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Microcosm Study for Revegetation of Barren Land with Wild Plants by Some Plant Growth-Promoting Rhizobacteria

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Abstract Growth promotion of wild plants by some plant growth-promoting rhizobacteria (PGPR) was examined in the microcosms composed of soils collected separately from a grass-covered site and a nongrass-covered site in a lakeside barren area at Lake Paro, Korea. After sowing the seeds of eight kinds of wild plants and inoculation of several strains of PGPR, the total bacterial number and microbial activity were measured during 5 months of study period, and the plant biomasses grown were compared at the end of the study. Acridine orange direct counts in the inoculated microcosms, 1.3–9.8×10^9 cells·g soil^{-1} in the soil from the grass-covered area and 0.9–7.2×10^9 cells·g soil^{-1} in the soil from the nongrass-covered site, were almost twice higher than those in the uninoculated microcosms. The number of *Pseudomonas* sp., well-known bacteria as PGPR, and the soil dehydrogenase activity were also higher in the inoculated soils than the uninoculated soils. The first germination of sowed seeds in the inoculated microcosm was 5 days earlier than the uninoculated microcosm. Average lengths of all plants grown during the study period were 26% and 29% longer in the inoculated microcosms starting with the grass-covered soil and the nongrass-covered soil, respectively, compared with those in the uninoculated microcosms. Dry weights of whole plants grown were 67–82% higher in the inoculated microcosms than the uninoculated microcosms. Microbial population and activity and growth promoting effect by PGPR were all higher in the soils collected from the grass-covered area than in the nongrass-covered area. The growth enhancement of wild plants seemed to occur by the activities of inoculated microorganisms, and this capability of PGPR may be utilized for rapid revegetation of some barren lands.

Key words: Revegetation, barren land, microcosm, plant growth-promoting rhizobacteria

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Although there may be some artificial and trivial effects on plant growth promotion induced by the inoculation of some soil bacteria, the overall evidence showing significant plant growth effects induced by rhizosphere microorganisms is overwhelming [11, 15]. This enhancement of plant growth by many plant growth-promoting rhizobacteria (PGPR) has been utilized for several decades [27]. The mechanisms of plant growth promotion by nonpathogenic, plant-associated bacteria have not been completely elucidated, but the important mechanisms include direct phytohormonal action, plant disease suppression, increase of plant nutrient availability, and the enhancement of other plant beneficial microorganisms [12, 19, 23].

To date, many studies on the introduction of PGPR have focused on some economically important agricultural crops [4, 7, 21] and trees [7, 18], but wild flora has not been considered as an important research target. Recently, PGPR application to three grass species was reported for phytoremediation of creosote-contaminated soil [13]. Although some harsh or semiarid regions without vegetation have been covered with plants as a result of the forestation policy, many barren land areas have newly appeared owing to various reasons, such as deforestation and construction works. 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 has been exposed. These depleted areas spoil the beauty of the lake landscape; moreover, bare lakeside lands are prone to erosion and collapse. Natural or artificial revegetation of such lakeside land is difficult, for geological, topographical, and biological reasons. In this study, the enhancement of the growth of wild plants sowed and revegetation in lakeside soil by the introduction of some PGPR, previously shown to promote growth of indigenous plants in the soil microcosms

composed of soil collected from nearby barren area [14], were examined.

MATERIALS AND METHODS

Microorganisms

Bacterial strains used in this study were *Pseudomonas fluorescens* strains MC07, B16, and M45, which had been isolated by Dr. C. Park in the Department of Agricultural Biology at Gyeongsang National University, from the rhizosphere of monocotyledonous plants at mountainous areas and have shown plant growth promotion [16, 29]; and *Bacillus megaterium* and *Azotobacter vinelandii*, which had been isolated from forest soils in Kangwon-do, Korea, and have also shown plant growth promotion [14]. All bacteria were cultivated in Nutrient Broth medium (Difco Lab., U.S.A.) on a rotary shaker (160 rpm, 30°C), harvested and washed with sterile distilled water, and utilized as inoculum.

