

Note

A Simple and Rapid Method for Functional Analysis of Plant Growth-promoting Rhizobacteria Using the Development of Cucumber Adventitious Root System

Yeoung-Seuk Bae^{1*}, Kyungseok Park¹, Young-Gee Lee¹ and Ok-Hee Choi²

¹Plant Pathology Division, National Institute of Agricultural Science and Technology, RDA, Suwon 441-707, Korea

²Department of Applied Biology and Environmental Science, Gyeongsang National University, Jinju 660-701, Korea

(Received on July 1, 2007; Accepted on August 11, 2007)

Many plant growth-promoting rhizobacteria (PGPRs) have been known for beneficial effects on plants including biological control of soilborne pathogens, induced systemic resistance to plant pathogens, phytohormone production, and improvement of nutrient and water uptake of plants. We developed a simple and rapid method for screening potential PGPR, especially phytohormone producing rhizobacteria, or for analyzing their functions in plant growth using cucumber seedling cuttings. Surface-sterilized cucumber seeds were grown in a plastic pot containing steamed vermiculite. After 7 days of cultivation, the upper part 2 cm in length of cucumber seedling, was cut and used as cucumber cuttings. The base of cutting stem was then dipped in a microcentrifuge tube containing 1.5 ml of a bacterial suspension and incubated at 25°C with a fluorescent light for 10 days. Number and length of developed adventitious roots from cucumber cuttings were examined. The seedling cuttings showed various responses to the isolates tested. Some isolates resulted in withering at the day of examination or in reduced number of roots developed. Several isolates stimulated initial development of adventitious roots showing more adventitious root hair number than that of untreated cuttings, while some isolate had more adventitious root hair number and longer adventitious roots than that of untreated control. Similar results were obtained from the trial with rose cuttings. Our results suggest that this bioassay method may provide a useful way for differentiating PGPR's functions involved in the development of root system.

Keywords : cucumber seedling cuttings, plant growth-promoting rhizobacteria, adventitious root development

Microorganisms living in the plant rhizosphere interact with each other and with plant roots in several ways that affect plant growth and development (Asghar et al., 2002; Glick, 1995; Jetiyanon and Kloepper, 2002; Kloepper et al., 1989). The effects of soil microorganisms can be divided into beneficial to harmful, but most of interactions are considered to have no effect on plants. Beneficial free-living soil bacteria are often referred to plant growth-promoting rhizobacteria (Kloepper et al., 1989). Numerous studies have been reported on PGPR as biological agents to promote plant growth or control soilborne plant pathogens by introducing into soil or on plant seeds (Bae et al., 2004; Jetiyanon and Kloepper, 2002; Jeun et al., 2004; Kloepper and Schroth, 1981; Maurhofer et al., 1992).

A number of different bacteria have been known as PGPR such as species of *Azotobacter*, *Azospirillum*, *Pseudomonads*, *Acetobacteria*, *Burkholderia* and *Bacillus* (Brown, 1974; Glick, 1995; Kloepper et al., 1989). Mechanisms involved in plant growth promotion by PGPR have been known as indirect or direct effects. Indirect effect of PGPR on plant growth occur when PGPR suppress deleterious soil microorganisms or plant pathogens by the production of iron chelating siderophores (Kloepper et al., 1986; 1988) or antibiotics (Gardner et al., 1984; Maurhofer et al., 1992). Direct effects of PGPR occur when PGPR produce certain substances such as phytohormones (Dubeikovskiy et al., 1993; Janzen et al., 1992; Srinivasan et al., 1996) to plants that directly stimulate plant growth. For example, Patten and Glick (2002) reported that indoleacetic acid producing *P. putida* increased the length of canola seedling roots on average 35 to 50% longer than the roots from seeds treated with IAA-deficient mutant and the roots from uninoculated seeds.

We designed a simple and rapid method for screening potential phytohormone(s) producing rhizobacteria or for analyzing PGPR's functions in this study. This was achieved by analysing the development of adventitious root system on cucumber seedling cuttings, which were dipped in rhizobacterial suspensions.

The cucumber seedling cuttings were prepared as follows.

*Corresponding author.

