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NOTE

Revegetation of a Lakeside Barren Area by the Application of Plant Growth-promoting Rhizobacteria

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The growth stimulation of wild plants by several bacterial species showing plant growth-promoting capabilities was examined in a barren lakeside area at Lake Paro, Korea. Microbial numbers and activities in the field soil were monitored for 73 days after inoculation of the bacteria. The acridine orange direct counts for the total soil bacterial populations ranged between 2.0-2.3×10^9 cells/g soil and 1.4-1.8×10^9 cells/g soil in the inoculated and uninoculated soils, respectively. The numbers of *Pseudomonas* spp., which is known as a typical plant growth-promoting rhizobacteria, and the total microbial activity were higher in the inoculated soil compared to those in the uninoculated soil. The average shoot and root lengths of the wild plants grown in the inoculated soil were 17.3 cm and 12.4 cm, respectively, and longer than those of 11.4 cm and 8.5 cm in the uninoculated soil. The total dry weight of the harvested wild plants was also higher in the inoculated soil (42.0 g) compared to the uninoculated soil (35.1 g). The plant growth-promoting capabilities of the inoculated bacteria may be used for the rapid revegetation of barren or disturbed land, and as biofertilizer in agriculture.

***Keywords*:** plant growth promoting rhizobacteria, revegetation, barren area, *Pseudomonas*

Root colonizing bacteria, or rhizobacteria, that exert beneficial effects on plant development are defined as plant growth-promoting rhizobacteria (PGPR). The enhancement of plant growth by many PGPR has been utilized for several decades, and the mechanism has been extensively studied since the 1990s (Vessy, 2003; Lucy *et al*., 2004). Although there may be some artificial and trivial plant growth-promoting effects induced by the inoculation of certain soil bacteria, overall evidence shows that there are significant and overwhelming effects induced by rhizosphere microorganisms (Gerhardson and Wright, 2002). The mechanisms for plant growth promotion by non-pathogenic, plant-associated bacteria have not been completely elucidated, however, several direct or indirect mechanisms have been described, including direct phytohormonal action, plant disease suppression, enhancement of plant nutrient availability, and the enhancement of other beneficial plant microorganisms (Lucy *et al*., 2004).

Many studies examining PGPR inoculation have focused on certain economically important agricultural crops (Kloepper *et al*., 1989; Bertland *et al*., 2001; Kokalis-Burelle *et al*., 2006) and trees (Chanway, 1997; Lucas García *et al*., 2004), but there are few reports on wild flora (Carrilo-Garcia *et al*., 2000). Although some semi-arid regions have been covered with plants as a result of afforestation policies, many barren lands have recently appeared for reasons such as deforestation and construction work. Lake Paro, which is a large artificial reservoir in Korea containing 900 million tons of oligotrophic water, was almost drained for many years because of a political situation in the Korean peninsula, and a large submerged area was exposed (Fig. 1). Such depleted areas spoil the beauty of the lake landscape; moreover, bare lakeside lands are prone to erosion and collapse. Tree planting is the most efficient means for the vegetation and afforestation of barren land areas. However, revegetation, either natural or artificial, may not be appropriate in certain areas, like the steep

Fig. 1. Bare lakeside lands at Lake Paro, Korea

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rocky lakeside of Lake Paro. Therefore, PGPR could be a candidate for revegetating such unproductive lands. In this study, the revegetation of a lakeside barren area and growth enhancement of wild plants were examined by inoculating PGPR that was previously shown to promote plant growth in microcosms composed of soil collected from the same area (Jeon *et al*., 2003).

The revegetation field study was performed in the bare lakeside of Lake Paro in Korea during a 73-day period from March to May, 2005. Large stones and organic and inorganic debris were removed from the surfaces of two adjacent 5 m^2 plots (2.5 m×2 m) selected as the inoculated and uninoculated control plots. A composite top soil sample (1 kg) was collected for physicochemical analyses that included soil texture, pH, and organic matter content. The predominant plant species in this area were common lespedeza (*Kummerowia striata*), water agrimony (*Bidens tripartita*), foxtail grass (*Setaria viridis*), a species of sedge (*Carex leiorhyncha*), a species of common millet (*Panicum bisulcatum*), a species of umbrella sedge (*Cypetus amuricus*), evening primrose (*Oenothera erythrosepala*), and hedge parsley (*Caucalis scabra*) (Ahn *et al*., 2004), and their seeds were collected from nearby areas in autumn of 2004. Five grams of each seed were evenly distributed on the soil surface and covered with a 1-2 cm layer of the same soil. Five kinds of PGPR were suspended in distilled water and sprayed on the soil surface with a strainer (10^6 cells/g soil/ml water for each strain). The bacterial strains used in this study were *Pseudomonas fluorescens* strains MC07, B16, and M45. These strains had been isolated by Dr. C. Park in the Department of Agricultural Biology at Gyeongsang National University from the rhizospheres of monocotyledonous plants in mountainous areas, and had previously shown plant growth-promoting capabilities (Yeom and Park, 1995). The other PGPR used were *Bacillus megaterium* and *Azotobacter vinelandii* that had been isolated from forest soils in Kangwon-do, Korea, and had also shown plant growth promotion (Jeon *et al*., 2003). The bacteria were cultivated in Nutrient Broth medium (Difco, USA) on a rotary shaker (160 rpm, 30°C) for 48 h. The bacterial cells were harvested by centrifugation, washed with sterile distilled water, and utilized as an inoculum.