Microcosm Study for Growth Promotion of Wild Plants

Plant growth promotion was investigated in the microcosms containing surface soils from a bare lakeside area of Lake Paro, Korea. Surface soils (15 cm depth) were collected separately from a site covered with some wild plants and a neighboring nongrass-covered site, and sieved through a mesh with 4 mm diameter to remove large organic and inorganic debris, and the physicochemical properties (texture, pH, moisture content, and field capacity) were analyzed [25]. All soils were sandy with low organic content (1.2% in the nongrass-covered soil and 1.9% in the grass-covered soil) measured by loss-on-ignition method [2]. Ten kg of sieved soils was poured into plastic containers [45 (W)× 35 (L)×30 (H) cm]. The seeds of eight kinds of wild plants (0.1 g each) that had been collected from nearby areas in previous autumn were evenly distributed on the soil surface and covered with a 1–2-cm layer of the same soil. Sowed plants, *Kummerowia striata*, *Bidens tripartita*, *Setaria viridis*, *Carex leiorhyncha*, *Panicum bisulcatum*, *Cypetus amuricus*, *Oenothera erythrosepala*, and *Caucalis scabra*, were the predominant species in the lakeside area of Lake Paro [1]. The washed bacteria suspended in distilled water were sprayed on the surface soil with a strainer (10^6 cells·g^{-1} soil· ml^{-1} water for each strain). The inoculated and uninoculated control microcosms were maintained in a greenhouse in which the air temperature was set at 20°C, and water was sprayed regularly to adjust the soil moisture content to 60% of field capacity.

The triplicate microcosms were established in November, 2005, and soil microorganisms and plant growth were monitored along a 5-month period. About 10 g of composite soil samples was collected from the undisturbed surface layer of each microcosm immediately after inoculation and on every 30 days, and the followings were determined; acridine orange direct count (AODC), viable count of *Pseudomonas* sp. on *Pseudomonas* Isolation Agar medium (Difco Lab., U.S.A.), and total microbial activity as determined by a dehydrogenase assay using INT [2(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride]. The experimental methods have been previously described in detail [14]. After 5 months of growth, all the plants grown in the microcosms were cautiously harvested without root loss, and washed with distilled water to remove the remaining soil particles. Plants were dried in an oven (80°C) for 24 h, and the entire length of each plant and dry weight of whole plants were measured. All experiments were carried out in triplicate, and the mean values are presented.

RESULTS

Changes of Microbial Populations and Activity in Soil Microcosm

Total bacterial population measured by AODC in the nongrass-covered and grass-covered soils at the beginning of the experiment were 4.5×10^8 and 8.3×10^8 cells·g^{-1} soil, respectively, and these numbers in the uninoculated microcosm soils increased to $4.1-4.5\times10^9$ cells·g^{-1} soil (Fig. 1). AODCs in the microcosms containing the soils from the nongrass-covered and grass-covered sites became 9.1×10^8 and 1.3×10^9 cells·g^{-1} soil, respectively, right after inoculation of PGPR, continuously increased thereafter, and reached to 7.2×10^9 and 9.8×10^9 cells·g^{-1} soil, respectively. Bacterial populations in both inoculated microcosms composed of soils from the grass-covered and nongrass-covered areas were about 2 times higher than those in the uninoculated microcosms. The microcosm containing the grass-covered soil showed a slightly higher level of AODC compared with that of the nongrass-covered soil.

