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Short Communication

Effect of Wild-Type and Mutant Plant Growth-Promoting Rhizobacteria on the Rooting of Mung Bean Cuttings

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Abstract. Mung bean cuttings were dipped in solutions of wild type and mutant forms of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 and then incubated for several days until roots formed. The bacteria *P. putida* GR12-2 and *P. putida* GR12-2/*aux*1 mutant do not produce detectable levels of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, whereas *P. putida* GR12-2/*acd*36 is an ACC deaminase minus mutant. All bacteria produce the phytohormone indole-3-acetic acid (IAA), and *P. putida* GR12-2/*aux*1 overproduces it. Treatment of cuttings with the above-mentioned bacteria affected the rates of ethylene production in the cuttings in a way that can be explained by the combined effects of the activity of ACC deaminase localized in the bacteria and bacterial produced IAA. *P. putida* GR12-2 and *P. putida* GR12-2/*acd*36-treated cuttings had a significantly higher number of roots compared with cuttings rooted in water. In addition, the wild type influenced the development of longer roots. *P. putida* GR12-2/*aux*1 stimulated the highest rates of ethylene production but did not influence the number of roots. These results are consistent with the notion that ethylene is involved in the initiation and elongation of adventitious roots in mung bean cuttings.

Key Words. Ethylene—Plant growth-promoting rhizobacteria—Adventitious roots—ACC deaminase—Indole-3-acetic acid—Mung bean

Abbreviations: PGPR, plant growth-promoting rhizobacteria; ACC, 1-aminocyclopropane-1-carboxylic acid; IAA, indole-3-acetic acid; DF, Dworkin and Foster.

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Beneficial free-living soil bacteria, often referred to as plant growth-promoting rhizobacteria or PGPR (Kloepper et al. 1989), can affect plant growth in a number of different ways (Glick 1995). For example, a particular PGPR strain may affect plant growth and development by influencing the levels of plant hormones known to regulate the development of plants (Glick 1995, Patten and Glick 1996, Xie et al. 1996). Among the plant hormones that are active in promoting root development are ethylene and auxin (Nordstrom and Eliasson 1984, Riov and Yang 1989). In the case of one particular PGPR strain, *Pseudomonas putida* GR12-2, it has been postulated that the effect of the bacterium on plant ethylene production is brought about in two ways. The first, leading to a reduction in ethylene production, is the result of the bacterium sequestering the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) from the plant and then hydrolyzing it through the action of the enzyme ACC deaminase (Glick et al. 1994, 1998, Hall et al. 1996). The second way, leading to an increase in ethylene production, is the result of bacterial synthesis of indole-3-acetic acid (IAA) (Xie et al. 1996), which may stimulate ethylene production in the plant. The production of ethylene in the plant will depend on the interaction between the stimulation effect on one hand and the suppression effect caused by sequestration of ACC on the other hand. The availability of two related bacterial mutants allow insight to the mechanism involved in the bacterial influence on plant growth. *P. putida* GR12-2/*acd*36 is a nitrosoguanidine-induced ACC deaminase minus mutant of the wild type (Glick et al. 1994). In the absence of active ACC deaminase an increase in ethylene production is to be expected. *P. putida* GR12-2/*aux*1 is a transposon Tn*5* mutant of the wild type which over-

produces IAA (Xie et al. 1996). Because IAA can stimulate the activity of ACC synthase (Kende 1993), the bacterium can be used to study the effect of a higher level of bacterial auxin on ethylene concentration.

The stimulation effect of the bacterium *P. putida* GR12-2 on developing roots in canola seeds has been reported in numerous papers (e.g. Hall et al. 1996, Lifshitz et al. 1987, Tang et al. 1995). However, studies concerning the effect of this and other similar bacteria on the initiation and development of adventitious roots have not been reported. This is somewhat surprising because the rooting of cuttings is of major importance in horticultural practice. The aim of the present study was to evaluate the effect of PGPR on the formation of adventitious roots. Although mung bean is not a horticultural crop per se, it has nevertheless been used widely as a model plant in studies on the involvement of plant hormones, more specifically auxin and ethylene, in the initiation and development of adventitious roots (e.g. Jarvis et al. 1985). Treatment of mung bean cuttings with wild type and mutant plant growth-promoting bacteria is the first step in understanding how these organisms might be used in a horticultural setting to promote adventitious root formation.

