PROMOTION OF PLANT GROWTH BY SOIL BACTERIA THAT REGULATE PLANT ETHYLENE LEVELS

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ABSTRACT

One of the central the mechanisms used by many soil bacteria to directly promote plant growth is the production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. This enzyme facilitates plant growth as a consequence of the fact that it sequesters and cleaves plant-produced ACC (the immediate precursor of ethylene in plants), 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. Thus, 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. ACC deaminase-containing *Rhizobia* spp. are also significantly more efficient at nodulating their legume hosts than are rhizobial strains that lack the activity of this enzyme. The results of a wide range of studies using several different plants and soil bacteria are discussed in the context of a previously proposed model for the functioning of ACC deaminase-containing bacteria. In addition, the plant genes that are expressed as a consequence of the interaction with ACC deaminase-containing soil bacteria, and some of the bacterial regulatory factors involved are also discussed.

PLANT GROWTH-PROMOTING BACTERIA (PGPB)

Plant growth-promoting bacteria include both free living and symbiotic bacteria, typically found in the soil, that facilitate the growth and development of plants (Glick et al., 1999). This can occur in two different ways: either indirectly or directly. Indirect promotion of plant growth occurs when these bacteria decrease or prevent some of the deleterious effects of phytopathogenic organisms by any one or more of several different mechanisms including (but not limited to) the production of antibiotics or fungal cell wall-degrading enzymes. On the other hand, bacteria can directly promote plant growth either by providing the plant with a compound that is synthesized by the bacterium or by facilitating the uptake of nutrients from the soil. Thus, plant growth-promoting bacteria can directly facilitate the proliferation of plants by fixing atmospheric nitrogen; producing siderophores which can solubilize and sequester iron and provide it to plants; synthesizing phytohormones, such as auxin, cytokinin and gibberelin, which can enhance various stages of plant growth; solubilizing minerals such as phosphorus; and synthesizing enzymes that can modulate plant growth and development. A particular bacterium may affect plant growth and development using any one, or more, of these mechanisms. Moreover, many plant growth promoting bacteria possess several activities that enable them to facilitate plant growth and, of these, may utilize different ones at various times during the life cycle of the plant.

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A number of plant growth-promoting bacteria contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and this enzyme can cleave the plant ethylene precursor ACC and thereby lower the level of ethylene in a developing or stressed plant (Glick, 2005). 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. Thus, plant growth-promoting bacteria 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.

Stress Ethylene and Plant Growth

Ethylene, which is produced in almost all plants, mediates a range of plant responses and developmental steps. 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)). 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. For example, when plants are infected by pathogens, a large portion of the damage that occurs to the plant is due to autocatalytic ethylene synthesis and not from direct pathogen action. In this regard, not only does exogenous ethylene often increase the severity of a pathogen infection; but, as well, inhibitors of ethylene synthesis can significantly decrease the severity of that infection.

One model that explains the seemingly paradoxical effects of stress ethylene on plants is based on the fact that in stressed plant tissues there is an initial small peak of ethylene close in time to the onset of the stress, and then a second much larger peak some time later (Stearns and Glick, 2003). The size of the first ethylene peak is only a small fraction of the magnitude of the second peak and is thought to initiate a defensive response by the plant, such as induced systemic resistance or transcription of pathogenesis-related genes (Van Loon and Glick, 200. The second peak is so large, however, that processes such as senescence, chlorosis and abscission are initiated, the overall effect of which is often inhibitory to plant growth and survival. The first small wave of ethylene production generally consumes the existing pool of ACC within plant tissues, after which ACC synthase genes are transcribed and more ACC begins to accumulate to fuel the second wave of ethylene production. This occurs as a direct consequence of the regulation of the transcription of ACC synthase genes by environmental and developmental cues with the subsequent enhancement of its enzymatic action during stress conditions.

Many of the symptoms of a diseased plant arise as a direct result of the stress imposed by the infection. That is, a significant portion of the damage to plants infected with fungal phytopathogens occurs as a result of the response of the plant to the increased levels of stress ethylene. And, not only does exogenous ethylene often increase the severity of a fungal infection; but, as well, inhibitors of ethylene synthesis can significantly decrease the severity of a fungal infection. Moreover, the evidence indicates that in terms of the synthesis of stress ethylene, plants respond similarly to abiotic stresses as they do to biotic stresses such as phytopathogens.

Model of ACC Deaminase Action

A model was previously proposed to explain how ACC deaminase-containing plant growth-promoting bacteria can lower plant ethylene levels and in turn stimulate plant growth (Glick et al., 1998), especially under stress conditions. In this model, the plant growth-promoting bacteria 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 plant growth-promoting bacteria 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 of ACC synthase to produce ACC. 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 (Penrose and Glick, 2001). ACC may be cleaved by ACC deaminase to form ammonia and α-ketobutyrate, compounds that are readily further metabolized by the bacteria. 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, plant growth-promoting bacteria 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. And, 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 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.