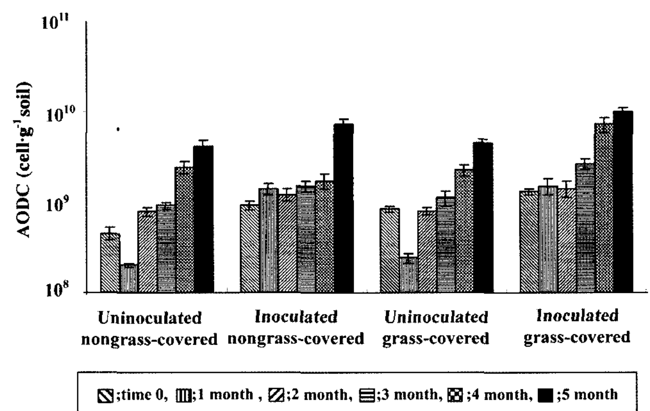

Fig. 1. Change of acridine orange direct count (AODC) in the uninoculated and inoculated microcosms composed of soils from the grass-covered and nongrass-covered areas.

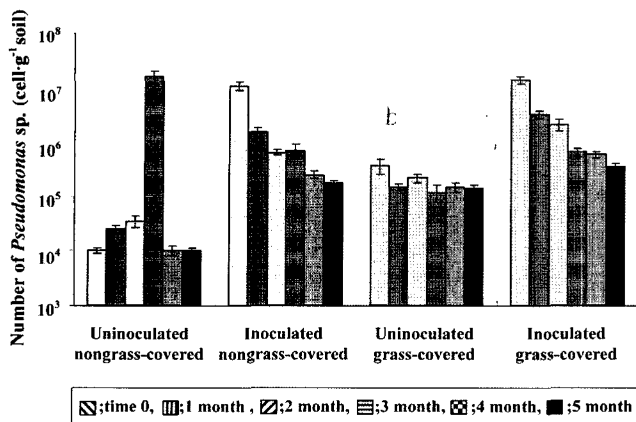

Fig. 2. Change of number of *Pseudomonas* sp. in the uninoculated and inoculated microcosms composed of soils from the grass-covered and nongrass-covered areas.

Of the inoculated microorganisms, change in the population of *Pseudomonas* sp. was monitored. The initial numbers of *Pseudomonas* sp. were 1.0×10^4 and 3.7×10^5 CFU·g^{-1} soil in the microcosm with the nongrass-covered soil and grass-covered soil, respectively, and they were not significantly changed in the inoculated microcosm soils along the period studied (Fig. 2). However, *Pseudomonas* counts greatly increased to $1.1-1.4\times10^7$ CFU·g soil^{-1}, immediately after the introduction of bacterial inoculum containing 3 strains of *P. fluorescens* applied at 10^6 cells·g soil^{-1} for each strain. This elevated number reduced continuously to $1.8-3.5\times 10^5$ CFU·g soil^{-1} in 5 months, which was still higher than those in the uninoculated microcosm soil. As like the AODC result, the microcosms containing soil from the grass-covered area showed a higher level of populations of *Pseudomonas* sp. compared with those with soil from the nongrass-covered site.

Microbial activity measured by the electron transport system assay (dehydrogenase activity) was 2.9 and 20.0 μg

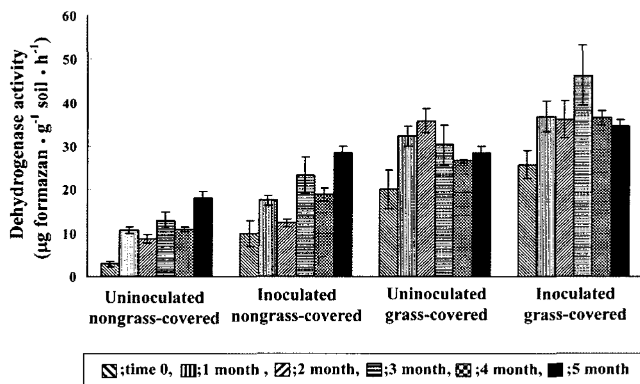

Fig. 3. Change of total microbial activity measured by dehydrogenase assay in the uninoculated and inoculated microcosms composed of soils from the grass-covered and nongrass-covered area.