Since plant growth seems to be partially affected by the direct and indirect stimulation of indigenous and/or introduced microorganisms, the microbial community of the field soil in the study area was analyzed by measuring bacterial numbers and activities. Approximately 50 g of composite soil samples were collected from each plot initially and then again at 38 and 73 days after inoculation. The soil suspensions were diluted in a 10-fold series, and the properly diluted suspensions were used for enumeration of the soil bacteria. For the direct counting of total bacteria (acridine orange direct count, AODC), a 10 μl amount of soil suspension was filtered through black polycarbonate membrane filters (pore size 0.2 μm, dia. 25 mm, Nuclepore, USA), and stained with acridine orange (final con. 0.01%) (Hobbie *et al*., 1977). The stained bacterial population was counted under an epifluorescent microscope (Olympus BX60, Japan). The initial numbers of bacteria (AODC) in the uninoculated plot were $1.6\times10^9 \pm 0.3\times10^9$, and ranged between $1.4\text{-}1.8\times10^9$ cells/g soil during the study period. In the inoculated soil, the bacterial number was higher throughout the study period ($2.0\text{-}2.3\times10^9$ cells/g soil), and this may be due to PGPR inoculation and a subsequent increase of organic materials provided from enhanced plant growth. The increase of total bacterial populations by the introduction of PGPR was also reported in a forest soil community (Shishido and Chanway, 1998). Of the inoculated microorganisms we monitored for change in the number of *Pseudomonas* spp., which are known as a typical PGPR (Misko and Germida, 2002; Gamalero *et al*., 2003). The viable count of *Pseudomonas* sp. was determined on Pseudomonas Isolation Agar medium (Difco, USA) using the diluted soil suspension. Previously, it was reported that *Pseudomonas* sp. showed various plant growth-promoting capabilities and occupied 35% of the rhizobacterial population (Misko and Germida, 2002; Gamalero *et al*., 2003; Çakmakçi *et al*., 2006). The enumeration method for *Pseudomonas* sp. used in this study showed a high correlation with the fluorescent in situ hybridization method of the previous report (Jeon *et al*., 2003). The initial number of *Pseudomonas* sp. was $3.5\times10^3 \pm 0.6\times10^3$ CFU/g soil and it did not change significantly in the uninoculated soil during the 73-day study period. The *Pseudomonas* counts greatly increased to 1.3×10^6 CFU/g soil in the inoculated soil immediately after the introduction of *P. fluorescens*, when the bacterial inoculum was applied at 10^6 cells/g soil. This high number decreased to 10^4 CFU/g soil at 38 and 73 days, however, counts were still higher than in the uninoculated plot. The survival and population change of the introduced strains should be further investigated.

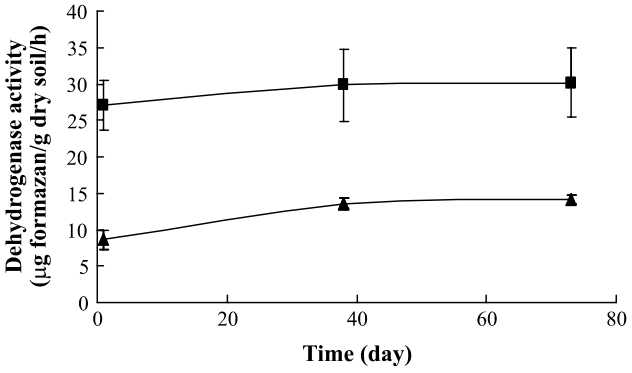

Fig. 2. Change of total microbial activity measured by dehydrogenase assays in the uninoculated soil (▲) and inoculated soil (○).