ACC Deaminase-Containing PGPB Decrease plant Stress Ethylene levels

Treatment of plant seeds or roots with ACC deaminase-containing bacteria typically reduces ACC and ethylene levels about 2- to 4-fold. Notwithstanding the often small reduction in ACC and ethylene levels, the protection afforded stressed plants through the action of ACC deaminase is often quite dramatic (and depending on the plant utilized and its age, ACC deaminase-containing plant growth-promoting bacteria can reduce growth inhibition by 25-500%). For example, in laboratory experiments, treatment of a variety of plants (tomato, canola, lettuce, Indian mustard, the common reed, and tomato) with ACC deaminase-containing plant growth-promoting bacteria protects the plants against damage from cold temperatures (Glick et al., 1997), drought (Mayak et al., 2004a), flooding (Grichko and Glick, 2001a), high salt (Mayak et al., 2004b), phytopathogens (Wang et al., 2000), polyaromatic hydrocarbons (Reed and Glick, 2005) and several different metals including nickel (Burd et al., 2000), copper (Reed and Glick, 2005) and lead (Burd et al., 2000). Most of the experiments where a protective effect of ACC deaminase-containing plant growth-promoting bacteria on plant biomass and growth has been demonstrated have been conducted in either a greenhouse or growth chamber setting. However, more recently, significant protection of plants against flooding (Farwell et al., submitted for publication), the presence of high levels of nickel (Farwell et al., submitted for publication), and polycyclic aromatic hydrocarbons (Greenberg et al., unpublished observations) has recently been observed in field trials.

Most of the experiments in which ACC deaminase-containing plant growth-promoting bacteria have been shown to reduce growth inhibition by various stressors were conducted with (ethylene sensitive) dicots including canola, tomato, tobacco, Indian mustard and mung bean. These bacteria are also effective (although to a lesser extent) with monocots such as wheat, rye and rice (all of which are somewhat less ethylene sensitive than the dicots).

ACC Deaminase-Containing Transgenic Plants have lower Stress Ethylene levels

ACC and/or ethylene levels are generally reduced to a similar extent in transgenic plants that express a bacterial ACC deaminase under the control of either the *35S* (constitutive) or *rolD* (root-specific) promoter as treatment with ACC deaminase-containing bacteria, although ethylene levels have been reported to be decreased by more than 95% in some ripening 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 (Robison et al., unpublished observations), flooding (Grichko and Glick, 2001b), high salt (Sergeeva et al., 2006), phytopathogens (Robison et al., 2001 a & b) , arsenic (Nie et al., 2002), and several different metals (Grichko and Glick, 2000; Czarny et al. 2005). In all instances, transgenic plants, in which ACC deaminase was under the control of the *rolD* promoter, performed significantly better than the non-transformed 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 non-transformed 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 behaviour of non-transgenic plants treated with ACC deaminase-containing plant growth-promoting bacteria. However, the performance of plants treated with ACC deaminase-containing plant growth-promoting bacteria 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.

ACC Deaminase-Containing Rhizobia are more efficient nodulators of their host plants

Ethylene inhibits nodulation in various legumes. However, through the action of ACC deaminase, which is present in some strains of Rhizobia (Ma et al. 2003a), ethylene biosynthesis in plants can be reduced. Thus, mutants of *R. leguminosarum* bv. viciae that cannot synthesize ACC deaminase show decreased nodulation efficiency (~30%) compared to the parental strain (Ma et al., 2003b) and transformation of *S. meliloti* (which does not have an ACC deaminase gene) with the ACC deaminase gene from *R. leguminosarum* bv. viciae resulted in increases of ~35% in both nodule numbers and biomass in alfalfa plants infected with the transformed strain (Ma et al., 2004).

Future Prospects

Given the current reluctance of many consumers worldwide to embrace the use as foods of genetically modified plants, it may be advantageous to use plant growth-promoting bacteria 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. In addition, given the large number of different plants, the various cultivars of those plants and the multiplicity of genes that would need to be engineered into plants, it is not feasible to genetically engineer all plants to be resistant to all pathogens and environmental stresses. Rather, it is much simpler to select or engineer plant growth-promoting bacteria to do this job.

Finally, while ethylene signaling is required for the induction of systemic resistance elicited by rhizobacteria, a significant increase in the level of ethylene is not. Hence, lowering of ethylene levels by bacterial ACC deaminase does not appear to be incompatible with the induction of systemic resistance.

LITERATURE CITED

Abeles FB, PW Morgan, and ME Saltveit Jr. 1992. Regulation of ethylene production by internal, environmental and stress factors. *In* Ethylene in Plant Biology, 2nd Edition, pp. 56-119, Academic Press, San Diego.

Burd GI, DG Dixon, and BR Glick. 2000. Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can. J. Microbiol.46: 237-245.