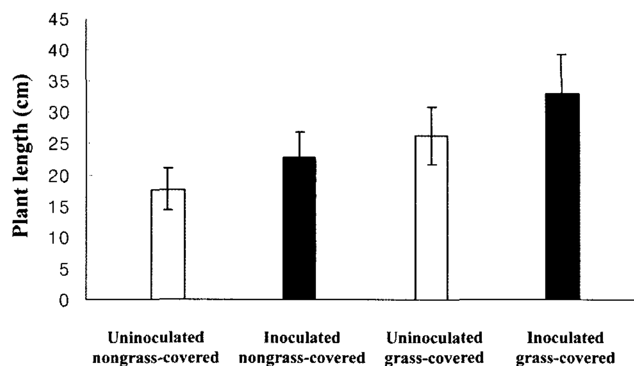

Fig. 4. Comparison of average of entire plant length grown in the uninoculated (open bar) and inoculated (closed bar) microcosms composed of soils from the grass-covered and nongrass-covered area.

formazan· g^{-1} soil·h^{-1} in the nongrass-covered and grass-covered soils, respectively at the beginning of the experiment (Fig. 3). Although there were some fluctuations, they increased over 50% during the rest of the study period. Dehydrogenase activities in the inoculated microcosms were higher than those in the uninoculated soils along the entire period studied, and the activities in the microcosms with the soil from the grass-covered area were always higher compared with those of the soil from the nongrass-covered area.

Growth of Wild Plants in Soil Microcosm

Germinated plants in the inoculated soils appeared about 15 days after establishing the microcosms, and the germination of plants in the uninoculated microcosms was delayed approximately 5 days. During the study period, plant growth in the inoculated microcosms seemed to be more favorable than that in the uninoculated microcosms. After the 5-month study period, the average lengths of wild plants grown were 17.7 cm and 26.2 cm in the uninoculated microcosms

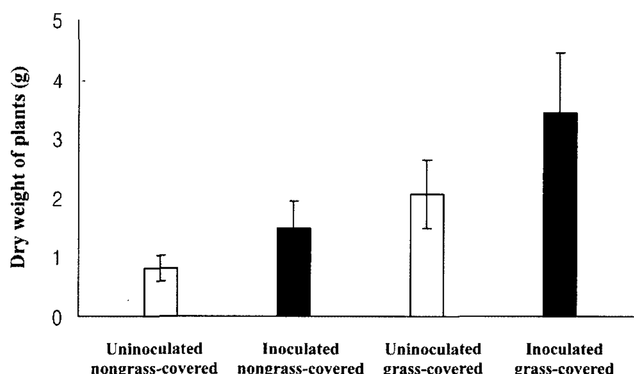

Fig. 5. Comparison of the dry weights of wild plants grown in the uninoculated (open bar) and inoculated (closed bar) microcosms composed of soils from the grass-covered and nongrass-covered areas.

from the nongrass-covered and grass-covered soil, respectively (Fig. 4). About 26% and 29% enhancement of elongation of wild plants occurred by the inoculation of PGPR in 5 months in the microcosms containing the grass-covered soil and nongrass-covered soil, respectively.

Total dry weights of the plant biomass grown were 0.82 and 2.06 g in the uninoculated microcosms composed of the nongrass-covered and grass-covered soils, respectively (Fig. 5). Introduction of PGPR augmented the plant biomass by 67% and 82% in the microcosms containing the grass-covered soil and nongrass-covered soil, respectively. Many wild plants grew in the microcosms, and the dominant species were *Bidens tripartita* and *Setaria viridis* in the microcosm from the nongrass-covered soil, and *Setaria viridis* and *Carex leiorhyncha* in the microcosm with the grass-covered soil, although the quantitative analysis was not performed.