The total soil microbial activity was measured by dehydrogenase assay using INT [2(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride] (Prosser, 1997). One gram (wet wt.) of a soil sample was mixed with 1 ml of sodium phosphate buffer (60 mM, pH 7.6) and 0.5 ml of 0.2% INT solution and incubated for 1 h in a water bath (30°C). After centrifugation of the incubated sample (6400×g, 10 min), the precipitated soil was mixed with 1 ml of methanol, mixed vigorously, and centrifuged again. The OD of the supernatant was measured at 480 nm. The dehydrogenase activity was 8.6±1.4 μg formazan/g soil/h at the beginning of the experiment and increased over 50% in the uninoculated soil during the study (Fig. 2). This increase might have been induced by an increase in the activity of other soil organisms

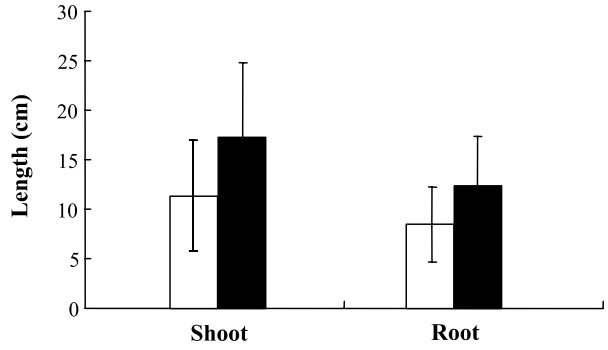

Fig. 3. Comparison of average shoot and root lengths of wild plants grown in the uninoculated soil (open bar) and inoculated soil (closed bar) in a barren lakeside area at Lake Paro.

occured with some indigenous plants during spring time. The dehydrogenase activitiy of the inoculated plot was higher than that of the uninoculated plot over the period studied and seemed to be directly and/or indirectly responsible for plant growth, and accounted for the increased numbers of AODC and *Pseudomonas* sp. A similar pattern of microbial activity was observed in a soil microcosm of similar soil type (Jeon *et al*., 2003). Strigul and Kravchenko (2006) suggested that the most effective PGPR inoculation can be expected in organic and mineral poor soils or stressed soils. The top soil in our study area was a very coarse sand with low organic matter (1.2% measured by the loss-on-ignition method), and these poor physicochemical characteristics were likely favorable to the survival of the introduced bacteria. In addition, the lakeside at Lake Paro is unproductive land due to the leaching of colloidal minerals and organic matter by the lake water, resulting in a low water holding capacity.

Plant germination began approximately 2 weeks after establishing the experimental plots. The plant growth in the inoculated plot appeared to be more favorable than that in the uninoculated site. After a 73-day period of growth, all the plants grown in the plots were harvested without root loss, and washed with distilled water to remove the remaining soil particles. Plants were dried in an oven at 80°C for 24 h, and the shoot and root lengths and dry weights of the whole plants were measured. The average shoot lengths of the plants were 17.3 cm and 11.4 cm and the root lengths were 12.4 cm and 8.5 cm in the inoculated and uninoculated plots, respectively (Fig. 3). After 73 days there was an approximate 50% enhancement of root and shoot elongation in the wild plants resulting from inoculation. Also, the total dry weight of the combined biomasses of all plants grown in the inoculated plot (42.0 g) was higher than that of the uninoculated plot (35.1 g). The PGPR used in this study were reported to produce auxin and related phytohormones and soluble phosphatase (Jeon *et al*., 2003); moreover, these bacteria can produce gibberellins and siderophores (data not shown). All these activities may have been utilized in the growth promotion of the wild plants in this study. The increasing rate of dry weight in the wild plants was lower than the elongation rates for the plant shoots and roots. Differences in growth stimulation between plant length and biomass have been observed frequently (Lucas García *et al*., 2004; Çakmakçi *et al*., 2006), which may be partly due to the water content of plants. Bashan and de-Bashan (2005) suggested that fresh weight determination should not be used to evaluate the effects of PGPR on plants.

Ultimately, hundreds of wild plants grew in the barren lakeside study area, but the most frequent species were evening primrose (*Oenothera erythrosepala*), *Youngia sonchifolia* (indigenous to Asia), hedge parsley (*Caucalis scabra*), dayflower (*Commelina communis*), a species similar to hogweed (*Ambrosia trifida*), a species similar to bindweed (*Calystegia japonica*), *Hemistepta lyrata* (indigenous to Asia), *Ixeris polycephala* (indigenous to east Asia), and poor man's pepper (*Lepidium apetalum*). Among the eight plant species sowed, only *Oenothera erythrosepala* and *Caucalis scabra* grew well in the study plot. PGPR may alter plant species differently due to specific interactions between the species and bacterial strains (Lucas-García *et al*., 2004; Lucy *et al*., 2004), and the bacterial strains used in this study may not have been compatible with the 6 plant species that did not grow well. Other factors such as climate, the soil organic matter content and physicochemical characteristics, and biotic factors may modify the effects of PGPR on plants (Chanway, 1997; Çakmakçi *et al*., 2006). Here, *Caucalis scabra* was the predominant species of the species that were sown, although a quantitative analysis was not performed.