Glick BR, C.L. Patten, G Holguin, and DM Penrose. 1999. "Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria", Imperial College Press, London, 270 pages.

Glick BR. 2005. Modulation of plant ethylene levels by the enzyme ACC deaminase. FEMS Microbiol. Lett. 251: 1-7.

Glick BR, C Liu, S Ghosh, and EB Dumbroff. 1997. The effect of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 on the development of canola seedlings subjected to various stresses. Soil Biol. Biochem. 29: 1233-1239.

Glick BR, DM Penrose, and J Li. 1998. A model for the lowering of plant ethylene concentrations by plant growth promoting bacteria. J. Theor. Biol. 190: 63-68.

Grichko VP, B Filby, and BR Glick. 2000. Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb and Zn. J. Biotechnol. 81: 45-53.

Grichko VP, and BR Glick. 2001a. Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiol. Biochem. 39: 11-17.

Grichko VP, and BR Glick. 2001b. Flooding tolerance of transgenic tomato plants expressing the bacterial enzyme ACC deaminase controlled by the 35*S*, *rolD* or PRB-1*b* promoter. Plant Physiol. Biochem. 39: 19-25.

Hyodo H. 1991. Stress/wound ethylene. *In* The Plant Hormone Ethylene, eds. A.K. Mattoo and J.C. Suttle, pp. 65-80, CRC Press, Boca Raton.

Ma W, S Sebestianova, J Sebestian, GI Burd, F Guinel, and BR Glick. 2003a. Prevalence of 1-aminocyclopropaqne-1-carboxylate in deaminase in *Rhizobia* spp. Anton. Van Leeuwenhoek 83: 285-291.

Ma W, FC Guinel, and BR Glick. 2003b. The *Rhizobium leguminosarum* bv. *viciae* ACC deaminase protein promotes the nodulation of pea plants. Appl. Environ. Microbiol. 69: 4396-4402.

Ma W, TC Charles, and BR Glick. 2004. Expression of an exogenous 1-aminocyclopropane-1-carboxylate deaminase gene in *Sinorhizobium meliloti* increases its ability to nodulate alfalfa. Appl. Environ. Microbiol. 70: 5891-5897.

Mayak S, T Tirosh, and BR Glick. 2004a. Plant growth-promoting bacteria that confer resistance to water stress in tomato and pepper. Plant Sci. 166: 525-530.

Mayak S, T Tirosh, and BR Glick. 2004b. Plant growth-promoting bacteria that confer resistance in tomato to salt stress. Plant Physiol Biochem. 42: 565-572.

Nie L, S Shah, GI Burd, DG Dixon, and BR Glick. 2002. Phytoremediation of arsenate contaminated soil by transgenic canola and the plant growth-promoting bacterium *Enterobacter cloacae* CAL2. Plant Physiol. Biochem. 40: 355-361.

Penrose DM and BR Glick. 2001. Levels of 1-aminocyclopropane-1-carboxylic acid (ACC) in exudates and extracts of canola seeds treated with plant growth-promoting bacteria. Can. J. Microbiol. 47: 368-372.

Reed MLE, and BR Glick. 2005. Growth of canola (*Brassica napus*) in the presence of plant growth-promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can. J. Microbiol. 51: 1061-1069.

Robison MM, S Shah, B Tamot, KP Pauls, BA Moffatt, and BR Glick. 2001a. Reduced symptoms of Verticillium wilt in transgenic tomato expressing a bacterial ACC deaminase. Molec. Plant Pathol. 2: 135-145.

Robison MM, M Griffith, KP Pauls, and BR Glick. 2001b. Dual role of ethylene in susceptibility of tomato to Verticillium wilt. J. Phytopathol. 149: 385-388.

Sergeeva E, S Shah, and BR Glick. 2006. Tolerance of transgenic canola expressing a bacterial ACC deaminase gene to high concentrations of salt. World J. Microbiol. Biotechnol. 22: 277-282.

Stearns J, and BR Glick. 2003. Transgenic plants with altered ethylene biosynthesis or perception. Biotechnol. Adv. 21: 193-210.

VanLoon LC. 1984. Regulation of pathogenesis and symptom expression in diseased plants by ethylene. *In* Ethylene: Biochemical, Physiological and Applied Aspects, eds. Y. Fuchs and E. Chalutz, pp. 171-180, Martinus Nijhoff/Dr W. Junk, The Hague.

VanLoon LC, and BR Glick. 2004. Increased plant fitness by rhizobacteria. *In* Molecular Ecotoxicology of Plants. (Ed.) H. Sandermann, pp. 177-205, Springer-Verlag, Berlin.

Wang C, E Knill, BR Glick and G Défago. 2000. Effect of transferring 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase genes into *Pseudomonas fluorescens* strain CHA0 and its *gacA* derivative CHA96 on their growth-promoting and disease-suppressive capacities. Can. J. Microbiol. 46: 898-907.