On the basis of physiologic response, anatomic characteristics of initiation and genetic control, there are four different types of roots (Zobel 1986). Radicle or tap roots are of embryonic origin, lateral roots grow from preexisting roots and develop from the pericycle layer, adventitious roots originate from non-root tissues, and basal roots originate from the pericycle of the lower hypocotyl and upper tap root (Zobel 1986). It is possible that the response of different types of roots to plant hormone may vary. Therefore it is essential to study each type of root specifically. In the present study, unlike all previous reports on the effects of PGPR on root development, only adventitious roots were considered.

Materials and Methods

P. putida GR12-2 was originally isolated from the rhizosphere of an arctic plant (Lifshitz et al. 1986) and was kindly provided by Dr. Gerry Brown (Agrium Inc., Saskatoon, Saskatchewan, Canada). This bacterium is an effective colonizer of canola (*Brassica campestris* cv. Tobin) seeds and roots and promotes canola root elongation under gnotobiotic conditions. The growth of the bacteria in liquid medium at room temperature (23 ± 1°C) was monitored by measuring the optical density of the cultures at 780 nm. *P. putida* GR12-2/*acd*36 is a nitrosoguanidine-induced ACC deaminase minus mutant of the wild type (Glick et al. 1994). *P. putida* GR12-2/*aux*1 is a transposon Tn*5* mutant of the wild type which overproduces IAA (Xie et al. 1996).

To prepare mung bean (*Vigna radiata*) cuttings, seeds were imbibed in running water for 24 h and then sown in vermiculite. After 7 days, the upper parts of the plants, approximately 8 cm in length, were cut and used as cuttings. The cuttings were placed in bacterial suspensions prepared in the following way. Bacterial cells were maintained on a solid DF (Dworkin and Foster 1958) salts minimal medium plus ammonium sulfate as a nitrogen source. Liquid DF salts minimal medium plus ammonium sulfate was inoculated with bacterial cells and incubated at 23 ± 1°C for 1–2 days. The bacteria were then pelleted by centrifugation, and the supernatant was discarded. The bacterial pellet was suspended in water and diluted to an optical density of 1.0 at 780 nm. Cuttings were placed in the resultant aqueous bacterial suspension and maintained for 5–10 days at a temperature of 23 ± 1°C and light intensity of 12.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ supplied continuously from cool white fluorescent lamps.

The effect of the bacterium on root initiation and root elongation was assessed by counting the number of roots of various sizes which developed on the cuttings, at the times indicated. Root length was measured using a magnifying glass. To analyze the association of the bacteria with the roots, sections were excised from the cuttings 3 days after inoculation, fixed, dehydrated, coated with gold, and observed with a scanning electron microscope.

Ethylene production rates were determined in 2-cm sections excised from the base of the cuttings. Five sections, one from each cutting, were excised and transferred to a 10-mL vial sealed with a rubber serum cap. Ethylene that had accumulated during the subsequent 1 h was determined by withdrawing a 2-mL gas sample with a hypodermic syringe and injecting it into a gas chromatograph equipped with an activated alumina column and a flame ionization detector.

Table 1. Effect of wild type and mutant forms of the PGPR *P. putida* GR12-2 on the number of roots on treated mung bean cuttings. Roots were counted 7 days after inoculation with the bacteria. In each case $n = 10 \pm$ S.E..

Treatment	Roots/cutting
Water	10.1 ± 1.1
P. putida GR12-2	19.8 ± 2.8
P. putida GR12-2/*acd*36	20.1 ± 2.3
P. putida GR12-2/*aux*1	12.4 ± 1.3

Results

The effect of wild type and mutant forms of the PGPR *P. putida* GR12-2 on adventitious root development in mung bean cuttings was assessed in several ways. First, the total number of roots per cutting was compared for the four different treatments, i.e. water, *P. putida* GR12-2, *P. putida* GR12-2/*acd*36, and *P. putida* GR12-2/*aux*1, on the 7th day after the cuttings had been inoculated with the bacteria (Table 1). The data indicate that treatment with either wild type *P. putida* GR12-2 or the ACC deaminase minus *P. putida* GR12-2/*acd*36 significantly increased the number of adventitious roots on the 7th day after treatment. The bacterium *P. putida* GR12-2/*aux*1 did not affect the number of roots.