Phone) +82-31-290-6827, FAX) +82-31-290-6839

E-mail) ysbae@rda.go.kr

*Present address: Ginseng Division, Ginseng & Medicinal Plants Research Institute, Eumseong 369-873, Korea

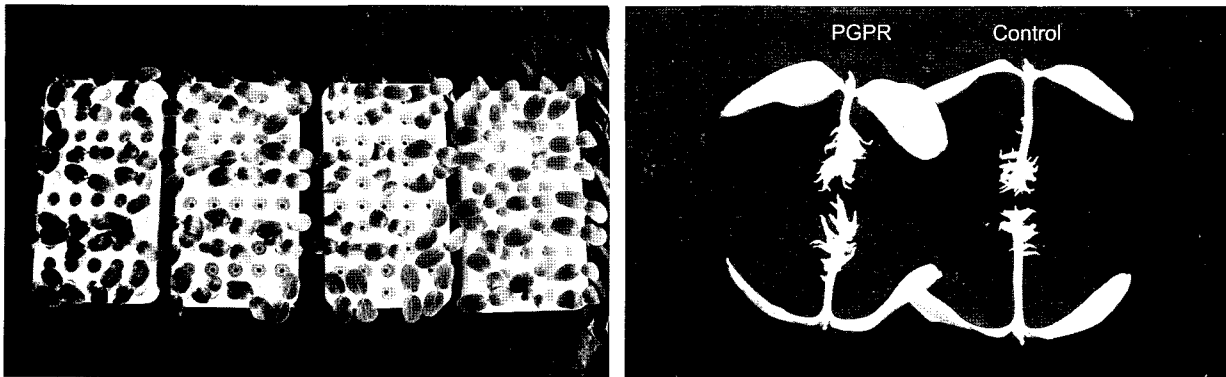


Fig. 1. Bioassay for the selection of potential plant growth-promoting rhizobacteria (left) and effect of a rhizobacterium on the development of adventitious root in cucumber cuttings (right).

Surface-disinfected cucumber seeds (*Cucumis sativus* L.) cv. Eunsung-bakdadaki (Semini Co., Korea) were planted in plastic pots containing vermiculite steamed. The plastic pots were placed in a greenhouse at 27°C for 7 days with watering at every day. The upper part of cucumber seedling, 2 cm long in the length of stem, was cut and used as cucumber cuttings.

Total 380 rhizobacteria isolates isolated from the rhizoplane of various plants were cultured on tryptic soy agar (TSA, Difco) at 27°C for 3 days. Each bacterium was resuspended in microcentrifuge tubes containing 1 ml of sterile distilled water at the final concentration of 5×10^6 cfu/ml. The cucumber cutting was placed in each bacterial resuspension and incubated at 25°C with 12 hours fluorescent light. At 10 days after incubation, number and length of adventitious roots developed from cucumber seedling cuttings were examined. From 3 independent experiments, 5 isolates (*Enterobacter* sp. strain B4, *Bacillus* sp. strain B70, *Bacillus lentimorbus* strain B145, *Pseudomonas chloraphis* strain B202, and *Pseudomonas putida* strain B351) were selected for promoting the development of adventitious root system, or for inhibiting the development of adventitious root system. Among 5 isolates, 4 isolates (B4, B70, B202, and B351) were tested for the development of root systems on rose cuttings in a greenhouse. Branches (5 cm long in length) of rose (*Rosa red-sandra* L. cv. Red Sandra) with a bud were dipped in 100 ml of each bacterial suspension (1×10^8 cfu/ml) and planted in a plastic pot (15-cm diameter \times 10-cm depth) containing perlite. The development of adventitious roots was investigated after 70 days of cultivation.

The cucumber seedling cuttings showed various responses to the isolates tested. Some isolates resulted in withering at the day of examination (Fig. 1), and other treatments showed the normal growth of adventitious roots. Treatment of *B. lentimorbus* B145 strongly inhibited the development of seedling roots without wilting of leaves, measured by

