (PGPR) which colonize on roots in the rhizosphere. The effect of PGPR on plant growth can be direct or indirect. Disease caused by pathogenic microbes often results in loss of crop productivity. It is also well known that plant growth is inhibited when plants are infected by pathogens, although the underlying mechanism is poorly understood. Some PGPR are found to protect colonizing plants from pathogen attack by directly killing parasites. These types of PGPR are shown to produce antibiotics such as HCN, phenazines, pyoluteorin, and pyrrolnitrin. Some rhizobacteria can induce plant resistance to pathogenic microbes, which is called induced systemic resistance (ISR). ISR is in general different from systemic acquired resistance (SAR) in that it depends on the plant jasmonic acid (JA) and ethylene signaling rather than salicylic acid (SA) signaling (Haas and Keel 2003). These are indirect effects of PGPR on plant growth.

The second group of PGPR can stimulate plant growth directly in the absence of pathogens by providing plant-helpful substances. *Rhizobium* fixes gaseous N_2 into ammonia that can be used by legume plants as a nitrogen source. Some PGPR help plants grow by providing soluble phosphate converted from insoluble phosphorus. Growth-promoting plant hormones such as auxin, cytokinin, and gibberellins can also be synthesized by some soil bacteria using plant-secreted precursors. These bacteria-derived hormones subsequently facilitate plant growth. Removal of soil contaminants, which normally induce plant stress responses and inhibit plant growth, by soil bacteria can also help plants grow better. In many cases, environmental stresses by soil pollutants stimulate ethylene production in plants, which subsequently retards plant growth. Many PGPR possess an interesting enzyme called ACC (1-aminocyclopropane-1-carboxylate) deaminase that can hydrolyze ACC, the precursor of ethylene, into 2-oxobutanoate and ammonia (Glick 2005). Glick et al. (1998) previously proposed that ACC might be exuded from plant roots and that soil bacteria containing ACC deaminase could convert this for their growth. In the interactions between plants and ACC deaminase-expressing PGPR, more ACC molecules would be secreted from plants to maintain equilibrium when metabolized by the PGPR. As a result, the bacterial growth would be enhanced by the hydrolyzed ACC products. Taken together, the ACC deaminase function seems to be mutually beneficial between plants and PGPR, because ethylene levels in plants can be reduced by continuous ACC secretion and degradation by bacteria, and bacteria can use metabolized ACC.

Suppression of ethylene production in plant roots by bacterial ACC deaminase and plant growth

Ethylene is well known to function in various physiological events such as triple responses of seedlings, senescence including leaf abscission, and fruit ripening (Abeles et al.

1992). Ethylene is also well known to work as a stress hormone. Various harmful chemicals, extremely high temperatures, chilling or freezing, water stress, flooding, high-energy light, herbivorous animals, disease, and mechanical wounding give rise to stressful conditions to plants. In response to these stimuli, plants produce more ethylene than under normal conditions, which results in retardation of their growth (Morgan and Drew 1997).

It has been shown that plants produce ethylene at two different phases in response to stressful stimuli (Glick et al. 2007). In the first phase, ethylene is evolved from pre-existing ACC and the amount is small. This ethylene is known to play a role in protecting plants by promoting activities of stress-related genes. In the second phase, ethylene production occurs relatively late (1–3 days after stimulus application), but the amount is higher than that produced in the first phase. This second and larger amount of ethylene is known to cause problems such as inhibition of growth and harmful effects on plants including senescence, chlorosis, and abscission.

Metabolic pathways of ethylene have so far been well established (Abeles et al. 1992). In higher plants, S-Adenosyl L-methionine (S-AdoMet) is produced from methionine. Using S-AdoMet, ACC synthase biosynthesize ACC, and ACC is converted to ethylene by ACC oxidase. Among enzymes involved in the biosynthetic pathway, ACC synthase is known to play a rate-limiting step. Ethylene production in plants is controlled by the regulation of expression of ACC synthase and ACC oxidase genes (Kim et al. 2001). Ethylene can be catabolized to ethylene glycol or ethylene oxide but this pathway may not be essential since ethylene molecules are easily diffused from plant cells. Another catabolic pathway is performed in the bacterial cell expressing ACC deaminase, which can be used for bacterial growth.

Until recently, bacterial strains that contain ACC deaminase have been identified in a wide range of genera such as *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, and *Rhizobium* (Blaha et al. 2006; Vessey 2003). Although there might be various kinds of ACC deaminase among a lot of bacterial strains, a model of the action of the enzyme (Fig. 1) has been suggested and now approved widely (Glick et al. 2007). Using tryptophan excreted by plant roots to the rhizosphere, PGPR synthesize indole-3-acetic acid (IAA). Then, IAA molecules are secreted by bacterial cells and can be transported into plant cells. These auxin molecules seem to have dual roles. One is to participate in plant cell growth and the other is to promote ACC synthase activity resulting in increases in ethylene titer. Like IAA molecules, stress can induce an increase in the level of ACC. Increased ACC molecules can be diffused from plants and imported into PGPR cells where the molecules are subjected to the action of ACC deaminase. Due to these microbes, plants can be more tolerant to stress-induced growth inhibitions mediated by ethylene, which results in growth of plant roots.

There are several ways to apply ACC deaminase when plants are under stressful conditions. One is to supply plants with PGPR containing the enzyme in the field or laboratory and another is to make transgenic plants that contain the gene. Examples are as follows (see also Table 1). Transgenic tomato plants that contain ACC deaminase showed resistance to Verticillium-induced wilting and flooding-caused abnormalities of leaves (Robison et al. 2001). In the presence of pathogens, PGPR prevented pathogen-caused growth inhibition (Wang et al. 2000). *Achromobacter peichaudii* ARV8 cells prevented stress-induced growth inhibition in the presence of high concentrations of salt (Mayak et al. 2004). Such positive effects of PGPR containing ACC deaminase was shown when plants were exposed to stresses such as drought, heavy metals, and rhizobium infection as listed in Table 1. These kinds of effects were also exhibited in the field especially when heavy metals were added to plants (Reed and Glick 2005).