DISCUSSION

The microbial community in the microcosm soil was analyzed by population enumeration and activity measurement to examine the effect of PGPR introduction. The total bacterial number measured by acridine orange direct counting was lower in soils from the nongrass-covered area than soils from the grass-covered area (Fig. 1), and it might be due to the difference of organic content. The textures of both soils utilized in this study were almost similar [a coarse sand soil (sand 88%, silt 10%, clay 2%) with pH of 5.2], but the grass-covered soil had a higher organic content (1.9%) compared with that in the nongrass-covered soil (1.2%). Some other growth factors present in the soil (type of carbon substrate, inorganic nutrients, etc.) from the grass-covered site could affect soil microbial population. AODC in the uninoculated soils continuously increased after 1 month of study, and it was likely coincident with the germination and growth of wild plants. Introduction of PGPR into the microcosms almost doubled the bacterial population in both soils. This increase was likely due to synergistic effects on indigenous soil bacteria by inoculated PGPR. Part of the increase may be ascribed to the regular moisture addition to microcosm soil that increased the biodegradation of indigenous substrates in soil. The increase of AODC continued thereafter, and populational jump occurred especially during the last 2 months, which seemed to be by seasonal change from winter to spring and resulting rapid plant growth. The cells of PGPR, their metabolites, and cell components might increase the total bacterial count. Increase of total bacterial population by introduction of PGPR was also reported in a forest soil community [24].

Among the inoculated PGPR species, change in the population of *Pseudomonas* sp. known as a typical PGPR was monitored. *Pseudomonas* sp. showed various plant growth-promoting capabilities and occupied 35% of the rhizobacterial population [6, 10, 20]. The enumeration method for *Pseudomonas* sp. used in this study showed a high correlation with the fluorescent *in situ* hybridization method in the previous report [14]. The initial number of *Pseudomonas* sp. was 1.0×10^5 and 3.7×10^5 CFU·g^{-1} soil in the nongrass-covered and grass-covered soils, respectively, and they showed some fluctuations in the uninoculated microcosms. However, *Pseudomonas* counts greatly increased right after the introduction of bacterial inoculum containing 3 strains of *P. fluorescens*, and the elevated numbers higher than 10^6 CFU·g^{-1} soil might be as a result of concentration of inoculum in a surface soil layer. They reduced continuously until the end of the study, but were still higher than those in the uninoculated microcosms. Similar pattern of change was reported in the soil collected from a nearby site at Lake Paro [14]. Strigul and Kravchenko [26] suggested that the most important factor for survival and effects of PGPR was expected in organic and mineral poor soils or stressed soils. The soil utilized in this study was a coarse sand with low organic matter, and these poor physicochemical characteristics were likely favorable to survival of the introduced bacteria. The survival and population changes of the introduced strain itself should be further investigated.

Dehydrogenase activity, which has been often used to detect total microbial activity in soil [22], was 2.9 and 20.0 μg formazan·g^{-1} soil·h^{-1} in the nongrass-covered and grass-covered soils, respectively at the beginning (Fig. 3). Although AODCs in the nongrass-covered soil were not much lower than those in the grass-covered soil, the dehydrogenase activity in the nongrass-covered soil was far less than the grass-covered soil, and this result seemed to be from the large proportion of dead and dormant bacteria in the unproductive nongrass-covered soil. The increase of dehydrogenase activity after establishment of the microcosm was likely due to the maintenance of better environmental conditions in the microcosm, such as moisture content and temperature, and the resulting increase of activity of many soil organisms including plants. Dehydrogenase activities were also increased by inoculation of PGPR in both soils, and they were higher than those in the uninoculated microcosm during the entire study period, which seemed to promote directly and indirectly plant growth together and account for the increased numbers of total bacteria and PGPR including *Pseudomonas* sp. Similar pattern of microbial activity measured by fluorescein diacetate hydrolysis assay was also observed in the soil from a nearby site [14].