Lake Paro is an environmentally sensitive area and usage of chemical fertilizers and pesticides should be limited at lakeside areas to prevent water pollution. This lakeside territory at Lake Paro is an unproductive land with some steep rocky slopes. In such a sensitive and barren area, PGPR may be a good candidate for use in revegetation because its inoculation can be most effective in stressed soils, when development of the resident microorganisms is inhibited (Strigul and Kravchenko, 2006). The selection of appropriate microorganisms and plant species, as well as the roles and formulation method of PGPR should be further investigated for plant growth promotion via the microbial inoculation of barren ground areas.

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References

Ahn, T., H. Song, and J. Park. 2004. Rehabilitation of naked shoreline of lakes and reservoirs. Report No. 1403013-1-1. Korea Institute of Environmental Science and Technology.

Bashan, Y. and L.E. de-Bashan. 2005. Fresh-weight measurements of roots provide inaccurate estimates of the effects of plant growth-promoting bacteria on root growth: a critical examination. *Soil Biol. Biochem.* 37, 1795-1804.

Bertland, H., R. Nalin, R. Bally, and J.C. Cleyet-Marel. 2001. Isolation and identification of the most efficient plant growth promoting bacteria associated with canola (*Brassica napus*). *Biol. Fertil. Soils* 33, 152-156.

Çakmakçi, R., F. Dönmez, A. Aydin, and F. Şahin. 2006. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biol. Biochem*. 38, 1482-1487.

Carrilo-Garcia, Á., Y. Bashan, E. Rivera, and G. Bethlenfalvay. 2000. Effects of resource-island soils, competition, and inoculation with *Azospirillum* on survival and growth of *Pachycereus pringlei*, the giant cactus of the Sonoran desert. *Restoration Ecol*. 8, 65-73.

Chanway, C.P. 1997. Inoculation of tree roots with plant growth promoting soil bacteria: An emerging technology for reforestation. *For. Sci*. 43, 99-112.

Gamalero, E., L. Fracchia, M. Cavaletto, J. Garbaye, P. Frey-Klett, G. Varese, and M. Martinotti. 2003. Characterization of functional traits of two fluorescent pseudomonads isolated from basidiomes of ectomycorrhizal fungi. *Soil Biol. Biochem*. 35, 55-65.

Gerhardson, B. and S. Wright. 2002. Bacterial associations with plants: Beneficial, non N-fixing interactions, p. 79-103, *In* K. Sivasithamparam, K. Dixon, and R. Narrett (eds.), Microorganism in plant conservation and biodiversity. Kluwer Academic Press, London, UK.

Hobbie, J., R. Daley, and S. Japer. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33, 1225-1228.

Jeon, J.S., S.S. Lee, H.Y. Kim, T.S. Ahn, and H.G. Song. 2003. Plant growth promotion in soil by some inoculated microorganisms. *J. Microbiol*. 41, 271-276.

Kloepper, J., R. Lifshits, and R. Zablotowicz. 1989. Free living bacterial inocula for enhancing crop productivity. *Trends Biotechnol*. 7, 39-44.

Kokalis-Burelle, N., J. Kloepper, and M. Reddy. 2006. Plant growth-promoting rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. *Appl. Soil Ecol*. 31, 91-100.

Lucas-García, J., J. Domenech, C. Santamaría, M. Camacho, A. Daza, and F. Gutiérrez-Mañero. 2004. Growth of forest plants (pine and holm-oak) inoculated with rhizobacteria: relationship with microbial community structure and biological activity of its rhizosphere. *Environ. Exp. Botany* 52, 239-251.

Lucy, M., E. Reed, and B. Glick. 2004 Application of free living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek* 86, 1-25.

Misko, A. and J. Germida. 2002. Taxonomic and functional diversity of pseudomonads isolated from the roots of field-grown canola. *FEMS Microbiol. Ecol*. 42, 399-407.

Prosser, J.I. 1997. Microbial processes within the soil, p. 183-213. *In* J. van Elsas, J. Trevors, and E. Wellington (eds.), Modern Soil Microbiology. Marcel Dekker, Inc., New York, USA.

Shishido, M. and C. Chanway. 1998. Forest soil community responses to plant growth-promoting rhizobacteria and spruce seedlings. *Biol. Fertil. Soils* 26, 179-186.

Strigul, N. and L. Kravchenko. 2006. Mathematical modeling of PGPR inoculation into the rhizosphere. *Environ. Model Softw*. 21, 1158-1171.

Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* 255, 571-586.

Yeom, J.R. and C.S. Park. 1995. Enhancement of plant growth and suppression of damping-off of cucumber by low temperature growing *Pseudomonas fluorescens* isolates. *Kor. J. Plant Pathol*. 11, 252-257.