The wild type and the mutant forms also affected root

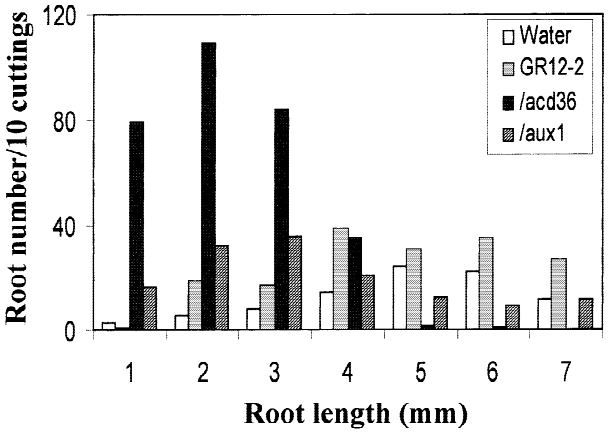

Fig. 1. Effect of treating mung bean cuttings with water, *P. putida* GR12-2, *P. putida* GR12-2/*acd*36, or *P. putida* GR12-2/*aux*1 on length distribution. The roots were counted on the 9th day after the beginning of treatment. Each treatment included 10 cuttings. The experiment was repeated three times with similar results.

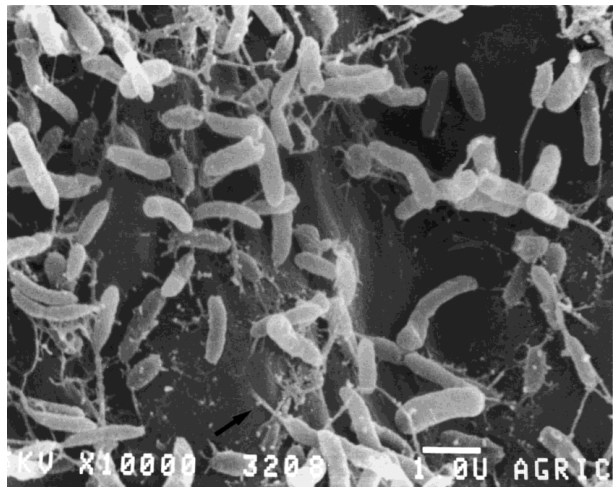

Fig. 2. Scanning electron microscope of the association of the bacterium *P. putida* GR12-2/*acd*36 with the surface of a mung bean cutting. The *arrow* points to a filament connecting the bacterium with the cutting. The magnification is × 10,000.

elongation. In this experiment, mung bean cuttings were treated with bacterial suspensions, and roots were sorted according to length and counted after 9 days. Clearly, in cuttings treated with *P. putida* GR12-2, roots were longer compared with roots developed in control cuttings (Fig. 1). At the same time, shorter roots developed in cuttings treated with *P. putida* GR12-2/*acd*36. Cuttings incubated in *P. putida* GR12-2/*aux*1 also yielded a greater number of shorter roots compared with control cuttings.

The association of the bacterium *P. putida* GR12-2/*acd*36 with the surface of mung bean cuttings is visualized in Fig. 2. The filaments extending from the bacteria to the plant cell wall and weaving into the epidermal layer are of particular interest (for example, see the *arrow*).

To study the pattern of bacteria-induced ethylene production in the cuttings, the production of ethylene by the basal sections of the cuttings was monitored at various times after the cuttings had been placed in bacterial suspensions. Ethylene production rates in cuttings incubated in water were relatively low and did not change during the entire experimental period (Fig. 3). Ethylene production rates in sections of cuttings treated with bacterial suspensions peaked 3 h after the initiation of the bacterial treatment and then declined gradually (Fig. 3 and *inset* therein). The production rates in cuttings exposed to the various treatments differed, however; cuttings treated with GR12-2/*aux*1 produced the highest level followed by a slightly lower level in cuttings treated with GR12-2/*acd*36. Still a lower ethylene production level was measured in cuttings treated with GR12-2.