Conclusion

It is possible to use chemicals for control of endogenous level of ethylene or ethylene action in plants (Abeles et al.

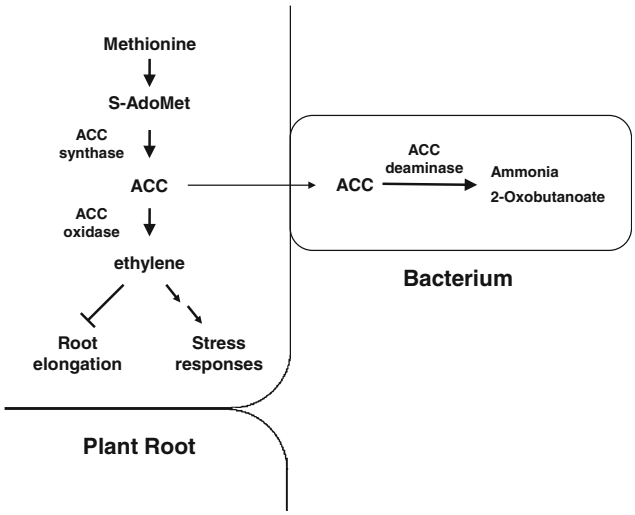

Fig. 1 A possible mechanism of how stress controller bacteria reduce ethylene levels in the plant root using bacterial ACC deaminase. ACC synthesized in plant tissues by ACC synthase is thought be exuded from plant roots and be taken up by neighboring bacteria. Subsequently, the bacteria hydrolyze ACC to ammonia and 2-oxobutanoate. This ACC hydrolysis maintains ACC concentrations low in bacteria and permits continuous ACC transfer from plant roots to bacteria. Otherwise, ethylene can be produced from ACC and then cause stress responses including growth inhibition. *S-AdoMet* S-adenosyl-L-methionine; *ACC* 1-aminocyclopropane-1-carboxylate

Table 1 PGPR containing ACC deaminase exert their roles in response to various kinds of stresses such as pathogens, high salt, flooding, and heavy metals

Stresses	Symptoms	Plants	Treatment methods	Results	References
Pythium ultimum	Root growth inhibition	Cucumber	Addition of PGPR	Increased survival rate and root fresh weight	Wang et al. (2000)
Erwinia carotovora	Rotting of tubers	Potato	Addition of PGPR	Protection of potato tuber against soft rot	Wang et al. (2000)
Verticillium	Wilting	Tomato	Transgenic plants	Increased disease tolerance	Robison et al. (2001)
High salt	Growth inhibition	Tomato	Addition of PGPR	Increased weight of seedling	Mayak et al. (2004)
Flooding	Leaf epinasty, chlorosis, necrosis	Tomato	Transgenic plants and PGPR	No curvature change Promotion of chlorophyll synthesis	Grichko and Glick (2001)
Nickel, copper and aromatic hydrocarbon	Growth inhibition	Canola	Addition of PGPR	Increased shoot length	Stearns et al. (2005)
				Increased root and shoot biomass	Reed and Glick (2005)
Plant-produced ethylene	Inhibition of nodulation	Alfalfa	Addition of PGPR	Enhanced nodulation	Ma et al. (2004)
		Pea	Addition of PGPR	Enhanced nodulation	Ma et al. (2003)
	Growth inhibition	Rape	Addition of PGPR	Increased root lengths	Belimov et al. (2001)
	Growth inhibition	Soybean	Addition of PGPR	Early root growth	Cattelan et al. (1999)
	Growth inhibition	Mungbean	Addition of PGPR	Development of longer roots	Mayak et al. (1999)

Responses are mostly promotion of plant growth that was inhibited under stressful conditions

1992; Sisler and Serek 1997). In some aspects, these chemicals can be used in the field in order to increase crop production. Aminooxyaceticacid (AOA) and L-α-(amino-ethoxyvinyl)-glycine (AVG), a synthetic analog of rhizobitoxin, are well-known action inhibitors of ACC synthase. Silver thiosulfate and 1-methylcyclopropene (1-MCP) are known to block ethylene action. An agonist, 2-chlorethylphophoric acid (ethephon), a liquid ethylene, was also known to release ethylene. Among these, 1-MCP is well known to be commercially available such as in storage of post-harvest fruits and transportation of cut flowers. However, it needs to be noted that the application range of the chemical is too limited. Furthermore, AVG and silver thiosulfate are reported to be very toxic in foods. But, most of all, they are not so far readily prepared to be used in the field under conditions of various stresses.

As mentioned above (see Table 1), transgenic tomato plants containing ACC deaminase should be under examination for use in the fields. More transgenic plants will appear soon since it involves just a single kind of gene, i.e., ACC deaminase gene, which is subjected to manipulation. Future work to be carried out are testing of the developed transgenic plants in the field under various stressful conditions. Considering the problems mentioned above, it would also be advantageous for agriculture to use PGPR in the field looking at both environmental and economic aspects. In order to use PGPG as stress controllers, there are several technological problems to be solved. First is screening of bacterial strains that shows strong activity of ACC deaminase through mutagenesis. The screening should be performed under specific conditions regarding host plants, soils, climates, and pathogens. Second is optimizing of bacterial inoculum formulation in the presence of biotic and/or abiotic factors. In addition, appropriate carriers such as peat or alginate should be developed.

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