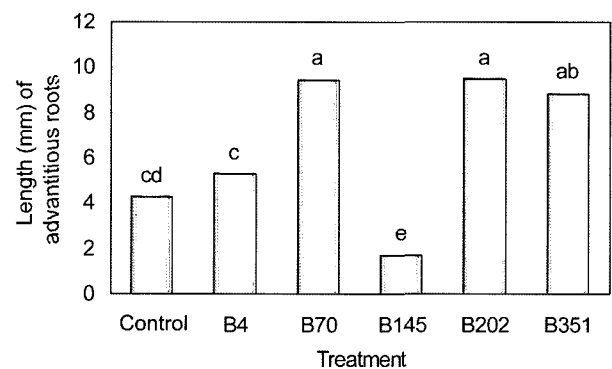


Fig. 2. Effect of rhizobacteria on the development of adventitious root length on cucumber cuttings. Control; untreated control, B4; *Enterobacter* sp., B70; *Bacillus* sp., B145; *Bacillus lentimorbus*, B202; *Pseudomonas chloraphis*, and B351; *Pseudomonas putida*. Means followed by the same letter are not significantly different at $p=0.05$.

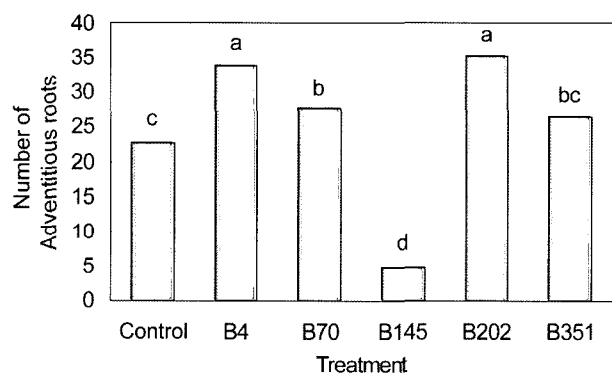


Fig. 3. Effect of rhizobacteria on the development of adventitious root number on cucumber cuttings. Control; untreated control, B4; *Enterobacter* sp., B70; *Bacillus* sp., B145; *Bacillus lentimorbus*, B202; *Pseudomonas chloraphis*, and B351; *Pseudomonas putida*. Means followed by the same letter are not significantly different at $p=0.05$.

means of number and length of adventitious roots. Treatment of most isolates (strain B4, B70, and B351)

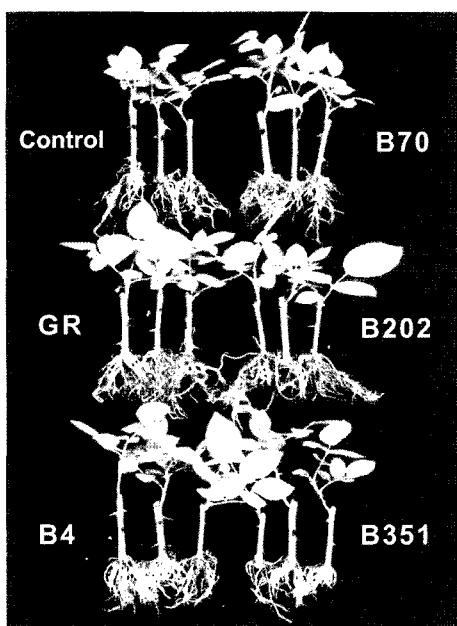


Fig. 4. Effect of rhizobacteria on the development of adventitious roots on rose cuttings. Control; untreated control, GR; sodium-5-mononitro-guaiacol, B4; *Enterobacter* sp., B70; *Bacillus* sp., B202; *Pseudomonas chloraphis*, and B351; *Pseudomonas putida*.

resulted in stimulated effect on only one way, that is more numbers or higher lengths of adventitious roots. However, treatment of *Pseudomonas chloraphis* strain B202 revealed stimulated effects on both adventitious root number and length developed from cucumber seedling cuttings compared to those of others (Fig. 2 and 3). However, there was no difference on the growth of stem or leaf between treatment during the experimental period (data not shown). Similar results were also obtained from the trial on rose cuttings. Treatment of *Pseudomonas chloraphis* strain B202 showed both more adventitious root number and longer adventitious roots than those of untreated control, while *Enterobacter* sp. strain B4 had only more adventitious root number (Fig. 4).

Our results suggest that the bioassay method may provide a useful way for screening potential PGPR and for differentiating PGPR's functions involved in the development of root system. Further work will be needed to understand the mechanisms related to different responses of plant growth by rhizobacteria.

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