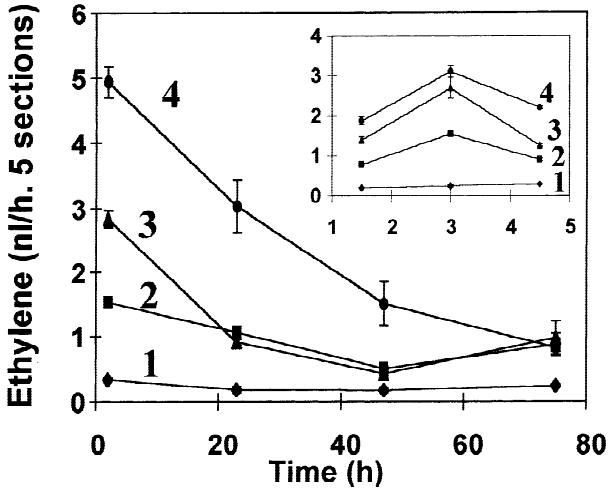

Fig. 3. Time course of ethylene production in sections of mung bean cuttings treated with various bacterial suspensions; *1,* water (control); *2, P. putida* GR12-2; *3, P. putida* GR12-2/*acd*36; *4, P. putida* GR12-2/*aux*1. At various times after the cuttings had been placed in the bacterial suspension, basal sections of 2 cm were excised, and their ethylene production rates were determined as outlined in the Materials and Methods section. The *inset* is of a similar experiment, except that the sampling times were at 1.5, 3, and 4.5 h. Values are means of five replicates of five sections each ±S.E.

Discussion

It has been well documented that PGPR can bind to either seeds or roots, both in growth pouches and in soil, under either gnotobiotic conditions or in the presence of other microorganisms, and subsequently stimulate root

elongation (Glick 1995, Hall et al. 1996, Tang et al. 1994). The effect of some PGPR on root initiation and elongation is caused by the combined effect of auxin on growth promotion and inhibition of root elongation by ethylene (Jackson 1991). The level of ethylene is influenced by the bacteria in two contradicting ways. The bacterial IAA that is taken up by the plant stimulates the activity of ACC synthase, resulting in increased synthesis of ACC (Jackson 1991), and consequently an elevated level of ethylene is expected. Indeed, higher levels of ethylene production were measured in cuttings treated with the different bacterial mutants (Fig. 3). The level of ethylene, however, was also influenced by the presence or absence of activity of ACC deaminase. The wild type *P. putida* GR12-2 containing active ACC deaminase induced a lower level of ethylene production than that induced by the ACC deaminase minus mutant, *P. putida* GR12-2/*acd*36. Because of higher levels of auxin provided by the auxin-overproducing bacteria *P. putida* GR12-2/*aux*1, the highest levels of ethylene were measured in this treatment (Fig. 3).

It is well accepted that both auxin and ethylene (Nordstrom and Eliasson 1984, Riov and Yang 1989, Robbins et al. 1985, Soffer et al. 1989) have the ability to stimulate the development of adventitious roots. In addition to root initiation, auxin promotes root elongation, whereas ethylene inhibits it (Jackson 1991). The results of the present study support the above data. Mung bean cuttings treated with either the wild type *P. putida* GR12-2 or *P. putida* GR12-2/*acd*36 developed more roots than cuttings incubated in water. The ACC deaminase minus mutant that induced higher ethylene production rates suppressed root elongation compared with the roots developed on cuttings treated with the wild type bacteria (Fig. 1). The higher amounts of auxin as may be provided by the bacterium mutant *P. putida* GR12-2/*aux*1 had a limited effect on the number of roots (Table 1). This may be the result of an excessive concentration of auxin. There is another possibility that cannot be ruled out: the high auxin-induced excessive concentration of ethylene which in turn inhibited root elongation, and thus the roots could not be observed and counted (Riov and Yang 1989).

The scanning electron microscope analysis was aimed at elaborating the nature of the association between the added bacterium and the surface of the mung bean cuttings. The results presented in Fig. 2 suggest a physical attachment between the bacteria and the cell wall of the mung bean epidermal layer. It is suggested that via these contact filaments, signals from the bacterium, possibly in the form of plant hormones, are transported to sites adjacent to the vascular bundles, thereby influencing the formation of root initials.

Taken together, the data presented in the present study indicate that PGPR such as *P. putida* GR12-2, which have been shown previously to be a suitable inoculant of seeds to enhance root development in seedlings, may also be a suitable inoculant of cuttings. The data also strengthen the view that ethylene is involved in the initiation and development of roots. Auxin-induced ethylene at a lower concentration promotes initiation of roots, but at a higher concentration it acts to suppress the emergence and elongation of roots.

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