Plant growth began about 15 days after establishing the inoculated microcosm, but it was delayed 5 days in the uninoculated microcosm. PGPR not only could help the germination of plant seed and consequent root elongation [9], but also shortened the time required for germination [13]. The time reduced for germination of Tall fescue was

very similar to that in this study, although it was germinated in the presence of creosote [13]. In this study, plant growth in both the inoculated microcosms appeared to be more favorable than those in the uninoculated microcosms, and the average of the entire length and dry weight of all plants grown were higher in the inoculated microcosms (Figs. 4, 5). Increasing rate of dry weight of wild plants was higher than that of plant length. Difference of growth stimulation between plant length and biomass was observed frequently [6, 18], and it seemed to be different among plant species grown. Gamalero *et al.* [10] also observed the higher increasing rate of fresh weight than the length of cucumber by PGPR inoculation.

The PGPR utilized in this study were reported to produce auxin and related phytohormones, solubilize insoluble phosphates, and excrete phosphatase [14]; moreover, these bacteria could produce gibberellins, siderophore, and hydrogen cyanide (data not shown). All these activities would be utilized in the growth promotion of wild plants in this study. The increasing rates of entire length and dry weight of wild plants by application of PGPR were higher in the nongrass-covered soil (29 and 82%, respectively) than those in the grass-covered soil (26 and 67%, respectively) (Figs. 4, 5). Strigul and Kravchenko [26] also suggested that the effects of PGPR was expected to be more effective in organic and mineral-poor soils or stressed soils when development of the resident microflora was inhibited. The soils collected from a lakeside area at Lake Paro was an unproductive soil owing to the leaching of colloidal minerals and organic matter by repetitive fluctuation of the water level and resulting low water holding capacity, and thus showed the higher plant growth enhancing effects by PGPR introduction. Although Çakmakçi *et a*l. [6] reported the higher growth stimulation of sugar beet by PGPR application in high organic matter (15.9%) soil than in the low organic matter (2.4%) soil, their 15.9% of organic content was much higher than those in this study. Not only content, but also various properties of soil organic matter may influence PGPR and their plant growth-promoting activity.

Many species of wild plants grew up in the microcosms; however, precise study on the species diversity could not be performed because of incomplete plant growth. Tentative dominant species, *Bidens tripartita* and *Setaria viridis*, in the microcosm from the nongrass-covered soil, and *Setaria viridis* and *Carex leiorhyncha* in the microcosm from the grass-covered soil were all sowed plants. The PGPR effect may occur differently among plant species because of some specific interactions between plant species and bacterial strain such as plant-bacteria compatibility and bacterial colonization capacity [18, 19], and the bacterial strains used in this study may not be compatible with some plant species sowed. Although some strains in this study have several functions together for plant growth promotion, the creation of transgenic strains that combine multiple mechanisms of action was suggested for improvement of PGPR strains [5, 8]. Other factors, such as climate, organic matter content, and physicochemical characteristics of soil and biotic factors may influence the PGPR effect [6, 7].

To date, plant growth promotion by PGPR has been mainly applied to some economically important agricultural crops and trees [17, 18]. However, PGPR application to wild flora may be utilized for some specific purposes, such as phytoremediation of heavily contaminated soils [13]. Tree planting is the most efficient way of vegetation and forestation of barren land areas, and there are some other means of revegetation including latticed block pitching measure and netting measure [28]. However, it may not be applicable to some areas like a rocky region, wasteland, and contaminated site, and then wild plants and PGPR can be a candidate for revegetation of such unproductive lands. Moreover, PGPR inoculation may be most effective in stressed soils and harsh conditions, when indigenous soil microorganisms are inhibited [3, 26]. PGPR can be also applied in environmentally sensitive areas, such as lakeside and riverside territories in which usage of chemical fertilizers and pesticides is limited. Selection of appropriate plant/microorganism species and the roles and the formulation method of PGPR should be further investigated for the application of plant growth promotion by microbial inoculants to barren grounds.

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